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Stem Cells as A Double-Edged Sword in Treatment of Liver Diseases

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ABSTRACT

The liver is an organ known to have tremendous regenerative capacity. Liver pathologies affect millions of patients worldwide. Stem cells are promising tools as a regenerative medicine for the treatment of degenerative disorders, inborn errors of metabolism, and organ failure. Although stem cells have a desired role in treatment of various diseases, it has an undesirable role in generation and progression of tumor cells.

Under the right conditions in the body or laboratory, stem cells divide to form more cells called daughter cells. Hematopoietic stem cells (HSCs) are the predominant population of stem cells within bone marrow and express CD34 as the cell surface marker; they can renew themselves and differentiate into progenitor cells. Mesenchymal stem cells (MSCs) are a rare population in bone marrow which is capable of self-renewal and differentiation into hepatocytes-like cells as well as cell types of mesenchymal origin. MSCs based therapy has been suggested as an attractive therapeutic option for treatment of liver cirrhosis and fibrosis due to immune modulatory properties.

On the other side, liver cancer stem cells (LCSC) have an important role in occurrence and development of liver cancer as in hepatocellular carcinoma. The development of cancer recurrence, metastasis, and chemo- and radioresistance in a solid tumor is attributed to the presence of CSCs. Accumulating evidence demonstrated the existence of a small subset of cancer cells with stem cell properties and several cancer stem cells (CSCs) markers have been identified. Therapies targeting these cells may have great potential for clinical treatment and prognosis of liver cancer result in eradication of liver CSCs. Therefore**,** the aim of this study is to focus on the recent advances in understanding of the biology of liver CSCs, and the development of strategies for their treatment.

Keywords: *Stem cells, Mesenchymal stem cells, hepatocellular carcinoma, inflammation*

1. Introduction

With high morbidity and mortality, liver disease presents a major threat to human health. The liver is essential for maintaining life. It is responsible for bile production, metabolism of nutrients, removal of toxins, blood purification,

and immune responses. There are more than 100 different kinds of liver diseases, including fascioliasis, hepatitis, alcoholic liver disease, fatty liver disease, hereditary disease, cirrhosis, etc. These diseases may lead to liver failure and result in an irreversible liver damage. Although the liver can repair itself, when the damage is beyond repair it is dangerous and fatal to patients (Michalopoulos & DeFrances, 1997). According to statistics, the liver disease is among the top 10 leading causes of death in the world (Sentürk, Yücedag, Polat, & Technologies, 2018) Currently, the most effective treatment option for both chronic and acute liver failure is liver transplant. However, the donor organs are limited, and the cost of surgery is high (A. T. Song et al., 2014). In addition, the recipient immune system may reject the transplanted organ (Soltys et al., 2017). Therefore, the development of alternative therapeutic strategies for patients with serious liver diseases is an urgent need. The liver could heal itself to maintain its important functions. Hepatocytes are the type of cells that make up 70–85% of the liver mass, which will self-renewal after injury. However, this ability will be impacted under the conditions that cause severe liver injury. A possible therapeutic strategy to repair the liver function is to transplant hepatocytes. However, lots of hepatocytes (approximately 5×10^9 cells) are needed for transplantation therapy and these cells are difficult to expand in culture (Forbes, Gupta, & Dhawan, 2015). Recent advances in the cell biology show the therapeutic potential of stem cells (Volarevic, Nurkovic, Arsenijevic, & Stojkovic, 2014). Compared with hepatocytes, stem cells are easier to culture and expand **in vitro**. In addition, they could differentiate into hepatocytes and other liver cell types. Due to these properties, stem cells are an attractive option for liver tissue regeneration. Lots of studies have shown that a variety of stem cell types are promising candidates for liver cell replacement (Kopp, Grompe, & Sander, 2016). This review summarizes current stem cell-based therapies for the treatment of liver diseases and discusses their potentials.

1.1. Sources of stem cells

Several studies have reported the successful isolation of MSCs from different tissues with similar *in vitro* properties, including synovial membrane (De Bari, Dell'Accio, Tylzanowski, & Luyten, 2001) adipose tissue (AT) (Zuk et al., 2001) umbilical cord blood (UCB) (O. K. Lee et al., 2004) amniotic fluid (AF) (Antonucci et al., 2011) and placenta. Umbilical cord tissue (UC) has been a particularly promising source of MSCs – cells can be isolated from several compartments within UC, including umbilical vein, umbilical arteries, umbilical cord perivascular tisssues, MSCs isolated from UC tissue are believed to be more primitive than other cells isolated from other tissues and are found in higher numbers, ensuring this source is gaining prominence(Fukuchi et al., 2004).

