



# Research Repository UCD

<b>Title</b>	Advances in mesenchymal stem cell-mediated gene therapy for cancer
<b>Authors(s)</b>	Dwyer, Roisin M., Khan, Sonja, Barry, Frank P., et al.
<b>Publication date</b>	2010
<b>Publication information</b>	Dwyer, Roisin M., Sonja Khan, Frank P. Barry, and et al. "Advances in Mesenchymal Stem Cell-Mediated Gene Therapy for Cancer." Springer (Biomed Central Ltd.), 2010. <a href="https://doi.org/10.1186/scrt25">https://doi.org/10.1186/scrt25</a> .
<b>Publisher</b>	Springer (Biomed Central Ltd.)
<b>Item record/more information</b>	<a href="http://hdl.handle.net/10197/5001">http://hdl.handle.net/10197/5001</a>
<b>Publisher's statement</b>	The final publication is available at <a href="http://www.springerlink.com">www.springerlink.com</a>
<b>Publisher's version (DOI)</b>	<a href="https://doi.org/10.1186/scrt25">10.1186/scrt25</a>

Downloaded 2025-12-04 22:53:21

The UCD community has made this article openly available. Please share how this access benefits you. Your story matters! (@ucd\_oa)



© Some rights reserved. For more information

**Title: Advances in Mesenchymal Stem Cell (MSC) Mediated Gene Therapy for Cancer**

**Authors:** RM Dwyer<sup>1,2</sup>, S Khan<sup>1</sup>, FP Barry<sup>2</sup>, T O'Brien<sup>1,2</sup> and MJ Kerin<sup>1</sup>

**Author Affiliations:** School of Medicine<sup>1</sup> and Regenerative Medicine Institute<sup>2</sup>, National University of Ireland Galway (NUIG), Galway, Ireland.

**Corresponding author:**

Roisin M Dwyer,

Discipline of Surgery, Clinical Science Institute, National University of Ireland Galway, Galway, Ireland.

E-mail: [roisin.dwyer@nuigalway.ie](mailto:roisin.dwyer@nuigalway.ie)

## **Abstract**

Mesenchymal Stem Cells (MSCs) have a natural tropism for tumors and their metastases, and are also considered immunoprivileged. This remarkable combination of properties has formed the basis for many studies investigating their potential as tumour-specific delivery vehicles for suicide genes, oncolytic viruses and secreted therapeutic proteins. The aim of this review is to discuss the range of approaches that have been used to exploit the tumour-homing capacity of MSCs for gene delivery, and highlight advances required to realize the full potential of this promising approach.

## **Introduction**

Despite significant advances in the field of gene therapy for cancer, two major obstacles remain which continue to limit the clinical potential of this approach: lack of tumour tropism of vectors, and stimulation of an immune response. These barriers preclude systemic administration of current vectors to efficiently target metastatic disease. The combination of cellular therapy and gene delivery is an attractive option as it will potentially protect the vector from immune surveillance, and support targeted delivery of a gene or therapeutic protein to the tumour site.

## **Mesenchymal Stem cells (MSCs)**

Mesenchymal Stem Cells (MSCs) are non-haematopoietic stem cells that have generated a significant amount of interest in this context, as a result of their apparent ability to home to the tumour site following systemic delivery. MSCs have an inherent ability both to self-renew and differentiate into multiple lineages including osteoblasts, chondrocytes and adipocytes [1]. They are readily isolated from the stromal compartment of bone marrow, along with a number of other sources including adipose tissue, trabecular bone, and skeletal muscle [2]. Although a single marker for MSCs has not been isolated, a panel of specific antigens has been identified, including expression of CD105, CD73 and CD90 in greater than 95% of the culture, and absence of CD14, CD34, CD19, HLA-DR and CD45 [3]. When introduced systemically to healthy animals, MSCs have been shown to home preferentially to lung, liver and bone, and were found to a lesser extent in other tissues. However, upon injury, the migratory pathway changes to preferentially target sites of injury [4]. Although MSCs have potential uses in regenerative medicine and a number of different disease models, this review will specifically focus on their potential for

