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REVIEW ARTICLE

The therapeutic potential of bone marrow-derived mesenchymal stromal cells on hepatocellular carcinoma

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Keywords
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Abbreviations
AT, adipose tissue; BM, bone marrow; CAFs, cancer-associated fibroblasts; CB, umbilical cord blood; CD, cytosine deaminase; CFUs-F, colony forming units-fibroblasts; CM, conditioned medium; CRAds, conditionally replicating oncolytic adenoviruses; EPCs, endothelial progenitor cells; HCC, hepatocellular carcinoma; HLSCs, human liver stem cells; HSCs, activated hepatic stellate cells; HUCPVCs, human umbilical cord perivascular cells; IBSP, integrin binding sialoprotein; INF-β, interferon beta; ISCT, International Society for Cellular Therapy; MAPCs, multipotent adult progenitor cell; M-CSF, monocyte colony stimulating factor; MMP, metalloproteinase; MSCs, mesenchymal stromal cells; NIS, sodium iodide symporter; PB, peripheral blood; PDGF, platelet-derived growth factor; PEDF, pigment epithelium-derived factor; PFs, portal fibroblasts; TAMs, tumour-associated macrophages; TGF-β, transforming growth factor beta; TIMP, tissue inhibitors of metalloproteinases; TK, tyrosine kinase; TRAIL, tumour necrosis factor-related apoptosis-inducing ligand; VEGF, vascular endothelial growth factor; WJ, Wharton's jelly.

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Abstract
Mesenchymal stromal cells (MSCs) are more often obtained from adult and extraembryonic tissues, with the latter sources being likely better from a therapeutic perspective. MSCs show tropism towards inflamed or tumourigenic sites. Mechanisms involved in MSC recruitment into tumours are comprehensively analysed, including chemoattractant signalling axes, endothelial adhesion and transmigration. In addition, signals derived from hepatocellular carcinoma (HCC) tumour microenvironment and their influence in MSC tropism and tumour recruitment are dissected, as well as the present controversy regarding their influence on tumour growth and/or metastasis. Finally, evidences available on the use of MSCs and other selected progenitor/stem cells as vehicles of antitumourigenic genes are discussed. A better knowledge of the mechanisms involved in progenitor/stem cell recruitment to HCC tumours is proposed in order to enhance their tumour targeting which may result in improvements in cell-based gene therapy strategies.

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The occurrence of non-haematopoietic stem cells in the bone marrow (BM) was first suggested in the 19th century by Julius Cohnheim (1), who proposed that bone marrow could be the source of fibroblasts contributing to wound healing in different tissues (2). However, it was not until the 70s when Friedenstein isolated for the first time adherent and spindle-shaped cells from the BM with clonogenic potential, which were named as 'colony forming units-fibroblasts' (CFUs-F) (3). Additional studies by Friedenstein and Owen demonstrated their adipocyte and osteocyte differentiation potential (4, 5). Since then, several research groups named such cells as BM stromal cells, mesenchymal stem cells and, more recently, mesenchymal stromal cells (MSCs) (3, 6, 7). MSCs were subsequently isolated from a wide variety of tissues (see below).

Mesenchymal stromal cells constitute a heterogeneous population of cells, but a subset of them has been shown to contain multipotent stem cells (8, 9). Despite the lack of a specific marker, the International Society for Cellular Therapy (ISCT) proposed minimal criteria to define human MSCs: adherence to plastic in culture, multipotent differentiation potential and a characteristic cell surface protein expression profile. Thus, there is now a general consensus in that MSCs express CD105, CD73 and CD90, and lack haematopoietic markers such as CD45, CD34, CD14 or CD11b, CD79α or CD19, as well as HLA class II (7). This phenotype may vary among species, tissue sources and culture conditions (10, 11). Regarding their differentiation potential, MSCs must be able to differentiate *in vitro* into osteoblasts, adipocytes and chondroblasts (12). Additionally, it has been reported that MSCs might have the ability to differentiate *in vitro* to cardiomyocytes, vascular endothelial cells, neurons, hepatocytes and/or other epithelial cells (13–16) or eventually could express some of their specific markers.