1.2. Characteristics of liver stem cells (LSCs)

LSCs are adult stem cells found in the liver, mainly including hepatic oval cells (HOC) and small hepatocyte-like progenitor cells (SHPC). An oval shape, small cell body, large nucleus, and small cytoplasm characterize HOC, which can differentiate into hepatocytes or bile duct Epithelial cells in both directions (Miyajima, Tanaka, & Itoh, 2014). The morphology of SHPC is between that of stem cells Hepatocytes. LSCs are located around the portal vein and in the Hering duct of the terminal bile duct (B. Wang, Zhao, Fish, Logan, & Nusse, 2015). A study had identified a population of proliferating and self-renewing cells adjacent to the central vein in the liver lobule. These cells can differentiate into hepatocytes and replace all hepatocytes along the liver lobule during homeostatic renewal (Herrera et al., 2013). Liver stem Cells are ideal for treating liver diseases because of their directional differentiation into Hepatocytes or cholangiocytes, and lake of ethical concerns. Still, there are rare reports about human liver stem cell lines at home and abroad (Bi, Liu, et al., 2019; Herrera et al., 2010). After years of exploration, research groups (Bi et al., 2019) and (S. Li et al., 2019) had successfully developed and isolated human liver stem cell line from the adult liver. Human Liver stem cells can be maintained for 50 generations, and two mouse liver stem cell lines can be passed for more than 100 generations. At present, all these cells have been applied in experimental animal research.

1.3. Method of Stem Cell Transplantation

Autologous transplantation: uses the patient's own stem cells. These cells are removed, treated, and returned to his or her own body after a conditioning regimen (Bi, Li, et al., 2019).

Allogeneic transplantation: uses stem cells from a donor. A donor may be a family member or someone who is not related to the patient. (Bi, Li, et al., 2019). MSCs can be isolated from umbilical cord or cord blood, placenta, and amniotic fluid. They have been shown to be more potent than BM-MSCs (Hass, Kasper, Böhm, & Jacobs, 2011). With respect to the involvement of MSCs in liver regenerative medicine, many studies have demonstrated that the infusion of human umbilical cord- or cord blood-derived MSCs via a vein or multiple direct injections into the liver effectively relieves liver cirrhosis and improves the survival rate in rodent models (Almawi et al., 1991). These studies also suggest that multiple infusions show better clinical outcomes as compared to a single infusion of allogeneic hematopoietic stem cell transplantation (allo-HSCT) inorder to prevent graft versus host disease

(GVHD) and associated organ-tissue lost. Donor T cells recognize different histocompatibility antigens of the host cells as foreign. As a result of antigen recognition, activated T cells secrete cytokines such as interleukin (IL) -1, IL-2, tumor necrosis factor (TNF), and gamma-interferon, so that GVHD process begin(Antin & Ferrara, 1992) GVHD is classified as acute and chronic according to time of onset. Classically acute GVHD occurred within 100 days after transplantation. Rarely, patients may present acute GVHD findings after than 100 days which is named persistent, recurrent, late onset acute GVHD'. 'Overlap syndrome' is another clinical scenario which may carry both acute and chronic GVHD features at any time after HSCT (Filipovich et al., 2005). Numerous risk factors have been identified for development of acute GVHD, one of the most important one is acute GVHD prophylactic regimen used. Immunosuppressive agents for prophylaxis and treatment of GVHD are evaluated in two major categories: Non-specific immunosuppressives and specific T cell mediated drugs; antibodies and other drugs (Gale et al., 1987) and (L. L. Shi, Liu, & Wang, 2011).

1.4. Non-specific immunosuppressive drugs

Corticosteroids: agents that are preferentially used, combined with other immunosuppressive in acute GVHD treatment and thought to reduce T cell count (Almawi et al., 1991).

Methotrexate: folic acid antagonist that inhibits dihydrofolate reductase In GVHD prophylaxis, it is thought to reduce activated T cell proliferation (Almawi et al., 1991).

1.5. Specific T cell immunosuppressive drugs

Antibodies: Sirolimus (rapamycin) is a macrolide that is currently approved by the FDA used only in solid organ transplantation in order to prevent organ rejection, tacrolimus, and mycophenolate.

Other drugs; Rituximab and Ruxolitinib (Matsuda & Koyasu, 2000).

1.6. Bone Marrow-Derived Mesenchymal Stem Cells (BM-MSCs) in Autologous Liver Cirrhosis Therapy

Mesenchymal stem cells possess unique biological properties. They are not only able to differentiate into abroad range of mesodermal lineages but also other embryonic lineages. These cells amplify their clinical utility through other innate properties such as their self-renewing capacity, homing and migration ability, immunosuppressive potential, and paracrine effects such as anti-inflammation, anti-apoptosis, anti-fibrosis/anti-scarring (matrix re-modeling) and angiogenesis (Newman, Yoo, LeRoux, & Danilkovitch-Miagkova, 2009). These features make MSCs the most potent and preferred therapeutic tool in regenerative medicine.

Several experimental and pre-clinical studies demonstrate that MSCs seem to have a crucial role in the improvement of liver regeneration in many liver disease models, especially in cirrhosis. Most clinical studies utilizing MSCs to treat liver cirrhosis are in phase I or II trials. Much of the focus is to evaluate the safety, feasibility, and efficacy of the treatment. While several clinical trials have used various sources of MSCs for liver cirrhosis treatment, BM-MSC-based therapy has successfully entered phase III trials. Since phase III clinical trials involve many patients, they provide more robust evidence of the potency of BM-MSCs in treating liver cirrhosis (W. H. Liu et al., 2015).