targeted gene delivery in the context of cancer. This is an exciting area of research that has gained considerable momentum in recent years, with studies reporting engineered MSCs specifically targeting multiple tumor types followed by local secretion of therapeutic proteins (IFN- $\beta$  [5-7], IL-2 [8-9], IL-12 [10-12], PEDF [13], NK4 [14], TRAIL [15-18]), expression of prodrug activating suicide genes (HSV-tk [19-21], CD [22]), and delivery of replicating oncolytic viruses [16, 19, 23-25]. A major advantage of MSCs in this setting is that they are considered immunoprivileged, possibly due to low expression of Ag (HLA) MHC class 1, and no expression of CD40, CD80 & CD86 [4]. They are also known to secrete prostaglandin, TGF $\beta$  and hepatocyte growth factor, which regulate the T cell immune response, thereby decreasing probability of a cytotoxic T cell response to transduced cells [17]. Resident MSCs suppress both transient and continuous immune surveillance, which aims at facilitating the healing process [26]. However, this immune privilege in the context of cancer has the potential to support tumour progression. Djouad et al [27] reported growth of B16 melanoma cells in allogenic animals only in the presence of MSCs, suggesting that protection from the host immune response supported tumour establishment [27]. Further studies by the same group revealed that MSCs administered in low numbers with Renca adenocarcinoma cells actually induced tumour rejection [28]. MSCs were also shown to inhibit outgrowth of colon carcinoma in rats, with complete inhibition seen when the number of MSCs were at least equal to the number of tumor cells. Tumour establishment using the mixed cell population was found to induce more infiltration of monocytes and granulocytes than the individual populations alone [29]. This may be explained by the fact that high numbers of MSCs have been shown to suppress alloreactive T cells, with very low numbers found to stimulate lymphocyte proliferation [30]. Indeed additional evidence suggests that the context with which MSCs are introduced in vivo may influence their immune phenotype [26].

### **Tumour Tropism**

Tumour specific migration of MSCs is not completely understood, but appears to be dependant upon the biological properties of the tumour microenvironment, as well as the native tropism of selected cells. Integration of MSCs into the tumour stroma is thought to be mediated by high local concentrations of inflammatory chemokines and growth factors. The tumour microenvironment is considered a site of chronic

inflammation [31]. This environment may mediate MSC migration through secretion of soluble factors such as epidermal growth factor (EGF), vascular endothelial growth factor-A (VEGF-A), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), stromal-derived growth factor-1 $\alpha$  (SDF-1 $\alpha$ /CXCL12), IL-8, IL-6, granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), Ang1, monocyte chemoattractant protein-1 (MCP-1/CCL2), haematopoietic growth factor (HGF), transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) and urokinase-type plasminogen activator (uPA) [32-37].

The process of MSC mobilization to the tumour is thought to be regulated similarly to leukocyte migration through integrins and adhesion molecules [38]. Molecules involved in leukocyte trafficking, such as tethering, rolling, adhesion and transmigration from the bloodstream to the tissue are expressed on MSCs. These include integrins, selectins and chemokine receptors. Both P-selectin and VCAM-1 have been found to influence the adhesion of MSCs in endothelium [39].

MSCs express a wide range of molecules including growth factors, chemokines, adhesion molecules and toll-like receptors (TLRs) on their surface [38-44]. MSCs are known to functionally express chemokine receptors CCR1, CCR4, CCR7, CCR9, CCR10, CXCR4, CXCR5, CXCR6, CX3CR1, and c-met, which has been increasingly linked to tumour tropism [40-43]. The mechanism of MSC migration is however still not fully elucidated.