Hundreds of clinical trials have been carried out using MSCs. A striking feature of these cells is their ability to be cultured and expanded *in vitro,* together with their apparent self-renewal properties, low inherent immunogenicity (17), trophic activity (18), high capacity to promote vascularization (19, 20) and eventually broad differentiation potential. All these particular and, at the same time, complex properties have prompted their experimental use in the regenerative medicine field (21, 22) as well as in the treatment of myocardial ischaemia/infarction (23), cerebral injury (24, 25), bone diseases and muscular dystrophy (26). In this regard, several studies have postulated that endocrine signals released by injured tissues and organs induce selectively migration of MSCs (20, 27–31). Furthermore, MSCs exhibit increased motility towards inflamed regions as well as tumourigenic sites (32, 33). This phenomenon would be expected since tumours are considered as unresolved wounds (34), and their microenvironment is characterized by an increased local production of inflammatory mediators and chemoattractants (35). The first report using MSCs as vehicles of therapeutic genes in cancer took advantage of their migratory and homing capacity towards tumours, showing that the delivery of Interferon-β (INF-β) was able to improve animal survival (32). After this work, similar approaches were explored in the context of experimental models of cancer diseases (33, 36–48) (see below).

Tissue sources for MSCs

Mesenchymal stromal cells have been isolated and expanded from a variety of tissues and most frequently from BM (12) (the most used and best characterized) and adipose tissue (AT) (49, 50). For instance, they were obtained from peripheral blood (PB), however their efficiency of isolation is low (51). In addition to adult tissue, MSCs can be derived from extraembryonic tissue after birth including placenta (52), amnion (53, 54) and umbilical cord. For the latter case, MSCs were isolated from whole umbilical cord (55), the Wharton's jelly (WJ-MSCs) (56), perivascular areas (human umbilical cord perivascular cells, HUCPVCs) (57) as well as from umbilical cord blood (CB-MSCs) (58, 59). A particular advantage of extraembryonic sources is their ready availability, which avoids the need of invasive procedures and eliminates other ethical concerns. In addition, MSCs of such origin may have improved proliferative capacity, life span and differentiation potential [reviewed by Ralf Hass 2011 (60)].

MSCs and their migration capability towards injured and inflamed sites

MSCs have been considered as likely to be one of the most powerful cells involved in human body repair mechanisms (61). Several studies have shown that MSCs preferably engraft in injured or inflamed tissues (62, 63). In physiological conditions, a low frequency of these cells circulate through peripheral blood (64, 65), and mainly reside in the BM niche (66). Once endocrine signals are released in response to injury, sometimes following an increase in plasma concentration of VEGF or G-CSF (67), MSCs mobilize into the blood stream and migrate towards the injured sites to promote tissue regeneration (65, 67). In healthy mice, MSCs intravenously injected are first retained in the capillary layer of lungs and then in the liver and spleen probably because of cellular size and their expression of adhesion molecules (68, 69). Mechanisms involved in MSC deceleration within the vasculature and extravasation under physiological conditions and after their infusion into different animal disease models are not yet fully understood. It is presumed that MSCs actively migrate from bloodstream towards tissue extracellular space using leucocyte-like cell adhesion mechanisms, including rolling and adhesion to endothelial cells mediated by selectins and integrins (70). It has been reported that the rolling of MSCs is dependent on endothelial cell

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expression of P-selectin, and that MSC adhesion and transmigration involve the VLA-4/VCAM-1 axis (71). MSC transmigration likely occurs in response to chemoattractant stimuli which involve PDGFR, VEGFR-1/2, IGF1R, CCR6, CXCR1 and CXCR4 [reviewed by Spaeth 2008 (72)]. Moreover, most of the ligands that bind to these receptors induce chemotaxis (72, 73), transendothelial migration (74–76), activation of adhesion molecules (77, 78) and metalloproteinase (MMP) activity (79, 80) in MSCs. Several reports indicate that MSC transmigration occurs by an integration mode in which endothelial cells retract allowing spreading and incorporation of MSCs into the endothelial monolayer, and finally the endothelial cells are re-localized on the top of MSCs, facing the endothelial lumen and leaving the endothelial layer intact (74, 81). In addition to this mechanism, MSCs can transmigrate by paracellular and transcellular diapedesis, such as described for leucocytes. However, a very recent study showed that in contrast to the latter cell types, MSCs are able to display dynamic non-apoptotic blebbing protrusions, instead of lamellipodia or invadosomes, which can exert forces on endothelial cells during early stages of transmigration (82).