1.7. Types of the most used stem cells; Mesenchymal and Hematopoietic stem cells

Mesenchymal stem cells: MSCs have been shown to exert beneficial effects in a range of clinical settings, including the treatment of degenerative and immune-mediated diseases while also being reported to ameliorate liver injury in the setting of both acute and chronic liver damage. In both *in vivo* and *in vitro* experiments, MSCs have demonstrated the ability to differentiate into hepatocyte-like cells with liver-specific morphology and function in the presence of cytokines and growth factors including HGF, fibroblast growth factor (FGF), oncostatin M (OsM), epidermal growth factor (EGF), leukemia inhibitory factor, and insulin-like growth factor (IGF) (Schwartz et al., 2002). This pluripotent capacity is demonstrated by the presence of human hepatocyte markers such as albumin, α-fetoprotein (AFP), CK18, and CK19 in liver tissue of cirrhotic rats after human UC- derived MSC administration (Zhang, Sun, Zheng, Guo, & Zhang, 2017). MSCs can be isolated easily from a wide variety of tissues and can be expanded in vitro without changing their properties (Hoogduijn et al., 2007). MSCs can be injected into patients by allogeneic transplantation because of their low immunogenicity. MSCs represent a heterogenous population of multipotent stemcells, which can be isolated from different tissues including heart,spleen,bonemarrow (Kern, Eichler, Stoeve, Klüter, & Bieback, 2006). One of crucial characteristics of MSCs is their ability to regulate immune response. It has been well documented that MSCs inhibit lymphocyte proliferation, suppress production of proinflammatory cytokines and alter the balance of Th1/Th2/Th17/Treg lymphocytes (Kong et al., 2009) and (Svobodova et al., 2012). In addition, the secretion of various growth and

trophic factors (Haynesworth, Baber, & Caplan, 1996) enables them to support tissue regeneration ,inhibit apoptosis and exert a cytoprotective effect (Gu et al., 2013). Together with their low immunogenicity, MSCs provide promising features for their use in treating many harmful immune reactions (Cejkova et al., 2013).

1.8. Differentiation of MSCs into Hepatocyte like Cells:

Most in vitro studies demonstrated that MSCs had the capacity to differentiate into hepatocyte-like cells with liver-specific morphology and function with the help of specific growth factors, such as HGF, EGF, FGF, and OSM (W. H. Liu et al., 2015). Furthermore, (Yan, Xue, Wu, Liu, & Hou, 2015) showed that by mimicking the microenvironment of liver fibrosis using 50 g/L rat fibrotic liver tissue extracts, hUC-MSCs were stimulated to differentiate into hepatocyte-like cells in a shorter period. (Kuo et al., 2008) study indicated that MSCs can differentiate into liver-like cells under the action of growth factors cytokines, hepatocytes or non-parenchymal hepatocytes, and participate in the immune regulation, cell proliferation and injury repair in liver diseases (W. H. Liu et al., 2015). MSCs are rich in sources, easily obtainable and cultured, have low immunogenicity. Based on these advantages, MSCs are expected to become an ideal source of seed cells for stem cell research in liver diseases.

1.9. MSC therapy for liver cirrhosis

Liver cirrhosis (LC) is a complication of liver disease that involves the loss of liver cells and irreversible scarring of the liver tissue.

1.9.1. Mechanism of the Anti-fibrotic Effect of MSC

Liver fibrosis is characterized by the deposition of extracellular matrix (ECM), including collagen I, collagen III and collagen IV (Martinez-Hernandez & Amenta, 1993). Multiple signaling pathways, such as TGF-β/Smad, Ras/ERK, Notch, and Wnt/β-catenin, are involved in HSC activation. Kupffer cell activation is an important factor that induces HSC activation during chronic liver injury (J. N. Wang et al., 2018). Kupffer cells are resident macrophages in the liver. Activated Kupffer cells release large amounts of soluble mediators, such as oxidants, cytokines, and proteinases, which can affect HSC proliferation, migration, and differentiation (Tacke, 2017).

1.9.2. Mechanism of the Immuno-modulatory Effects of MSC

Inflammatory reactions are widely detected in injured liver tissues and are considered the primary causes of fibrosis and hepatic function failure (Zhangdi et al., 2019). A study by (Zhou, Yamamoto, Xiao, & Ochiya, 2019) reported that MSC therapy can reduce inflammation in liver diseases through different mechanisms. MSCs exhibit immunomodulatory functions through paracrine mechanisms. MSCs release multiple immunosuppressive factors, such as IL-10, VEGF, and TGF-β that may be related to reductions in the differentiation potential and proliferation of MSCs. The study suggested that MSCs at earlier passages were more suitable for therapy than cells at later passages due to their stability and more potent anti-inflammatory properties. Another study documented that age reduced human MSC-mediated T cell suppression (Kizilay Mancini, Shum-Tim, Stochaj, Correa, & Colmegna, 2015).

1.9.3. Therapeutic mechanisms of MSCs in hepatic immune microenvironment

Effects of MSCs on innate immune response

Macrophages: There are two forms of macrophages: the activated M1 macrophage and the alternatively activated M2 macrophage. Imbalance in M1/M2 polarization could lead to HSC activation and hepatocyte injury (Kizilay Mancini et al., 2015).