The most documented chemokine receptor implicated in targeted homing of MSCs is CXCR4, which has potential in cell mobilization and homing [45]. A study by Wynn et al, reported that CXCR4 is highly expressed on MSCs, however mainly intracellularly (83-98 %) rather than on the surface [46]. Another study reported no detectable CXCR4 expression on MSCs [42]. It has been suggested that variable expression of CXCR4 on MSCs in different studies may be related to sensitivity of the trypsin digestion procedure used [44], differences in culture conditions, and heterogeneity of MSC populations. In vitro 3D culture of MSCs as spheroids was shown to increase SDF-1 $\alpha$  signaling, which restored functional expression of its receptor CXCR4 and homing potential that is crucial for therapeutic applications [47]. Although the tumour tropism of MSCs is generally accepted, it is certainly dependent on the tumour model. Variation in levels of MSC engraftment reported in different studies may be explained by differences in MSC isolation, culture conditions, and

experimental protocols used. However, within individual studies, variable levels of MSC engraftment have been reported in different tumour types, most likely due to differences in the microenvironment created by the tumour in question [48]. The proportion of MSCs engrafted was not found to be related to tumour size [48].

A recent study further highlighted the role that the degree of inflammation in a tumour microenvironment plays in the level of MSC recruitment [7]. In a study of MSC-IFN- $\beta$  mediated therapy of pancreatic cancer, treatment with an anti-inflammatory agent resulted in reduction of MSC engraftment in the tumour, and reversed the tumour inhibitory effects observed [7].

### **Enhancing Tumour Tropism of MSCs**

- **Modification of Tumour microenvironment**

The apparent role of inflammation in MSC-tumour tropism has also been harnessed to increase engraftment through tumour irradiation, which is associated with release of several cytokines from exposed tissue [48-49]. Klopp et al [49] found that low dose irradiation of the tumour microenvironment enhanced MSC tropism and engraftment at the tumour site. Irradiation resulted in apoptosis and increased release of inflammatory signals at the site of radiation, including tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), platelet-derived growth factor (PDGF), as well as chemokines CCL2 and CCR8 [49]. The effect of tumour radiotherapy on localisation of lentivirus-transduced MSCs in a variety of tumour types has also been reported [48]. Irradiation increased MSC localisation in LoVo, HT-29 (colon), MDA-231 (breast), but not UMSCC1 (head and neck) xenografts. This study also reported a modest elevation in CCL2 expression in irradiated tumours, although it was not found to correlate with MSC infiltration [48]. Inflammation plays a critical role in tumour progression [50] and so stimulation to support MSC homing to tumours would not be a viable option. However, radiotherapy is frequently a component of cancer therapy and so could work in combination with MSC based gene delivery to support improved targeting of MSCs to tumours.

- **Modification of MSC surface**

While variations in MSC engraftment have been observed in different tumour models, attempts are being made to improve tumour tropism and infiltration through

modification of the MSC surface. Cell rolling is a critical step of the adhesion cascade supporting rapid deceleration of cells from the blood stream, and is mediated by selectins expressed on the endothelium of the target organ. Immobilized Sialyl Lewis X (SLeX) on MSCs was shown to induce cell rolling on P selectin surface under dynamic shear flow conditions in vitro, and may have potential applications in improving MSC engraftment in vivo [51]. In one study, where native MSC tropism for the tumour of interest was not detected, MSCs were engineered to overexpress the Epidermal Growth Factor Receptor (EGFR) which binds TGF- $\alpha$  and EGF. Transduced MSCs had enhanced migratory properties towards GL261 gliomas or B16 melanoma in vivo [52]. Following establishment of improved engraftment, the cells were further engineered to secrete IFN- $\gamma$ , resulting in increased animal survival [52].

