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# Bone marrow mesenchymal stem cells in hepatocellular carcinoma

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## 1. ABSTRACT

Bone marrow mesenchymal stem cells (BMSCs) are non-hematopoietic multipotent stem cells capable of differentiating into mature cells. Studies in animal models have indicated that hepatocellular carcinoma (HCC) may originate from genetically mutated BMSCs. Moreover, it has been shown that BMSCs are influenced by and can modulate their micro-environment via secreted cytokines that promote tumor initiation, growth, and homing to tumor sites. Based on these features, BMSCs have been recognized as a putative target of molecular therapies to treat and prevent HCC. In this review we discuss the role of human BMSCs in HCC pathogenesis and their therapeutic potential.

## 2. INTRODUCTION

Human bone marrow mesenchymal stem cells (hBMSCs) are non-hematopoietic multipotent stem cells capable of differentiating into both mesenchymal and non-mesenchymal cell types, including osteoblasts, adipocytes, and chondrocytes (1-5). Many studies have shown that hBMSCs can stimulate tumor growth and metastasis *in vivo* by facilitating the expansion of tumor-associated fibroblasts, stimulating angiogenesis, suppressing the cytotoxic function of immune cells, and secreting chemokines (6-9). Interestingly, opposing effects of BMSCs on tumor cell growth have been observed according to the use of *in vitro* and *in vivo* experimental

systems; for example, exposure to BMSCs *in vitro* led to transient arrest of tumor cells in the G(1) phase of the cell cycle, while *in vivo* exposure led to increased tumor growth (10). This discrepancy between experimental systems may reflect the ability of BMSCs to interact with their environment and other endogenous factors to form a cancer stem cell niche in which tumor cells can preserve their potential to proliferate and sustain the malignant process. Such a dynamic mechanism may reveal several molecules and pathways that hold promise for therapeutic manipulation. To this end, hBMSCs are generally considered well suited for clinical application because they are easily obtained from patients, with their procurement posing no ethical concerns, and can be used in autologous transplantation (11,12). Moreover, the tropism of hBMSCs and tumors implies that such cells could potentially serve as gene delivery vectors in cancer gene therapy (13). Before the full clinical benefit of hBMSC-based therapies may be fully realized, however, a detailed understanding of the effects of unmodified hBMSCs on tumor progression must be achieved.

Hepatocellular carcinoma (HCC) is the fifth most prevalent cancer worldwide, and the third in terms of cancer-related deaths (14). In China, the mortality rate of HCC has steadily increased since the 1990s, emerging as the second leading cause of cancer deaths (15). While high incidences of recurrence and metastasis have been implicated as the primary causes of HCC's poor prognosis (16), the pathogenic underlying mechanisms have yet to be elucidated. Considering the collective findings indicating hBMSCs as an effective delivery vehicle, researchers have begun to investigate the possibility of using hBMSCs-mediated gene therapy to treat HCC. A recent study in nude mice demonstrated that hBMSCs may play a role in the pathogenic mechanisms of HCC, further suggesting their potential as effective therapeutic agents of HCC (17). In this review, we will discuss the dual roles of hBMSCs in HCC and the implications of these roles for developing more effective HCC prevention and treatment strategies.

## 3. BMSCs AND PROMOTION OF TUMOR GROWTH

The effects of BMSCs on the initiation, progression, and metastasis of certain tumor types have been demonstrated in various experimental and model systems (9,18,19), as has the involvement of their secreted paracrine factors (20-22). These studies have indicated that BMSCs participate in liver regeneration by migrating to the affected site and differentiating into hepatic precursor cells (oval cells) and hepatocytes. However, chronic conditions of liver injury, such as hepatitis virus infection, alcoholism and congenital fibrosis, overcome the normal regenerative mechanisms and cause extracellular matrix (ECM) remodeling, cirrhosis, and liver failure. Alterations in the ECM components of the liver tissues triggers a signaling cascade that inhibits the transactivation potential of liver-specific transcription factors, leading to the arrest of BMSC differentiation and poorly differentiated liver tissues that are characteristic of HCC (23-36).