Neutrophils: are found to exacerbate acute liver injury by inducing inflammatory mediators (e.g., IL-1β and TNF-α) and oxidative killing of hepatocytes. However, an antifibrotic role of neutrophils has been reported in CCl_4-induced liver fibrosis by producing MMP8 and MMP9, and depletion of neutrophils delay the regression of liver fibrosis (Saijou et al., 2018).

1.10. MSC therapy for liver failure

Liver failure is a major health problem worldwide due to the variety of acute or chronic injuries that are induced by alcohol consumption, hepatotoxic drugs, autoimmune attack of hepatocytes, or infection with viruses, such as hepatitis B virus (HBV) and hepatitis C virus (HCV). Several controlled trials demonstrated that MSCs play a supportive role in the treatment of liver failure and show satisfactory tolerability and beneficial effects on liver synthetic functions and hepatic fibrosis resolution (Gaude, Chaudhury, & Hattiholi, 2015).

1.11. MSC therapy for complications of liver transplantation

Orthotopic liver transplantation is the only curative measure for patients with end-stage liver failure. However, the risk of complications after liver transplantation is still high, even with increases in surgical expertise. Two of the most common complications following liver transplant are rejection and infection. MSCs offer new therapeutic opportunities to prevent and treat solid organ transplant rejection. A pilot study demonstrated that UC-MSC therapy can alleviate liver damage and improve allograft histology in liver transplant patients with acute graft rejection who did not respond to immunosuppressive agent dose adjustments. However, the study was not carried out long enough to determine whether decreased infection resulted from MSC infusion (M. Shi et al., 2017).

1.12. Disadvantages of stem cells

Although stem cell therapy is promising treatment for liver diseases, It also have number of disadvantages that we will be; embryonic stem cells can have high rejection rate, adult stem cells have a determined cell type, obtaining any form of stem cell is a difficult process, stem cell treatments are an unproven commodity, stem cell research is a costly process, we do not know if there are long-term side effects to worry about, there will always be some limitation to the research possibilities, research has been held back by factual contradictions, research opportunities are somewhat limited, especially in the United States, adults have very few stem cells, current embryonic stem cell harvesting requires the death of an embryo (Evans & Kaufman, 1981). In addition to these disadvantages there are some issues about using stem cells.

Clinical issues: The current difficulty is finding suitable stem cell donors. Also, there is difficulty in storing a patient's embryonic stem cells. The cells would have to be collected before birth; some clinics offer to store blood from the umbilical cord when a person is born. Mutations have been observed in stem cells cultured for several generations, and some mutated stem cells have been observed to behave like cancer cells. Cultured stem cells could be contaminated with viruses which would be transferred to a patient (Solter, 2006).

Ethical issues: A source of embryonic stem cells is unused embryos produced by in vitro fertilization (IVF). For therapeutic cloning, there is a question of whether it is right to create embryos for therapy and destroy them in the process. Embryos could come to be viewed as a commodity, not as an embryo that could develop into a person. At what stage of its development is an embryo to be regarded and treated as a person (Lo & Parham, 2010).

Social issues: The importance of educating the public about what stem cells can do and the benefits of stem cell use patient could be exploited by paying for expensive treatments and being given false hope of a cure, as stem cell therapies are only in their developmental stages. From all the above, we noticed that the importance of stem cell research because advantages are more than disadvantages but this shouldn't mean that the disadvantages are insignificant (Habets, van Delden, & Bredenoord, 2014).

1.13. Surface markers of liver cancer stem cells and targeted therapies for Hepatocellular carcinoma

Liver cancer nearly develops in the setting of chronic liver disease (CLD), in which continuous inflammation and hepatocyte regeneration occur (Fan et al., 2011). Pathophysiological changes take place during long-term inflammation/regeneration processes that work coordinately to initiate and/or promote liver cancer .

Liver cancer is the fifth most commonly diagnosed cancer and the second most frequent cause of cancer death in men worldwide (Jemal et al., 2011). Among primary liver cancers, hepatocellular carcinoma (HCC) represents the major histological subtype, accounting for 70%–85% of cases of primary liver cancer. Traditional therapy strategies currently available for HCC include surgical procedures, radioactive particle implantation, radiofrequency ablation, hepatic artery chemoembolization, and chemotherapeutics. Recent studies have indicated that these therapy strategies are still not fully efficient and have multiple drawbacks, including post-treatment relapse, chemotherapy drug resistance and metastasis (Bruix & Sherman, 2011). Immunohistochemical studies of stem cell markers suggest that HCC contain a subset of cells expressing a variety of stem cell markers (Cardinale et al., 2012). In HCC, Liver cancer stem cells (LCSCs) represent a small fraction of cells in HCC cancer tissues that possess the abilities of self-renewal, multi-directional differentiation, and indefinite proliferation, as well as high tumorigenic ability (Skubitz et al., 2013). As specific markers of CSCs, the CSC-specific overexpressed receptors may offer a new research direction as therapeutic targets for the diagnosis and treatment of tumors. Currently, potential clinical treatments targeting CSC include blocking signal transduction pathways in CSCs; inducing differentiation of CSCs; changing the microenvironment and inhibiting telomerase activity in CSCs; specific gene therapy targeting CSCs; specific compounds or drugs targeting CSCs; and ligands targeting CSCs (Iacopino et al., 2014).