### **MSC-mediated Virus Delivery**

A significant advantage of MSCs as cellular vehicles is their accessibility for genetic manipulation in vitro. Recent studies have incorporated the use of lentivirus [13, 16, 48, 53] retrovirus [10, 19-20, 22], or plasmid [21] mediated transduction, however the majority remain Adenovirus based [5-8, 11, 14-15, 17-18, 23-25, 54-55]. MSCs have a low coxsackie and adenovirus receptor (CAR), high integrin phenotype, which results in low transfection efficiency using wild-type adenoviruses. Modification of the adenovirus fiber or knob domain has been used to improve adenovirus mediated transgene expression. Incorporation of an arginine-glycine-aspartate (RGD) motif into the adenovirus fiber, or 5/3 knob domain of human adenovirus serotype 3 supports CAR independent transfer and improves MSC transduction efficiency [14, 23-25, 55]. This has evolved to include the use of conditionally replicating adenoviruses, which support delivery of an increased viral load specifically to the tumour site [23-25]. Clearly the timing is important here to avoid toxicity to MSCs prior to engraftment at the target site. The cycle of MSC adenovirus replication has been reported to have relatively slow kinetics which may allow time for MSCs to reach the target site before replication causes cell death [56]. The delivery of oncolytic viruses does not rely on long term survival and proliferation of cellular vehicles, as they are destroyed by viral replication. Capsid modified oncolytic adenoviruses have been coupled with the use of transcription specific promoters to limit ectopic viral amplification in non-target cells [55]. MSCs have also been engineered to express the Herpes Simplex Virus-

thymidine kinase (HSV-tk) followed by administration of the prodrug ganciclovir (GCV) for targeted cancer suicide gene therapy [19-21]. Based on similar principles, retrovirus transduction of adipose derived MSCs to express cytosine deaminase (CD), followed by systemic administration of the prodrug 5-fluorocysteine (5-FC), mediated a strong antitumour effect in vivo [22].

### **Localized Delivery of Therapeutic Proteins**

Along with their tumour tropism, MSCs have been shown to integrate into and persist in the tumour stroma [5]. This has supported their use as delivery vehicles for various biological agents, whose systemic administration is precluded due to their short half-life and toxicity at the doses required for therapy. MSCs can efficiently produce biological products at tumour sites and so have the potential to improve pharmacokinetics of secreted agents [5].

In a number of tumour models, MSCs expressing IFN- $\beta$  have been shown to result in decreased tumour burden and increased animal survival [5-7]. Increased systemic levels of IFN- $\beta$ , or secretion at sites distant from the tumour were not effective, indicating that regional secretion was required [5-7]. MSCs engineered to secrete IL-12 and embedded in a matrix adjacent to tumours were also reported to have a significant therapeutic effect [10]. Similar to findings in the case of IFN- $\beta$ , regional secretion was required, with no reduction in growth observed when the implant was placed in the opposite flank to the tumour [10].

MSCs expressing the HGF antagonist, NK4 in vivo, were also found to prolong animal survival by inhibiting tumour associated angiogenesis, lymphoangiogenesis and induction of cancer cell apoptosis [14]. Local secretion of pigment epithelium-derived factor (PEDF) in a model of hepatocellular carcinoma (HCC) through lentivirus transduction of MSCs similarly resulted in lower tumour volume, reduced lung metastases and improved survival through inhibition of tumour angiogenesis [13].

Further, MSCs secreting IL-2 [8-9] or IL-12 [10-11] were shown to elicit an immunological reaction, and stimulate inflammatory cell infiltration of the tumour tissue. The observed anticancer effect was shown to be immune mediated and absent in immunodeficient animals [10]. Delivery of MSC-IL-12 did not cause systemic toxicity and resulted in increased serum and tumour levels of IL-12. In contrast, administration of Ad-IL-12 only increased serum IL-12 levels and induced systemic



toxicity [11]. Therefore it appears that MSC mediated local delivery of a therapeutic agent may be better tolerated by the host without inducing an unacceptable immune response [11].

Tumour necrosis factor related apoptosis inducing ligand (TRAIL) induces caspase mediated apoptosis in tumour cells that overexpress the receptor. MSCs, like most healthy tissues, are resistant to TRAIL induced apoptosis due to very low levels of active receptors [17]. As a result of this, MSCs secreting TRAIL have been used in models of lung, breast, cervical and brain cancer in vivo, resulting in significant anti-tumour effects [15-18, 53]. In one study using a lentiviral vector, TRAIL expression was placed under the control of a *tet* promoter, supporting conditional activation using doxycycline [16]. In an animal model of lung metastases of breast cancer, this controlled, local delivery of TRAIL completely cleared metastatic disease in a selection of animals [16]. Interestingly, when MSC-TRAIL cells were coinjected with tumour cells for subcutaneous tumour formation, only doxycycline mediated activation on the day of tumour cell inoculation (D0) caused a significant decrease in tumour weight. Activation following tumour establishment (D25) did not result in a change in tumour burden [16].