Mechanisms and factors involved in MSC migration towards hepatocarcinoma (HCC)

The establishment and spread of a tumour is a complex process and involves an extensive cross-talk between cancer cells and tissue/tumour microenvironment (83). This interaction may result in tumour growth promotion, invasion, angiogenesis and metastasis [reviewed by Sheng-Di Wu 2012 (84)]. During tumour development, a sustained process of tissue destruction and subsequent repair leads to a state of unresolved wounds (34). In particular, the HCC environment is composed by sinusoid and tumour endothelial cells, activated hepatic stellate cells (HSCs), cancer associated fibroblasts (CAFs), portal fibroblasts (PFs), Kupffer cells, tumour-associated macrophages (TAMs), NK and NKT lymphocytes, dendritic cells and neutrophils (84). This HCC microenvironment contains several extracellular matrix components such as collagen, fibronectin and glycosaminoglycans (84). A recent report showed that not only the composition of the ECM but also matrix stiffness is able to regulate the proliferation and chemotherapeutic response of HCC cells (83). However, the mechanisms which govern the interactions between the different components of HCC milieu are not still completely elucidated. In addition, HCC cells are able to alter their surrounding microenvironment in order to promote their own growth and progression (85). To this end, HCC cells were shown to release cytokines, chemokines and growth factors such as vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF-BB), transforming growth factor beta (TGF-β), or monocyte colony stimulating factor (M-CSF), which recruit activated HSCs, CAFs, TAMs and endothelial cells; in turn, these cells respond to such signals by several mechanisms promoting HCC growth and invasion (86–88) (see below).

Homing of MSCs towards experimental tumours has been reported in several animal models including glioma (89, 90), melanoma (32), breast (33), colon (91), HCC (92–94) and liver metastasis of colon cancer (95). *In vivo* biodistribution assessment of MSCs after their intravenous administration in subcutaneous or orthotopic HCC models suggests that MSCs are localized first in lungs and thereafter in the liver parenchyma and spleen (92), following a similar temporal and spatial pattern to that described in mice free from inflammation or tumour events (63, 68). We have observed that one hour after i.v. MSC inoculation, the signal corresponding to transplanted cells is preferentially present in the lungs of animals bearing or not s.c. Hep3B tumours. Subsequently, from day 4 and at least up to day 14, such signal is found in liver and spleen and, in tumour-bearing mice, within the tumour (Fig. 1). We have previously shown that the hepatic tropism for i.v. injected MSCs is increased in tumour-bearing mice and that MSCs were able to migrate inside HCC tumours more efficiently when they were established in fibrotic livers, compared to when HCC tumours were established in non-fibrotic mice (92). This enhanced recruitment of MSCs towards the liver and HCC tumours might be explained, at least in part, by the activation of liver sinusoidal endothelial cells, likely be mediated by inflammatory cytokines and chemokines produced by cancer cells and its microenvironment, with a particular contribution of HSCs and Kupffer cells (96). In fact, it is considered that the cross-talk between tumour cells and their microenvironment could be critical for the recruitment of MSC to HCC. Factors such as VEGF, PDGF, TGF-β, MCP-1, IL-8, TNF-α, IL-1β, IL-6, SDF-1 or HGF, which are released by HCC cells and/or diverse tumour stromal components, have also been described as chemoattractants for MSCs (73, 80, 86–88, 96–122) (Fig. 2). However, no reports were published confirming the role of any of these factors in the recruitment of MSCs towards HCC tumours. We have recently showed that factors released by HCC cells and/or HSCs are able to induce migration of MSCs towards tumour tissue and to enhance adhesion and invasion capabilities of these cells in the context of endothelial cells, type IV collagen and fibronectin, with an observed induction in MMP-2 activity (92), a known required step for transmigration through the endothelial barrier (80, 123) (Fig. 2). In line with this, previous data suggest that incubation of MSCs with inflammatory cytokines such as TGF-β, TNF-α or IL-1β can enhance the invasive properties of MSCs through upregulation of MMP-1, MMP-2, MMP-3, MMP-9, membrane type 1 (MT1)–MMP and tissue inhibitors of metalloproteinases (TIMP-1 and TIMP-2) (73, 77, 80). Other cytokines that can also modulate the production of MMP/TIMPS are PDGF-BB and IL-6 (79). The *in vitro* migration of