1.14. LCSCS and their origin

Immense amount of endogenous stem cells in liver are responsible for its regenerative capacity. These cells are commonly derived from undifferentiated liver oval cells, known as hepatic precursor cells (HPC), and located in

the terminal bile canaliculi beside the interlobular bile duct (Turner et al., 2011). Oval cells have both the ability to differentiate into hepatocytes and bile duct cells, which, in human HCC, display the properties of stem cells. Additionally, most hepatic stem cell surface markers are the same as hepatic oval cell markers OV6, OV1, cytokeratin 7 (CK7) and CK19, α-fetoprotein (AFP), KIT proto-oncogene receptor tyrosine kinase (c-kit), and Thy-1 cell surface antigen (Thy-1). OV6 expression is a specific phenotype of oval cells that was originally identified in the livers of tumor-bearing rats, and is recognized as a surface marker of human liver progenitor cells (W. Yang et al., 2008). Yang et al., reported that overexpression of OV6 enhances the invasiveness and metastasis potential of HCC stem cells, and that increased numbers of OV6+ CSCs in patients with liver cancer indicate worst clinicopathological features and poorer prognosis (Yang et al., 2012). LCSCs can self-replicate, differentiate, and present strong drug resistance. (L. L. Liu, Fu, Ma, & Shen, 2011) hypothesized that CSCs are not derived from a specific source of cells in hepatitis-B (HBV)-associated HCC and may be derived either from hematopoietic stem cells (HSC) or from MSC (Fig. 1). The specific surface marker for HSCs is CD133, while the specific surface markers for MSCs are CD90 and CD44. Both HSCs and MSCs can differentiate into pluripotent stem cells (PSCs). PSCs can then differentiate into liver precursor cells/oval cells that express OV6 and epithelial cell adhesion molecule (EpCAM). PSCs and liver precursor cells can be induced into CSCs by the mechanism of 'maturation arrest', thus leading to the occurrence of liver cancer (L. L. Liu et al., 2011).

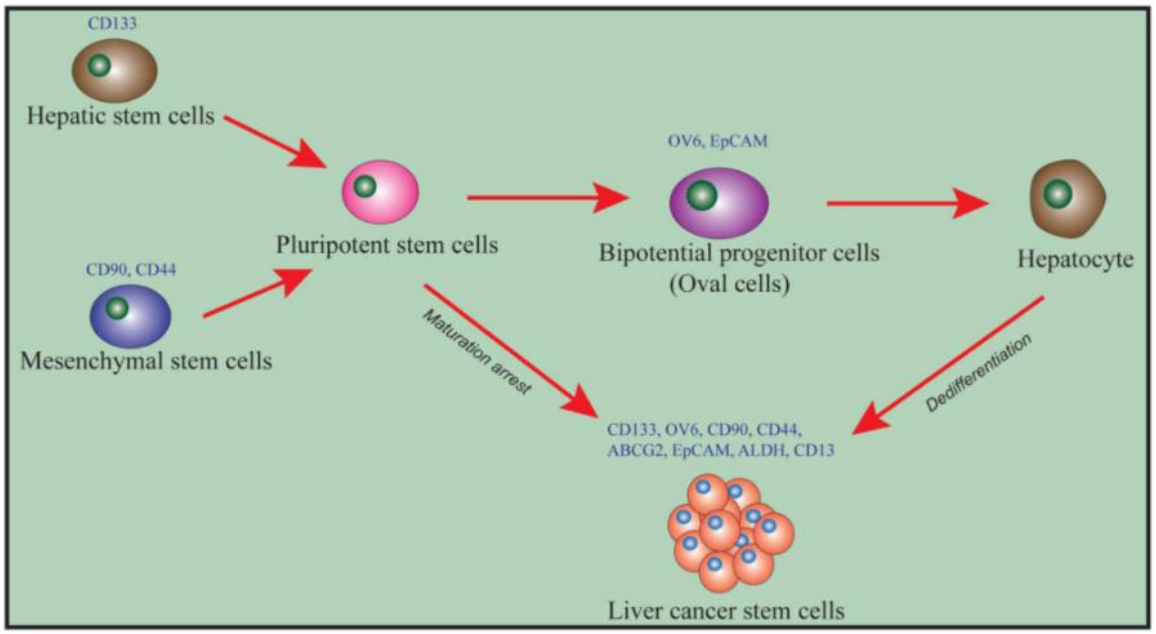

Fig. (1): Possible cellular origins and markers of LCSCs. HCC may arise from cells at various stages of differentiation in the hepatic stem cell lineage: Mature liver cells; liver progenitor cells or oval cells as bipotential stem cells; and bone marrow stem cells, including hematopoietic and mesenchymal stem cells as multipotent liver stem cells. HCC could originate from stem cells either due to 'maturation arrest' or to 'dedifferentiation of mature cells'. LCSCs, liver cancer stem cells; HCC, hepatocellular carcinoma; CD133, prominin-1; OV, oval cell marker antibody; EpCAM, epithelial cell adhesion molecule; ABCG2, ATP binding cassette subfamily G member; ALDH, aldehyde dehydrogenase (Qiu, Li, Fu, Chen, & Lu, 2018).