### **Potential role in Tumourigenesis**

Although beyond the scope of the current review, the potential role of MSCs in tumour initiation or promotion is a significant concern that must be addressed fully to allow MSC mediated therapy for cancer to realize its full potential. This remains a topic of continued debate. Expansion of MSCs in vitro will be required for therapeutic application and so their stability in culture is paramount. Spontaneous transformation of human MSCs has been reported following long term passage in vitro [57] [58], while Bernardo et al [59] found no evidence of hMSC transformation. Indeed the majority of studies have shown that human MSCs are stable, while murine MSCs are more prone to genetic transformation during in vitro culture, and may be capable of forming sarcomas in vivo [59-63]. Although transformation of human MSCs appears unlikely, and very rare, these studies certainly emphasise the importance of stringent monitoring of MSCs, including karyotyping, before application in the clinical setting. MSCs have also been implicated as tumor supportive when co-injected in the presence of a variety of tumour cell types, including breast [64-67], ovarian [68], melanoma [27], glioma [69-70] and colon [71-72]. However, the majority of these

studies used an equal or even excess number of MSCs over tumour cells. The data generated provides important information on interactions between MSCs and tumour cells, although the models are unlikely to reflect the *in vivo* situation. MSCs were shown to integrate into the tumour stroma and demonstrated to exert their effects at least partly through secretion of paracrine factors including CCL5, IL-6 and SDF-1 $\alpha$  [64-65, 68]. There is also evidence that MSCs may serve as precursors for carcinoma associated fibroblasts and/or pericytes, playing a potentially important role in tumour angiogenesis through differentiation and the release of proangiogenic factors [67-69, 71-76]. Additionally, as previously mentioned, the immunosuppressive qualities of MSCs may support tumour development and progression through protection of cancer cells from immune surveillance [27].

Conversely, co-injection of MSCs has also been shown to result in tumour suppression in a model of colon cancer [29], hepatoma [77], and melanoma [78].

In terms of MSC-mediated gene delivery, understanding the role of MSCs following engraftment at the site of a pre-established tumour is required. The majority of studies outlined here, using MSCs engineered to deliver therapeutic agents, have resulted in significant anti-tumour effects *in vivo*. Unmodified MSCs were also shown to result in tumour suppression in some cases [7-8, 79], with the majority showing no effect on tumour progression following engraftment at the site of an established tumour [13, 18, 23, 53, 55, 69, 75]. However, repeat IV administration of MSCs over three weeks was shown to stimulate increased tumour growth in a model of pancreatic cancer [21]. Similar to the level of MSC engraftment in tumors, it seems that the effect of MSCs following engraftment will be tumour specific, probably dependant on a range of factors including the method of MSC isolation and culture, the experimental model, the number of cells engrafted in the tumour, and the milieu of growth factors and inflammatory cytokines present within the tumour microenvironment.

## **Conclusion**

The studies outlined highlight very promising potential for MSC-mediated delivery of therapeutic agents directly to tumour tissue, with remarkable progress made in the past decade. Clearly MSCs have a number of advantages as cellular vehicles- they are relatively easy to isolate and expand, specifically target tumours and their metastases following systemic delivery, can be transduced efficiently with a range of vectors,

have immunosuppressive properties, the ability to express therapeutic proteins in secretory form, and can support amplification of oncolytic viruses. However, the potential for MSC-mediated tumour promotion must be addressed. Further understanding the biology of MSCs, and the specific combination of factors controlling their tumour-specific migration and persistence will support translation to the clinical setting.