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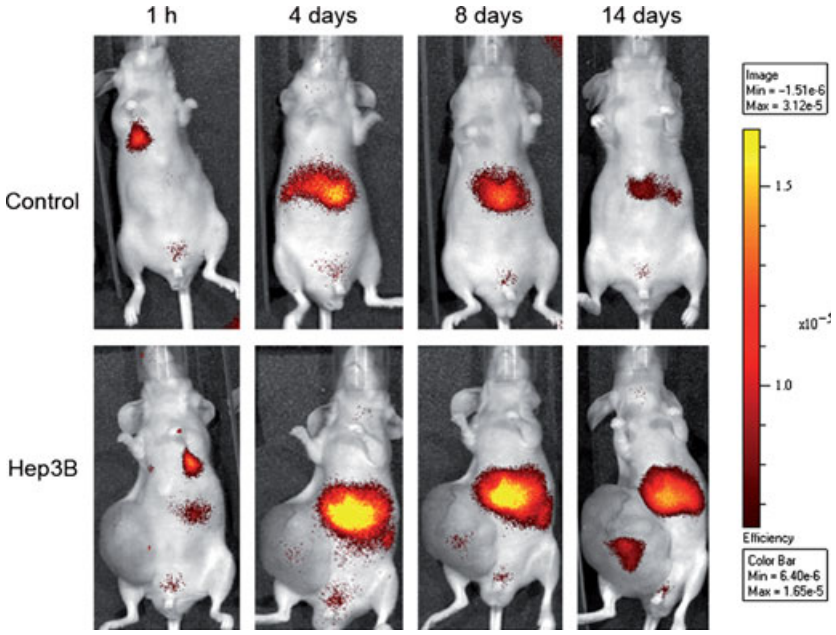

Fig. 1. *In vivo* non-invasive biodistribution of human MSCs. DiR-labelled human MSCs were i.v. administered and monitored at 1 h, and 4, 8 and 14 days after their infusion in healthy mice (control) and s.c. Hep3B-tumour bearing mice (Hep3B). Values correspond to total radiant efficiency [(p/s)/(μW/cm^2)].

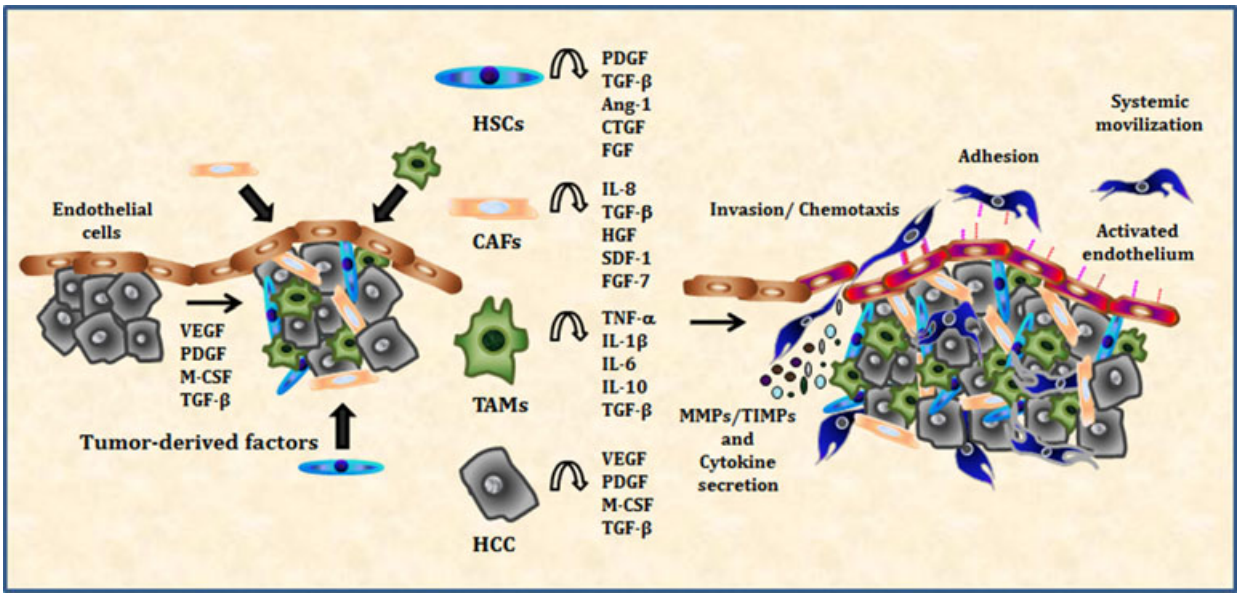

Fig. 2. Proposed model for recruitment of MSCs towards HCC. The HCC environment is composed by malignant cells (HCC), endothelial cells, activated hepatic stellate cells (HSCs), cancer-associated fibroblasts (CAFs) and tumour-associated macrophages (TAMs), among others. Factors secreted by the tumour and its microenvironment induce the activation of endothelial cells, allowing the adhesion of mobilized MSCs. For instance, TNF-α, HGF and IL-6 have been reported to upregulate chemokine receptors increasing chemotaxis in MSCs. Moreover, TNF-α and IL-1β have been shown to be involved in MSC adhesion by activation of VCAM-1, ICAM-1 and -4 and ITGβ3. MSCs in turn secrete cytokines and metalloproteinases (MMPs) and their inhibitors (TIMPs) in order to invade and reach the tumour. For example, IL-8, SDF-1, TNF-α and IL-1β induce the secretion of several cytokines with an autocrine function on MSCs. The upregulation of metalloproteinases (MMPs) and their inhibitors (TIMPs) in MSCs by available TNF-α, IL-1β, TGF-β, SDF-1, PDGF and IL-6 are able to increase MSC invasive properties.