Several studies demonstrated that LCSCs can originate from the 'blocked maturation' LSCs, because most HCCs consist of mixtures of mature cells and cells with a phenotype like HPCs. Immunophenotyping analysis of HCCs has further indicated that 28–50% of HCC cells express HPC surface markers, such as CK7 and CK19 (Durnez et al., 2006). These tumors also include intermediate cells between HPC and mature liver cells. Furthermore, Yeh *et al.* reported that the expression levels of CD133 were negatively correlated with the expression levels of HBV surface antigen (HbsAg) in HBV-associated liver cancer tissue samples, indicating that LCSCs more likely originate from blocked liver stem cells, rather than differentiated liver cells post-infection. Therefore, various

LCSC markers can be detected in HBV-associated clinical samples of HCC. There is also evidence suggesting that LCSCs may be derived from bone marrow stem cells and side population (SP) cells (Haraguchi et al., 2006).

1.15. LCSCS and their characteristics in HCC

Drug resistance is associated with the recurrence and metastasis of cancer. CSCs resist chemotherapy-induced cell death through various mechanisms, including intrinsic and external mechanisms. The intrinsic mechanism consists of the self-renewal ability of CSCs, the enhancement of DNA damage repair pathways, the high expression of drug efflux-related proteins, the overactivation of growth pathways and other stem-related pathways. The external mechanism refers to the influence of tumor microenvironment factors on CSC resistance, including hypoxia stimulation, epithelial-mesenchymal transition (EMT) signals, and angiogenesis abnormalities (Maugeri-Saccà, Vigneri, & De Maria, 2011). In HCC, SP cells or LCSCs expressing other molecular markers (including EpCAM, CD133, CD90, CD44 and CD13) exhibited resistance to radiotherapy and chemotherapy *in vitro* and *in vivo*. The mechanisms involved include increased expression of drug efflux-related proteins, activation of anti-apoptotic pathways, activation of stem cell-related pathways, and increased resistance and maintenance of a certain number of LCSCs (Xin et al., 2013). A previous study revealed that the viability, distant metastasis, and homing ability of LCSCs in the circulatory system were significantly higher than that of other tumor cells. This may be explained by the EMT status of LCSCs, which enables them to serve a leading role in the metastasis and invasion of HCC and to become the source of HCC recurrence (Fonsato et al., 2012). Theoretically, tumor recurrence may be effectively prevented if a method to eliminate CSCs could be developed, making CSCs a desirable diagnosis and treatment target for resistant tumors, including HCC. This would be especially true for cases with poor therapeutic effect by traditional methods. LCSC-targeted therapy is thus hypothesized to achieve excellent antitumor effects and to reduce the side effects of chemotherapy, providing novel more efficient strategies for the treatment of cancer (Philip et al., 2009).

1.16. LCSCS surface markers

With the identification of specific surface markers, LCSCs can be successfully separated and enriched through screening for these markers by fluorescence-activated and magnetic-activated cell sorting methods. If LCSC-specific molecular markers are targeted and blocked, the number of LCSCs may be reduced, potentially resulting to inhibited tumor growth and recurrence. To date, the commonly reported LCSC surface markers are EpCAM (also known as CD326), CD133, CD90 (also known as Thy-1), CD44, and CD13 (Yamashita et al., 2013). In addition, other surface markers have also been demonstrated to be involved, including OV6, K19, c-kit (also known as CD117), ATP binding cassette subfamily G member 2 (ABCG2), and aldehyde dehydrogenase (ALDH) (Mima et al., 2012).

1.17. Markers of HSCs

Prominin-1 (CD133): The HSC surface marker CD133 was used to isolate LCSCs in HCC. CD133 is expressed on the surface of stem cells in many solid tumors, including liver, colon, brain, lung, and prostate cancer, and in B16 melanoma. In human HCC cell lines, ~0–65% of cells are CD133+ cells. CD133 is considered one of the main LCSC markers, with self-renewal, multi-lineage differentiation and chemoresistance abilities (Xin et al., 2013). CD133± LCSCs were also resistant to interferon-induced autophagy. Therefore, the identification of targeted molecular markers is of great significance (J. Li et al., 2016).

CD13 (aminopeptidase N, zinc binding protein): It has 2 types CD13+ cells, exhibit features similar to that of stem cells, such as increased cell proliferation and tumor cell formation, and increased resistance to chemotherapy. CD13+ cells are resistant to adriamycin, fluorouracil (5-FU) and cyclophosphamide treatment, and expression of CD13 is enhanced by chemotherapy. The expression of the glutamate-cysteine ligase (GCLM) gene is significantly increased compared with other cells. GCLM catalyzes intracellular antioxidant glutathione synthesis, against reactive oxygen species induced by chemotherapy/radiotherapy, thereby protecting DNA from DNA damage, preventing apoptosis, and resulting in drug resistance (Bralet, Pichard, & Ferry, 2002).

CD13- cells, exhibit an increased response to oxygen clusters, leading to DNA breakage and cell death in the presence of chemotherapy drugs. CD13+ cells are predominantly in the G1/G0 phase of the cell cycle, suggesting that CD13 may be a marker of the dormant or semi-stationary status of LCSCs (Mima et al., 2012).