### 3.1. BMSC role in HCC tumor initiation and growth

The established association between cancer and chronic tissue injury, primarily related to the inflammatory response, suggests that cancer growth may represent the continuous operation of an unregulated state of tissue repair (37). Taken together, the well-known roles of the Hedgehog and Wnt signaling pathways in tissue regeneration, stem cell renewal, and cancer growth suggest that carcinogenesis proceeds by misappropriation of the homeostatic mechanisms that govern tissue repair and stem cell self-renewal (38). Malignant transformation of hepatocytes, therefore, may occur in the context of chronic inflammation and regeneration, which is in line with the potential role for BMSCs contributing to the eventual development of HCC (39).

*In vitro* experiments have demonstrated that HCC can be derived from genetically mutated rat BMSCs (40). When a rat hepatoma cell line was treated with the medium of cultured rat BMSCs, containing the full complement of secreted factors, cellular proliferation and cell division were markedly enhanced in a dose-dependent manner (41). Similarly, isolated hBMSCs were shown to be able to differentiate into hepatocyte-like cells that resembled poorly differentiated human hepatoma cell lines (42). Furthermore, when the hepatocyte nuclear factor-alpha (HNF-4□ transcription factor was overexpressed in the isolated hBMSCs, the expression levels of other hepatocyte-specific genes, liver-enriched transcription factor genes, and cytochrome P450 genes became markedly up-regulated, indicating that HNF-4□ plays a significant role in promoting the process of hBMSC differentiation towards the hepatocyte phenotype (42). In addition, primary BMSCs were shown to exhibit immunosuppressive properties following injection into mice (43). Taken together, these findings suggest that BMSCs promote tumor growth.

### 3.2. BMSCs home to sites with tumor cells

BMSCs' involvement in tumor invasion and angiogenesis (44-47), immunosuppression (48,49), and inhibition of apoptosis (10) has been demonstrated in multiple studies and various experimental systems. In addition, hBMSCs have been shown to selectively localize to xenotransplanted human gliomas following intravascular administration, where they successfully delivered anti-glioma agents (50,51). This capacity to localize to gliomas may reflect the intrinsic ability of BMSCs to home to solid tumors, regardless of the underlying cell type (52-55). However, recent studies of the BMSCs tumor homing mechanism indicated that this process may actually generate a microenvironment that is suitable for tumor cells, thereby promoting tumor growth and negating any potential therapeutic benefit (56). Focused studies of the tumor microenvironment revealed that BMSCs can induce formation of this cancer-related system and promote its maintenance by functionally interacting with cancer cells (57). Such a tumor microenvironment may contribute to HCC initiation by providing a concentrated locale of soluble factors, such as cytokines, that would stimulate BMSCs to differentiate into fibroblasts or vascular endothelial cells (58,59). Likewise, the BMSC secreted