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MSCs through matrigel in the presence of PDGF-BB is mediated by a reduction in TIMPs expression levels and an increase in MMP-2 activity, while IL-6 enhances MSC invasive properties by upregulating MMP-13 expression levels without modulating TIMPs (79). A recent microarray study revealed that IL-1β treatment induces in MSCs the upregulation of genes related with increased cell migration and adhesiveness such as chemokines (CCL20, CCL5, CXCL3 and CXCL1) and adhesion molecules (integrin binding sialoprotein (IBSP), ICAM1, ICAM4 and VCAM-1), among others (120). These factors can also modulate the interaction of the different ligands with their receptors; for example, TNF-α increases the sensitivity to SDF-1 and may induce the expression of the CC- but no CXC- chemokine receptors (73). Similarly, short-term stimulation of MSCs with Flt-3 ligand, SCF, IL-6, HGF and IL-3 was shown to increase surface expression of CXCR4 (124). Furthermore, IL-8, SDF-1, MCP-1, TNF-α or IL-1β may induce in MSCs the secretion of cytokines that in turn may, in a synergist autocrine mode, act promoting their migratory behaviour (120, 125–128) (Fig. 2).

Taking together, these data suggest that factors produced/released by HCC microenvironment are likely capable of inducing mechanisms leading to increase migratory and anchorage properties of MSCs towards HCC.

MSCs and its role in hepatocellular growth and metastasis

Since it is known that MSCs can migrate and anchor into tumours, several studies were aimed at elucidating whether MSCs can enhance or suppress tumour growth and metastasis in different animal models, with diverse and, sometimes, contradictory results (129). In this regard, MSCs were shown to stimulate secretion of several cytokines and extracellular matrix proteins, modulate apoptosis, stimulate endothelial cell proliferation, and modify immune responses against cancer cells (130–132). The effect of these cells in HCC regarding tumour growth and metastasis remains controversial (detailed in Table 1). For example, in an *in vitro* model it was shown that MSCs could inhibit tumour cell proliferation (133). Consistently, co-injection of MSCs with HCC cells in a subcutaneous tumour model resulted in a reduced tumourigenesis (41). In addition, co-administration of hepatoma cells with MSCs was found to reduce ascites formation (134); nevertheless, these findings remain to be confirmed by others. We have shown that soluble factors released by human MSCs can have different effects on HCC cells proliferation *in vitro,* depending on the cell line used; thus, conditioned media from MSCs was found to suppress Hep3B cells proliferation, while the opposite effect was achieved in PLC/PRF/5 cells and no changes were seen in HuH7 cells (92). In this line, systemic administration of MSCs in HuH7-tumour bearing mice was shown by us not to affect tumour growth, which was consistent with another study using different HCC models (40, 94). It is of note that Niess *et al.* using an orthotopic model with HuH7 cells showed that MSCs have a stimulatory effect in tumour growth, through enhancement of microvessel density (93). In addition, a pro-tumourigenic role for MSCs is suggested by results from application of these cells in a subcutaneous model using MHCC97-C cells: MSC-treated mice exhibited larger tumours, although a decreased number of lung metastases were observed; this effect seems to be related to TGF-β1 downregulation (135). Moreover, MSCs-derived conditioned media were shown to promote the tumour growth in an *in vivo* model using HepG2 cells (136). It was recently described that MSCs exposed to an inflammatory microenvironment may facilitate HCC metastasis through TGF-β-induced epithelial to mesenchymal transition in cancer cells (137). Thus, MSCs could be able to modulate tumour growth and metastasis through multiple mechanisms, depending on the type of the HCC tumour model and likely on their capability to reach tumour microenvironment.