CD90 (Thy-1): First was isolated from human T cell leukemia, CD90 overexpression was demonstrated to be associated with age in patients with HCC and HBV infection, tissue staging and high CD90 expression were associated with poor prognosis and has 2 types; CD90+, was isolated from HCC present increased tumorigenic abilities and indefinite proliferation compared to CD90-. Yang et al., noted that HCC tumor samples and most blood samples contain highly tumorigenic CD90+/CD45− cells, while samples from normal individuals or patients with chronic hepatitis do not. If the surface marker glycoprotein CD44 was also expressed in the CD90+ cells, the invasive phenotype was even stronger, with increased metastatic and self-renewal capacities. When CD44 was blocked by an inhibiting antibody, the tumor formation and metastasis abilities of CD90+ cells were decreased, and apoptosis was induced. CD90 has been shown to upregulate the expression of the molecular marker CD133, and this abnormal expression can promote tumor progression. CD45−/CD90+ cells also express other stem cell markers, including CD133, epithelial specific antigen (ESA), CXCR4, CD24, kinase insert domain receptor and CD44 (Z. F. Yang et al., 2008).

CD44: is a glycoprotein encoded by a single gene, and hyaluronic acid is its main receptor. As an important class of adhesion molecules, CD44 is widely distributed on the cell surface of various cell types, including lymphocytes, monocytes, and endothelial cells, and it is involved in intercellular cell adhesion and cell migration. CD44 may be associated with tumor cell invasion and metastasis of liver cancer (Noto et al., 2013). CD44 is overexpressed in tumor cells and mainly involved in heterotypic adhesion (the adhesion of tumor cells to the host cells and the host matrix), thereby promoting tumor cell invasion and metastasis. Its co-expression with other markers can better identify LCSC phenotypes. It was observed in a nude mouse model that the tumor formation rate of CD44+ cells was faster compared with CD44− cells. Compared with CD133+/CD44− cells, CD133+/CD44+ HCC cells were more prone to tumor formation and drug resistance and expressed more stem-associated genes. CD133+/CD90+ cells were more aggressive than CD44+ cells alone (Zhu et al., 2010).

EpCAM: It's transmembrane glycoprotein. It is currently used in research for various tumor types. EpCAM is expressed during the early liver development process, but not in normal mature liver cells. EpCAM is expressed in human epithelial tissue and tumors, as well as in precursor cells and stem cells. It is also present in liver stem cells and hepatoblasts. Nevertheless, the high expression of EpCAM is significantly associated with activation of cell proliferation. EpCAM is also expressed on the surface of LCSCs and pancreatic CSCs (Schmelzer & Reid, 2008). EpCAM+ HCC cells were significantly higher in Tumor formation and invasion compared with EpCAM− HCC cells. Liver stem cell surface markers were expressed in EpCAM+ cells, while the expression of mature hepatocyte markers was significantly increased in EpCAM− cells. CD90 and EpCAM expression obtained from HCC tumor cell lines were confirmed in human HCC samples (Alibolandi, Ramezani, Sadeghi, Abnous, & Hadizadeh, 2015).

CD47: **i**s a widely expressed integrin-related protein, was upregulated in LCSCs. Since CD47 acts as a ligand for signal-regulatory protein α (SIRPα), which is mainly expressed on phagocytic cells (including macrophages and dendritic cells), the activation of CD47 receptors can initiate a signal transduction cascade and inhibit macrophage cell phagocytosis (Barclay & Brown, 2006). The expression of CD47 to inhibit the innate immune monitoring and clearance of phagocytic cells, while CD47 is a widely expressed marker in all cancers that help tumor cells escape from phagocytosis and clearance. CD47 mRNA is preferentially expressed in CD133+/CD24+ LCSCs. In addition, the increased expression level of CD47 mRNA in HCC clinical samples is positively correlated with patient survival (T. K. Lee et al., 2014).

1.18. Targeting LCSCS for treatment of HCC

Prominin-1 (CD133): CD133 knockout may reduce the tumorigenicity and change the cell cycle distribution in these cells. Additionally, HCC patients with high CD133 expression in their tumors have poor prognosis and increased recurrence, indicating that CD133 expression may be associated with the prognosis of liver cancer (W. Song et al., 2008). Monoclonal antibodies are commonly used as ligands in CD133-targeted therapy. These antibodies can carry various drugs or toxins to the target to enhance the immune response of the human body towards the disease. Such methods have several advantages that are absent in traditional anticancer drugs, namely, relatively high target specificity, low molecular weight, less side effects, and better patient compliance. Currently reported antibodies against CD133 are AC133, 293C3 and AC141, among which AC141 and 293C3 are antibodies targeting CD133/2. CD133/2 is a variant of the CD133 antigen, (Prasad et al., 2015) prepared a compound antibody from CD133 and CD3 antibodies. This compound could specifically identify glioma stem cells and recruit T cells to kill these stem cells, demonstrating an excellent targeted therapeutic effect. (Smith et al., 2008) combined a

mouse anti-human CD133 antibody with the anti-microtubule cytotoxic drug monomethyl auristatin E and confirmed that this complex inhibited the growth of CD133+ LCSC-like cells *in vivo* and *in vitro.*

Transplanted tumor mice demonstrated that most of the 131I-CD133 mAb was deposited in the CD133+-transplanted tumor sites in the mice, while this was not observed in CD133− mice. The 131I-CD133 mAb may be therefore applied with high selectivity and high stability in the clinical diagnosis of LCSCs, as well as for immune imaging and radiation therapy of LCSCs, and clinical trials are currently ongoing. Despite these studies demonstrating that CD133 can be used for the isolation and identification of LCSCs in vitro as well as for targeted therapy, the application of a single surface marker remains limited (Christ, Stock, & Dollinger, 2011).