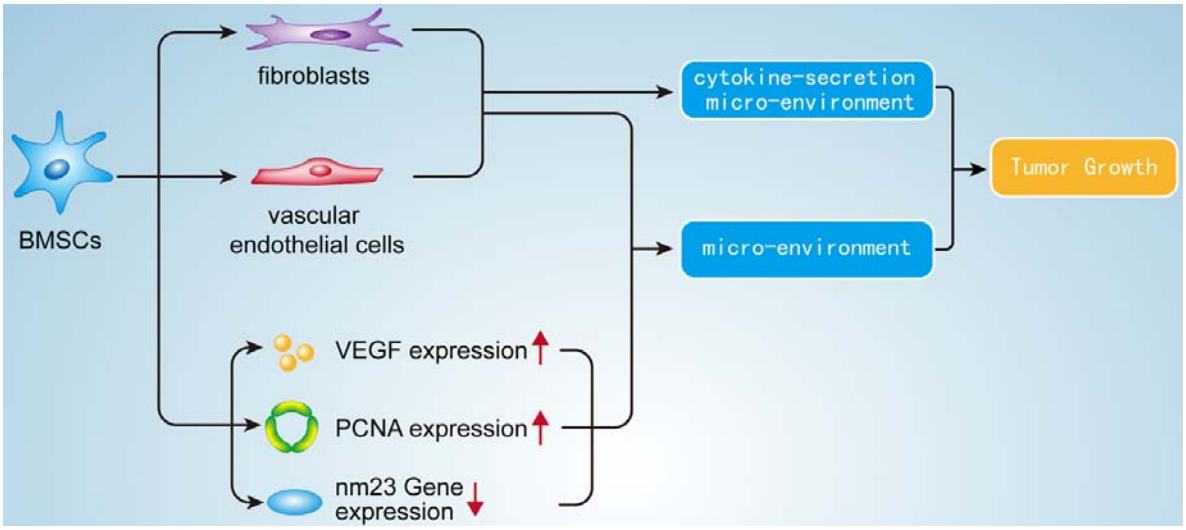


**Figure 1.** Bone marrow mesenchymal stem cells (BMSCs) promote tumor growth. BMSCs potentially participate in the formation of a tumor microenvironment and a secreted cytokine microenvironment via differentiation into fibroblasts or vascular endothelial cells, which are involved in the initiation of HCC. In addition, BMSC transplantation could increase the expression of vascular endothelial cell growth factor (VEGF) and proliferating cell nuclear antigen (PCNA), while decreasing the expression of the tumor metastasis inhibiting gene nm23 in hepatoma cells, resulting in the formation of the tumor microenvironment and promoting tumor growth.

factors in the microenvironment may induce HCC or promote its growth. Shao *et al.* reported that BMSC transplantation in a rat hepatoma model resulted in increased expression of vascular endothelial cell growth factor (VEGF) and of proliferating cell nuclear antigen (PCNA), but decreased expression of the tumor metastasis inhibiting gene nm23, and concluded that the consequent microenvironment supported the observed increase in tumor growth (60) (Figure 1). Although several studies have demonstrated that BMSCs can migrate to tumor and injury sites and to incorporate into the tumor stroma, the effects of the interactions between BMSCs and tumor cells and the mechanisms underlying these effects have yet to be elucidated.

On the other hand, hBMSCs and human hepatoma cells exhibit different responses to environmental stimuli, likely reflecting their unique cellular behavior and surface characteristics. The newly developed biodegradable, hydrophobic polyester poly(3-hydroxybutyrate-co-3-hydroxyhexnoate) (PHBHHx) has attracted the attention of tissue engineering researchers hoping to improve the distribution of hBMSCs *in vivo*. It has been proposed that the orientation of scars on the hBMSC surface would guide the intracellular growth direction of the actin cytoskeleton. In contrast, it has been demonstrated that the surface characteristics of the human hepatoma cell line C3A/HepG2 are obviously associated with their metabolic activity but not with their morphology. The C3A/HepG2 cells exhibit unique cellular characteristics when compared to the hMSCs/HepG2 cells which likely contribute to their differential responses to environmental stimuli (61). Therefore, it is possible that the microenvironment may exert different influence on BMSCs and hepatoma cells.

Recent studies have shown that BMSCs that home to tumors not only generate a suitable microenvironment for tumor cells, but also promote tumor metastasis (24,62,63). However, only a few reports to date have addressed the ability of BMSCs to promote HCC metastasis in this manner.

## 4. BMSCs AND ANTITUMOR ACTIVITY

MSCs can be expanded in culture for long periods of time without a loss of differentiation capacity. Furthermore, since MSCs are particularly amenable to genetic modification/correction, they can be harvested from a patient's own bone marrow even if the patient's liver disease were the result of an underlying genetic defect. Genetically corrected autologous MSCs could thus be propagated to generate a sufficient number of cells to achieve a meaningful level of engraftment following transplantation. *In vitro* studies have provided definitive evidence that BMSCs can, under appropriate conditions, differentiate into cells with all of the characteristics of functional hepatocytes (64-67). In addition, BMSC-exosomes have been proposed as another potentially manipulable regulatory mechanism of the paracrine action of BMSCs. Bruno *et al.* reported that BMSC-derived microvesicles can protect against acute tubular injury via horizontal transfer of mRNA (68). Thus, BMSCs appear to be able to exert beneficial effects in a wide range of injuries and disease states within the liver, including HCC. Du and colleagues demonstrated that IFN-□-stimulated hBMSCs were able to induce tumor cell apoptosis *in vitro* via tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) (69). Thus, these beneficial therapeutic effects coupled with the ability of BMSCs to home to sites of injury and tumors

further support the therapeutic potential of these cells for HCC (70,71).