MSC and gene therapy strategies

Results from preclinical studies using MSCs as carriers of therapeutic genes suggest their potential role in tumour therapeutic strategies. One of the first studies that exploited the combination of gene and cellular tools for the treatment of cancer demonstrated that MSCs expressing IFN-β were able to inhibit tumour growth in a melanoma model using A375SM cells (32). Since then, several approaches have been explored using MSCs engineered to secrete immune-stimulatory cytokines like IFN-α, IFN-β, IL-2 and IL-12 in different tumour models (33, 40, 43, 138). Furthermore, MSCs have also been genetically modified to express pro-apoptotic genes such as tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) or prodrug converting enzymes like tyrosine kinase (HSV-tk) or cytosine deaminase (CD) (39, 48, 139–141). Finally, MSCs have been used as carriers for the delivery of oncolytic viruses like Measles viruses or conditionally replicating oncolytic adenoviruses (CRAds) near the tumour, taking advantage of the capability of MSCs to accumulate at the tumour site and to protect the viruses from the neutralizing antibodies (142–144). In this regard, we have recently demonstrated using an experimental tumour model of melanoma that BM-MSCs preloaded with an oncolytic adenovirus are able to significantly inhibit tumour growth, overcoming the resistance of the tumour to non-vehiculized oncolytic viruses (143). Few studies have been performed using MSCs as vehicles for gene therapy against HCC (detailed in Table 2). For instance, MSCs genetically modified, using an adenovirus to secrete Interleukin 12 genes (Ad-IL12-MSC), were applied in a preventive protocol (36) with the result of important antimetastatic effects (40). Intravenous administration of Ad-IL12-MSCs generated higher

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Table 1. Studies on the effect of applying non-genetically modified MSCs in experimental HCC models

MSCs characteristics		Animal Model	Biological effect	Reference
Source	BALB/c BM-derived MSCs	i.p. injection of MSCs (5×10^5) in BALB/c mice with s.c. tumour (BNL cells)	Without effect on tumour development	X. Chen et. al. 2006 (36)
# Passage	6 to 9			
Surface markers	ND			
Source	BALB/c BM-derived MSCs	i.v. injection of MSCs (initial:2×10^6; then 1×10^6 every 5 days/20 days) in nude BALB/c mice with s.c. tumour (Hca cells)	Without effect on tumour growth	X. Chen et. al. 2008 (40)
# Passage	6 to 9			
Surface markers	$CD44^+$, $CD105^+$ and $CD73^+$			
Source	Dermis, foetal human: Z3 MSCs, immortalized cell line	s.c. co-injection of MSCs (1×10^7) with H7402 tumour cells in SCID mice	Inhibition of tumour growth	L. Qiao et. al. 2008 (41)
# Passage	ND			
Surface markers	$CD29^+$,$CD44^+$,$CD105^+$, $CD166^+$, $CD31^-$, $CD45^-$, hTERT+, $CD34^-$, vWF^- and $HLA\text{-}DR^-$			
Source	Mouse BM-derived MSCs	i.p. injection of MSCs (2×10^5/0, 3 and 10 days after tumour induction) in BALB/c mice with ascitogenous hepatoma (H22 cells).	Inhibition of tumour volume and ascites formation	Y. Lu et. al. 2008 (134)
# Passage	2 to 4			
Surface markers	$CD73^+$, $CD90^+$, $HLA\text{-}DR^-$			
Source	Human BM-derived MSCs	i.v. injection of MSCs (5×10^5/3 $\times$ week/4 weeks) in nude BALB/c mice with s.c. tumour (MHCC97-H cells)	Promotion of tumour growth. Inhibition of metastasis development	G. Li et. al. 2010 (135)
# Passage	5 to 8			
Surface markers	$CD44^+$ and $CD90^+$			
Source	Human BM-derived MSCs	i.v. injection of MSCs (1×10^6) in nude BALB/c mice with i.h. tumour (MHCC97-H cells)	Without effect on tumour growth	Y. Gao et. al. 2010 (94)
# Passage	3 to 4			
Surface markers	$CD105^+$,$CD29^+$,$CD90^+$, $CD45^-$, $CD34^-$ and $CD14^-$			
Source	Human BM-derived MSCs	i.v. injection of MSCs (5×10^5) in nude BALB/c mice with s.c. tumour (HuH7 cells)	Without effect on tumour growth	M. Garcia et. al. 2011 (92)
# Passage	4 to 6			
Surface markers	CD44+, CD49e+, CD73+, CD90+, $CD105^+$, $CD166^+$, $CD31^-$, $CD34^-$, $CD45^-$, $CD14^-$ and $CD79^-$			
Source	C57BL/6 $p53^{-/-}$ BM-derived MSCs	i.v. injection of MSCs (5×10^5/week/3 weeks) in nude BALB/c mice with i.h. tumour (HuH7 cells)	Promotion of tumour growth and angiogenesis	H. Niess et. al. 2011 (93)
# Passage	ND			
Surface markers	$CD73^+$, $CD105^+$,$CD34^-$, $CD14^-$, $CD45^-$ and $HLA\text{-}DR^-$			
Source	Human BM-derived MSCs	i.t. injection of CM-MSCs (100 μg/2 $\times$ week/3 weeks) in nude SCID mice with s.c. tumour (HepG2 cells)	Enhancement of tumour growth	C. Cavalliari et. al. 2012 (136)
# Passage	6			
Surface markers	$CD105^+$, $CD73^+$, $CD90^+$, $CD166^+$, $CD44^+$, $CD45^-$, $CD14^-$, $CD34^-$, $CD80^-$,$CD86^-$, $CD40^-$, $CD31^-$ and vWF^-			