### **Abbreviations**

MSC = Mesenchymal Stem Cells; EGF = epidermal growth factor; VEGF = vascular endothelial growth factor ; FGF = fibroblast growth factor; PDGF = platelet-derived growth factor; SDF-1 $\alpha$ /CXCL12 = stromal-derived growth factor-1 $\alpha$ ; IL = interleukin; GM-CSF = granulocyte-macrophage colony-stimulating factor; MCP-1/CCL2 = monocyte chemoattractant protein-1; HGF = hepatocyte growth factor; TGF = transforming growth factor; uPA = urokinase-type plasminogen activator VCAM = vascular cell adhesion molecule-1; SleX = Sialyl Lewis X; CAR = coxsackie and adenovirus receptor; HSV-tk = Herpes Simplex Virus-thymidine kinase; GCV = ganciclovir; CD = cytosine deaminase; 5-FC = 5-fluorocysteine; PEDF = pigment epithelium-derived factor; TRAIL = Tumour necrosis factor related apoptosis inducing ligand; IFN = interferon; MHC = major histocompatibility complex;

### **Competing interests**

The authors declare that they have no competing interests.

### **Acknowledgements**

Funding: National Breast Cancer Research Institute (NBCRI), Health Research Board of Ireland and Science Foundation Ireland.

### **Author details**

School of Medicine<sup>1</sup> and Regenerative Medicine Institute<sup>2</sup>, National University of Ireland Galway (NUIG), Galway, Ireland.

### **References**

1. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR: **Multilineage potential of adult human mesenchymal stem cells.** *Science* 1999, **284**:143-147.
2. Barry FP, Murphy JM: **Mesenchymal stem cells: clinical applications and biological characterization.** *Int J Biochem Cell Biol* 2004, **36**:568-584.
3. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop D, Horwitz E: **Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement.** *Cytotherapy* 2006, **8**:315-317.
4. Kidd S, Spaeth E, Klopp A, Andreeff M, Hall B, Marini FC: **The (in) auspicious role of mesenchymal stromal cells in cancer: be it friend or foe.** *Cytotherapy* 2008, **10**:657-667.
5. Studeny M, Marini FC, Champlin RE, Zompetta C, Fidler IJ, Andreeff M: **Bone marrow-derived mesenchymal stem cells as vehicles for interferon-beta delivery into tumors.** *Cancer research* 2002, **62**:3603-3608.
6. Nakamizo A, Marini F, Amano T, Khan A, Studeny M, Gumin J, Chen J, Hentschel S, Vecil G, Dembinski J, et al: **Human bone marrow-derived mesenchymal stem cells in the treatment of gliomas.** *Cancer research* 2005, **65**:3307-3318.
7. Kidd S, Caldwell L, Dietrich M, Samudio I, Spaeth EL, Watson K, Shi Y, Abbruzzese J, Konopleva M, Andreeff M, Marini FC: **Mesenchymal stromal cells alone or expressing interferon-beta suppress pancreatic tumors in vivo, an effect countered by anti-inflammatory treatment.** *Cytotherapy* 2010, [Epub ahead of print].
8. Nakamura K, Ito Y, Kawano Y, Kurozumi K, Kobune M, Tsuda H, Bizen A, Honmou O, Niitsu Y, Hamada H: **Antitumor effect of genetically engineered mesenchymal stem cells in a rat glioma model.** *Gene Therapy* 2004, **11**:1155-1164.
9. Stagg J, Lejeune L, Paquin A, Galipeau J: **Marrow stromal cells for interleukin-2 delivery in cancer immunotherapy.** *Hum Gene Ther* 2004, **15**:597-608.
10. Eliopoulos N, Francois M, Boivin MN, Martineau D, Galipeau J: **Neo-organoid of marrow mesenchymal stromal cells secreting interleukin-12 for breast cancer therapy.** *Cancer research* 2008, **68**:4810-4818.
11. Chen X, Lin X, Zhao J, Shi W, Zhang H, Wang Y, Kan B, Du L, Wang B, Wei Y, et al: **A tumor-selective biotherapy with prolonged impact on established metastases based on cytokine gene-engineered MSCs.** *Mol Ther* 2008, **16**:749-756.
12. Duan X, Guan H, Cao Y, Kleinerman ES: **Murine bone marrow-derived mesenchymal stem cells as vehicles for interleukin-12 gene delivery into Ewing sarcoma tumors.** *Cancer* 2009, **115**:13-22.
13. Gao Y, Yao A, Zhang W, Lu S, Yu Y, Deng L, Yin A, Xia Y, Sun B, Wang X: **Human mesenchymal stem cells overexpressing pigment epithelium-derived factor inhibit hepatocellular carcinoma in nude mice.** *Oncogene* 2010, **29**:2784-2794.
14. Kanehira M, Xin H, Hoshino K, Maemondo M, Mizuguchi H, Hayakawa T, Matsumoto K, Nakamura T, Nukiwa T, Saijo Y: **Targeted delivery of NK4 to multiple lung tumors by bone marrow-derived mesenchymal stem cells.** *Cancer Gene Ther* 2007, **14**:894-903.