### 4.1. Animal study findings of BMSCs' therapeutic potential

Studies based on HCC animal models have revealed the antitumor activity of BMSCs. However, the effects of unmodified BMSCs on tumor progression remain unclear. Some studies have suggested that BMSCs can induce tumor cell necrosis or suppress tumor growth. For example, Jiang *et al.* found that BMSCs not only engraft within the livers of carcinoma-bearing BALB/c mice but also differentiate to hepatocyte-like cells (74). And at the same time, BMSCs might induce tumor cells necrosis. In addition, administration of MSCs in chemically-induced HCC rats suppressed the tumor growth, as evidenced by marked down-regulation of Wnt signaling target genes that are associated with antiapoptosis, mitogenesis, cell proliferation and cell cycle regulation, as well as amelioration of liver histopathology and function (75). The BMSC-inhibited tumor growth was also shown to be correlated with increased overall survival of HCC rats (76). When rat BMSCs labeled with superparamagnetic iron oxide (SPIO) were transplanted into HCC rats, the tumor volume at post-transplantation weeks 1 and 2 was found to be significantly smaller than that in the control rats, as determined by magnetic resonance imaging (MRI).

BMSCs potentially control the growth of tumor cells in metabolism as one of the important factors to inhibit the proliferation of malignant cells. Qiao and colleagues found that the clonality and proliferation of hepatoma cells were inhibited upon culture in medium harvested from BMSC cultures; moreover, the inhibited hepatoma cells expressed significantly lower levels of nuclear transcription factor P8, the member of RhoGTP family CDC42EP and NK-kappaB2, but higher levels of metallothionein (MT) than the controls (77). All of these genes and cytokines are known to be involved in tumor cell metabolism. Recently, Lu and colleagues demonstrated that BMSCs exhibit potential inhibitory effects on tumor cell growth *in vitro* and *in vivo*, without inducing host immunosuppression, by triggering apoptotic cell death and arrest in the G(0)/G(1) phase (78). It is possible that these findings reflect BMSC-induced up-regulation of the cell cycle negative regulator p21 and/or the apoptosis-associated protease caspase 3 in tumor cells.

The tropism of hBMSCs toward tumors suggests that these cells may prove useful as gene delivery vectors for cancer gene therapy (79,80). Due to its dual role as a reporter and therapy gene, the sodium iodide symporter (NIS) allows non-invasive imaging of functional NIS expression by ($^{123}$)I-scintigraphy or ($^{124}$)I-PET imaging to be carried out prior to the application of a therapeutic dose of ($^{131}$)I. As such, NIS expression monitoring has provided a novel approach by which to evaluate mesenchymal stem cells (MSCs) as gene delivery vehicles for tumor therapy. Knoop *et al.* stably transfected bone marrow-derived CD34$^-$ MSCs with NIS cDNA, and three cycles of systemic BMSC-mediated NIS gene delivery followed by ($^{131}$)I application resulted in a significant delay in tumor growth (81). These results demonstrated the tumor-specific accumulation and therapeutic efficacy of radioiodine after BMSC-mediated NIS gene delivery in HCC tumors, and suggest that NIS-mediated radionuclide therapy of metastatic cancer using BMSCs as gene delivery vehicles may prove efficacious (81). In addition, the use of engineered BMSCs as therapeutic vehicles has been reported for the HCC xenografted mouse model. When isolated MSCs from bone marrow of C57/Bl6 p53$^{-/-}$ mice were injected, exogenous BMSCs were recruited to the growing HCC xenografts and this process was accompanied by activation of the CCL5 or Tie2 promoters within the injected BMSCs. Furthermore, stem cell-mediated introduction of suicide genes into the HCC xenografted tumor followed by administration of a routine drug regimen effectively resolved the HCC (82).