BM, bone marrow; CM, conditioned media; i.h., intrahepatic; i.p., intraperitoneal; i.t., intratumoural; i.v., intravenous; ND, no data; s.c., subcutaneous

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Table 2. Studies of applying genetically modified MSCs in experimental HCC models

MSCs characteristics		Animal model	Biological effect	Reference
Source # *Passage* *Surface markers*	BALB/c BM-derived MSCs 6 to 9 ND	i.p. injection of MSCs adenovirally engineered to secrete interleukin-12 (5×10^5) one week before of s.c. tumour implantation (BNL cells)	Prevention of tumour establishment	X. Chen et. al. 2006 (36)
Source # *Passage* *Surface markers*	BALB/c BM-derived MSCs 6 to 9 $CD44^+$, $CD105^+$, and $CD73^+$	Nude BALB/c mice with s.c. tumour (Hca cells). i.v. injection of MSCs adenovirally engineered to secrete interleukin-12 (initial: 2×10^6; then 1×10^6 every 5 days/20 days)	Suppression of tumour growth and antimetastatic effect	X. Chen et. al. 2008 (40)
Source # *Passage* *Surface markers*	Human BM-derived MSCs 3 to 4 $CD105^+$, $CD29^+$, $CD90^+$, $CD45^-$, $CD34^-$ and $CD14^-$	Nude BALB/c mice with i.h. tumour (MHCC97-H cells). i.v. injection of MSCs engineered to express hPEDF by lentiviral transduction (1×10^6)	Antiangiogenesis. Inhibition of tumour growth. Increased animal survival	Y. Gao et. al. 2010 (94)
Source # *Passage* *Surface markers*	C57BL/6 p53−/− BM-derived MSCs ND $CD73^+$, $CD105^+$, $CD34^-$, $CD14^-$, $CD45^-$ and HLA-DR^-	Nude BALB/c mice with i.h. tumour (HuH7 cells). i.v. injection of MSCs expressing HSC-TK gene under the promoter/enhancer for CCL5 or Tie2 (5×10^5/week/3 weeks) + GCV	Inhibition of tumour growth	H. Niess et. al. 2011 (93)
Source # *Passage* *Surface markers*	Human BM-derived MSCs (immortalized cell line) ND $CD73^+$, $CD105^+$, $CD34^-$, $CD14^-$, $CD45^-$ and HLA-DR^-	CD1 nu/nu mice with s.c. tumour (HuH7 cells). Three cycles of i.v. injection of MSCs expressing NIS (5×10^5) followed by ^{131}I application	Inhibition of tumour growth and reduction of tumour vessel density	K. Knoop et. al. 2011 (146)
Source # *Passage* *Surface markers*	Human BM-derived MSCs (UE7T-13, immortalized cell line) ND ND	Nude BALB/c mice with s.c. tumour (MHCC97-H cells). i.v. injection of MSCs expressing TRAIL (1×10^6) + i.p cisplatin (1.5 mg/kg, every 3 days/21 days)	Inhibition of tumour growth and reduction of tumour vessel density	B. Zhang et. al. 2012 (145)

BM, bone marrow; i.h., intrahepatic; i.p., intraperitoneal; i.t., intratumoural; i.v., intravenous; ND, no data; s.c., subcutaneous; GCV, ganciclovir.