15. Grisendi G, Bussolari R, Cafarelli L, Petak I, Rasini V, Veronesi E, De Santis G, Spano C, Tagliazzucchi M, Barti-Juhász H, et al: **Adipose-Derived Mesenchymal Stem Cells as Stable Source of Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand Delivery for Cancer Therapy.** *Cancer research* 2010, **70**:3718-3729.
16. Loebinger MR, Eddaoudi A, Davies D, Janes SM: **Mesenchymal stem cell delivery of TRAIL can eliminate metastatic cancer.** *Cancer research* 2009, **69**:4134-4142.
17. Mohr A, Lyons M, Deedigan L, Harte T, Shaw G, Howard L, Barry F, O'Brien T, Zwacka R: **Mesenchymal stem cells expressing TRAIL lead to tumour growth inhibition in an experimental lung cancer model.** *J Cell Mol Med* 2008, **12**:2628-2643.
18. Kim SM, Lim JY, Park SI, Jeong CH, Oh JH, Jeong M, Oh W, Park SH, Sung YC, Jeun SS: **Gene therapy using TRAIL-secreting human umbilical cord blood-derived mesenchymal stem cells against intracranial glioma.** *Cancer research* 2008, **68**:9614-9623.
19. Uchibori R, Okada T, Ito T, Urabe M, Mizukami H, Kume A, Ozawa K: **Retroviral vector-producing mesenchymal stem cells for targeted suicide cancer gene therapy.** *J Gene Med* 2009, **11**:373-381.
20. Matuskova M, Hlubinova K, Pastorakova A, Hunakova L, Altanerova V, Altaner C, Kucerova L: **HSV-tk expressing mesenchymal stem cells exert bystander effect on human glioblastoma cells.** *Cancer Lett* 2010, **290**:58-67.
21. Zischek C, Niess H, Ischenko I, Conrad C, Huss R, Jauch KW, Nelson PJ, Bruns C: **Targeting tumor stroma using engineered mesenchymal stem cells reduces the growth of pancreatic carcinoma.** *Ann Surg* 2009, **250**:747-753.
22. Kucerova L, Altanerova V, Matuskova M, Tyciakova S, Altaner C: **Adipose tissue-derived human mesenchymal stem cells mediated prodrug cancer gene therapy.** *Cancer research* 2007, **67**:6304-6313.
23. Dembinski JL, Spaeth EL, Fueyo J, Gomez-Manzano C, Studeny M, Andreeff M, Marini FC: **Reduction of nontarget infection and systemic toxicity by targeted delivery of conditionally replicating viruses transported in mesenchymal stem cells.** *Cancer Gene Ther* 2010, **17**:289-297.
24. Hakkarainen T, Sarkioja M, Lehenkari P, Miettinen S, Ylikomi T, Suuronen R, Desmond RA, Kanerva A, Hemminki A: **Human mesenchymal stem cells lack tumor tropism but enhance the antitumor activity of oncolytic adenoviruses in orthotopic lung and breast tumors.** *Hum Gene Ther* 2007, **18**:627-641.
25. Komarova S, Kawakami Y, Stoff-Khalili MA, Curiel DT, Pereboeva L: **Mesenchymal progenitor cells as cellular vehicles for delivery of oncolytic adenoviruses.** *Molecular Cancer Therapeutics* 2006, **5**:755-766.
26. Petrie Aronin CE, Tuan RS: **Therapeutic potential of the immunomodulatory activities of adult mesenchymal stem cells.** *Birth Defects Res C Embryo Today* 2010, **90**:67-74.
27. Djouad F, Plence P, Bony C, Tropel P, Apparailly F, Sany J, Noel D, Jorgensen C: **Immunosuppressive effect of mesenchymal stem cells favors tumor growth in allogeneic animals.** *Blood* 2003, **102**:3837-3844.