### 4.2. Clinical applications of BMSCs

The discovery of pluripotent stem cells made the prospect of cell therapy and tissue regeneration a clinical reality, particularly following the evidenced contribution of bone marrow-derived stem cells in hepatic regeneration. Since then, several research groups have aimed at developing effective and convenient clinical applications of BMSCs to treat HCC. Fürst *et al.* treated patients with malignant liver lesions using a combination of portal vein embolization (PVE) with BMSCs administration, and found that the method produced substantially more robust hepatic regeneration than PVE alone (83). In HCC patients who are otherwise unsuitable for resection, transplantation, ablation therapy or arterial chemoembolization, administration of stem cell differentiation stage factors have been shown to be effective (84). This finding suggests that cytokines secreted by BMSCs could potentially inhibit the growth of HCC by impacting the tumor microenvironment in patients.

Infusion of BMSCs prior to trans-arterial chemoembolization may help to promote liver regeneration, consequently increasing liver volume and the hepatic reserve, in patients with HCC. In a long-term follow-up clinical trial, 527 patients with hepatitis B virus (HBV)-related decompensated liver cirrhosis were found to experience improved hepatic function in the early period of treatment and lower tendency of HCC development in the long-term. Moreover, the long-term observation of these patients revealed no change in the incidence of HCC following the administration of BMSCs, suggesting the possibility of an improved survival rate associated with the BMSC treatment (85). The safety and efficacy of BMSCs in HCC have been reported by Ismail *et al.* (86). In that study, Child-Pugh class B patients with unresectable HCC were treated by transarterial chemoembolization and injected, during the same session, with autologous bone marrow mononuclear layer containing stem cells into the hepatic artery feeding the contralateral lobe of the liver. Results obtained at the 3 month follow-up indicated that BMSC infusion into the hepatic artery synchronized with transcatheter arterial chemoembolization (TACE) was safe and feasible for patients with chronic liver disease complicated with HCC, as evidenced by remarkable improvements in both biological and radiological

volumetric parameters (86). Although this study was carried out with only four patients, its findings indicate the promise of BMSCs in the therapy of HCC. Furthermore, a case report demonstrated that the combined treatment using autologous BMSC transplantation and TACE was an appropriate and sufficient alternative treatment for HCC patients who are unable to tolerate TACE due to hepatic dysfunction (87). Therefore, BMSCs may also benefit patients with advanced HCC, who can no longer tolerate invasive therapies due to the severe hepatic dysfunction. In the future, clinical studies involving a greater number of samples are needed to further clarify the efficacy of BMSCs in the therapy of HCC.

## 5. CONCLUSIONS

BMSCs play dual roles in HCC, promoting the initiation and progression of tumors and inhibiting tumor growth. As such, these cells may represent a useful target of HCC therapy or a delivery vehicle for antitumor agents. In animal models, HCC has been demonstrated to be derived from genetically mutated BMSCs. Moreover, BMSCs are potentially involved in the formation of microenvironments, such as those composed of secreted cytokines, that promote tumor growth and homing to tumor sites, whereas the BMSCs, in turn, may be influenced by the microenvironment itself to further support tumor development and growth. The initial efforts to develop BMSC-based therapies for HCC have shown promise. However, additional studies using larger sample size are needed to further clarify the efficacy and safety of BMSCs in clinical applications.

## 6. ACKNOWLEDGMENTS

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**Abbreviations:** BMSCs: Bone marrow mesenchymal stem cells; HCC: hepatocellular carcinoma; ECM: extracellular matrix; VEGF: vascular endothelial cell growth factor; PCNA: proliferating cell nuclear antigen; PHBHHx: poly (3-hydroxybutyrate-co-3-hydroxyhexnoate); MRI: magnetic resonance imaging; MSCs: mesenchymal stem cells; PVE: portal vein embolization; HBV: hepatitis B virus; TACE: transcatheter arterial chemoembolization

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