CD13: Combined treatment with Tegafur, a prodrug of 5-fluorouracil (5-FU), and cyclophosphamide, using a low-dose rhythmic administration, significantly reduced the number of tumor cells (Christ et al., 2011). Downregulation of CD13, by use of a CD13 neutralizing antibody or inhibitors, can induce apoptosis in the HCC cell lines Huh7 and PLC/PRF/5. When CD13+ hepatocytes were treated with 5-FU, which is directly targeted at CD13 molecules, the number of cells with tumorigenic and self-renewal abilities was significantly reduced. The combined application of CD13 and CD90 inhibitors significantly reduces tumor volume, compared with the application of each individual inhibitor alone. Reduction or inhibition of CD13 molecules on the surface of HCC cells by interfering techniques also affect, to a certain extent, the self-renewal and tumorigenic ability of LCSCs (Christ et al., 2011).

CD90: it was reported that cd45−/CD90+ cells may become a new target for diagnosis and treatment of liver cancer. The CD90/integrin/mechanistic target of rapamycin kinase (mTOR)/AMP-activated protein kinase (AMPK)/CD133 signaling pathway serves an important role in tumor formation, and inhibition of this pathway by the energy-limited simulant, OSU-CG5, reduced the proportion of CD90+ cells in fresh HCC specimens and inhibited tumor growth (Cho, Lee, Ha, Lee, & Yoon, 2015).

CD44: Blocking CD44 activity by use of a CD44-targeting antibody can induce the apoptosis of CD90+ cells in vitro and inhibit tumor formation of CD90+ cells in immunodeficient mice *in vivo* (Cho et al., 2015). IM7 is a murine monoclonal antibody specifically targeting CD44, which has a confirmed inhibitory effect on tumor growth. A study by (Cho et al., 2015) prepared a novel short peptide complex PDPP targeting CD44 by combining the short peptide with D-polylysine. The binding capacity of PDPP and CD44 was 4–10 times stronger than that of the CD44 antibody, suggesting that PDPP may serve as a probe for the diagnosis and treatment of cancer stem cells.

EpCAM: Targeted therapy towards the LCSC molecular marker EpCAM can effectively eliminate the expression of EpCAM in LCSCs. EpCAM antibodies currently available in preclinical or clinical studies include edrecolomab, adecatumumab, MT110 and catumaxomab, and they have been approved in the EU for patients with EpCAM+ malignant ascites (Kurtz & Dufour, 2010). *In vitro* experiments showed that the inhibitory effect of EpCAMAb-modified adriamycin-loaded micelles on LCSCs was significantly enhanced, with an IC50 of 0.051 mg/l, while the IC50 of the EpCAMAb-unmodified adriamycin-loaded micelles was 0.24 mg/l, which was 5 times that of the former. This targeted drug delivery system offers a significant therapeutic effect, indicating the feasibility of this antibody-mediated active CSC targeted therapy, as well as its potential value for the clinical treatment of cancer. Because RNA interference (RNAi) of EpCAM has been confirmed to significantly reduce the number of stem cells and their tumorigenic and invasive abilities (Yamashita et al., 2013).

CD47: (Willingham et al., 2012) proofed that that **a**nti-CD47 antibody in selectively targeting tumor cells and highlights the balance between phagocytosis and anti-phagocytosis in hepatocellular carcinoma immune escape blocked CD47 by use of targeting monoclonal antibodies and demonstrated that inhibition of macrophage phagocytosis by CD47 was relieved in in vitro experiments. Increased treatment duration extended survival in mice. Treatment of larger tumors with the anti-CD47 antibody inhibited tumor growth and metastasis. Anti-CD47 antibodies have potential effects on the treatment of smaller tumors, as well. Knockout of CD47 using lentivirus-based short hairpin RNA (shRNA) inhibited the characteristics of stem cells, suggesting that CD47 has a key role in regulating the stem cell characteristics of HCC (Willingham et al., 2012).

Conclusion

Taking all this in consideration ,it seems clear that stem cell therapy have been promising treatment for liver disease by different type Mesenchymal Stem Cells and Hematopoietic stem cell But there are still many problems to be addressed before clinical use of MSCsfor liver fibrosis/cirrhosis, including sufficient cell number, optimal time, and optimal delivery route for MSC transplantation there are two methods of transplantation allogeneic is

more viable than autologous transplantation immunosuppresive drug is important during allogeneic hematopioetic stem cell transplantation and for hepatocellular carcinoma current targeted therapy strategies are by different mechanism . Furthermore, direct targeting of liver cancer stem cell surface markers, stem cell therapy has been recently used in treatment of HIV and type 2 Diabetes. Treatment by stem cell is not clear and there are still many challenges and problems to be solved.

Disclosure

The authors report no conflicts of interest in this work.

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