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intratumoural levels of IL-12 when compared to Ad-IL12 treatment, without increase in systemic toxicity (40). In another study, MSCs were engineered to express the human antiangiogenic factor pigment epithelium-derived factor (PEDF) using a lentiviral vector; as a result of this strategy, significant suppression of tumour growth and pulmonary micrometastases were observed (94). Another approach was employed by Niess *et al.* who make use of MSCs expressing the HSV-TK gene under the control of *tie*-2 and CCL5 HCC specific promoters. Interestingly, TK gene was found to be expressed only once the MSCs reach the tumour microenvironment to convert the ganciclovir into a phosphorylated toxic compound that kills cancer cells (93). A recent study combined the application of MSCs expressing TRAIL with the chemotherapeutic agent cisplatin which reverses TRAIL resistance observed in HCC. Data indicated that the cotreatment inhibited tumour growth and reduced vessel density in an animal model of HCC (145). A new promising strategy recently reported consists in transducing MSCs with the sodium iodide symporter (NIS) gene, a transmembrane glycoprotein responsible for the accumulation of iodide inside cells. In this case, the therapeutic application of the radionuclide ^{131}I in a HCC xenograft mouse model resulted in a delayed tumour growth (146). These data demonstrate that MSCs can efficiently migrate into the HCC milieu and deliver therapeutic genes. However, despite of such promising results, the evaluation of factors involved in MSC migration towards HCC tumours can significantly add to achieve higher antitumoural and/or antimetastatic effects.

Other progenitor cells as potential vehicles for antitumoural genes

Regarding other progenitor/stem cells which could be of interest as vehicles of antitumoural genes, some reports have shown that liver stem cells have the ability to migrate to HCC both *in vitro* and *in vivo* (147, 148). The authors showed that stem cell administration through the portal vein results in the majority of cells being localized within tumour stroma, and only few cells in other organs such as kidneys, lungs or spleen (148). Recently, Cavallari *et al.* reported that intratumoural inoculation of conditioned medium from human liver stem cells (HLSCs-CM) was able to inhibit tumour growth in a subcutaneous HepG2 cell line model (136). The beneficial effect achieved with HLSCs-CM on HCC tumours was found to be mediated, at least in part, by the regulator of the nodal pathway, LEFTY, which was not found as component of BM-MSC conditioned media.

Other possible cell carrier candidates are the Multipotent Adult Progenitor Cells (MAPCs) that belongs to a plastic adherent progenitor cell population which can be isolated from the BM (149, 150) and have the ability to engraft in highly vascularized tumours as is the case of HCC (151). These cells have some similar phenotype and functional characteristics to those of MSCs, including the capacity to differentiate into connective tissue lineages and the presence of some MSCs surface markers (152). In addition, MAPCs are also considered as endothelial progenitor cells (EPCs) and their differentiation into functional endothelium both *in vitro* and *in vivo* has been described (16, 153, 154). Moreover, it was observed that after systemic administration in an orthotopic HCC model, undifferentiated MAPCs were recruited to the tumour and differentiated *in vivo* into endothelial cells, contributing to the tumour vasculature (151). Although other endothelial progenitor cells have the potential to incorporate into tumour vasculature, MAPCs can be more easily transduced with therapeutic genes and expanded *in vitro* (151, 155, 156). This settles MAPCs as an interesting alternative to MSCs, since they can spontaneously differentiate *in vivo* into endothelial cells and are thus potential vehicles for site-specific gene therapy.

Conclusions

This review summarized our current knowledge on the use of stem cells as carriers for therapeutic genes with a focus on factors mediating their recruitment to HCC. One of the challenges for the researchers involved in the gene therapy field is the poor transduction efficiency caused by the lack of tumour selectivity of viral and non-viral vectors [reviewed by Clare E (157)]. In addition, antiviral immunity as pre-existing immunity to parental wild type viruses remains a problem. In order to overcome them, a great interest is placed on the use of several cell types as vehicles for therapeutic genes. In particular, the use of MSCs for gene delivery appears to be a good candidate strategy for cancer therapy. In addition to the characteristics shared with other progenitor cells, such as the ability to selectively migrate towards injured areas and remodelling tissues, their abundance and accessibility coupled with their simplicity to be genetically manipulated make them a widely available candidate. Moreover, their ability to anchor into tumour may be improved by means of different strategies such as: (i) increasing the expression of certain cell surface receptors, i.e. by overexpressing one or more of them in MSCs; (ii) irradiating the tumour in order to increase migration and anchorage of MSCs because of the induction of tumoural cytokines/chemokines expression levels (158). However, a better understanding of the axes inducing MSC migration towards HCC would help increasing their specific recruitment and thus, their therapeutic efficacy. Despite the significant advances achieved in this field, several concerns remain about the use of MSCs as carriers for therapeutic genes. Among them, it is controversial whether or not they enhance tumourigenesis. It is also important to state that we should be cautious in extrapolating data from laboratory rodents to the clinical setting. These potentially useful strategies need to be tested in large mammalian models closer to the human. Finally,

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although potent antitumour effects have been observed using engineered MSCs in animal models, their isolation, characterization and expansion need to be standardized, with the aim of using them for therapeutic purposes in clinical trials against advanced HCC.

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