28. Djouad F, Bony C, Apparailly F, Louis-Plence P, Jorgensen C, Noel D: **Earlier onset of syngeneic tumors in the presence of mesenchymal stem cells.** *Transplantation* 2006, **82**:1060-1066.
29. Ohlsson LB, Varas L, Kjellman C, Edvardsen K, Lindvall M: **Mesenchymal progenitor cell-mediated inhibition of tumor growth in vivo and in vitro in gelatin matrix.** *Exp Mol Pathol* 2003, **75**:248-255.
30. Le Blanc K, Tammik L, Sundberg B, Haynesworth SE, Ringden O: **Mesenchymal stem cells inhibit and stimulate mixed lymphocyte cultures and mitogenic responses independently of the major histocompatibility complex.** *Scand J Immunol* 2003, **57**:11-20.
31. Dvorak HF: **Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing.** *N Engl J Med* 1986, **315**:1650-1659.
32. Feng B, Chen L: **Review of mesenchymal stem cells and tumors: executioner or coconspirator?** *Cancer Biother Radiopharm* 2009, **24**:717-721.
33. Nakamizo A, Marini F, Amano T, Khan A, Studeny M, Gumin J, Chen J, Hentschel S, Vecil G, Dembinski J, et al: **Human bone marrow-derived mesenchymal stem cells in the treatment of gliomas.** *Cancer Res* 2005, **65**:3307-3318.
34. Nakamura K, Ito Y, Kawano Y, Kurozumi K, Kobune M, Tsuda H, Bizen A, Honmou O, Niitsu Y, Hamada H: **Antitumor effect of genetically engineered mesenchymal stem cells in a rat glioma model.** *Gene Ther* 2004, **11**:1155-1164.
35. Studeny M, Marini FC, Dembinski JL, Zompetta C, Cabreira-Hansen M, Bekele BN, Champlin RE, Andreeff M: **Mesenchymal stem cells: potential precursors for tumor stroma and targeted-delivery vehicles for anticancer agents.** *J Natl Cancer Inst* 2004, **96**:1593-1603.
36. Wels J, Kaplan RN, Rafii S, Lyden D: **Migratory neighbors and distant invaders: tumor-associated niche cells.** *Genes Dev* 2008, **22**:559-574.
37. Dwyer RM, Potter-Beirne SM, Harrington KA, Lowery AJ, Hennessy E, Murphy JM, Barry FP, O'Brien T, Kerin MJ: **Monocyte Chemotactic Protein-1 (MCP-1) secreted by primary breast tumors stimulates migration of Mesenchymal Stem Cells (MSCs).** *Clin Cancer Res* 2007, **13**:5020-5027.
38. Chamberlain G, Fox J, Ashton B, Middleton J: **Concise review: mesenchymal stem cells: their phenotype, differentiation capacity, immunological features, and potential for homing.** *Stem Cells* 2007, **25**:2739-2749.
39. Ruster B, Gottig S, Ludwig RJ, Bistran R, Muller S, Seifried E, Gille J, Henschler R: **Mesenchymal stem cells display coordinated rolling and adhesion behavior on endothelial cells.** *Blood* 2006, **108**:3938-3944.
40. Ringe J, Strassburg S, Neumann K, Endres M, Notter M, Burmester GR, Kaps C, Sittinger M: **Towards in situ tissue repair: human mesenchymal stem cells express chemokine receptors CXCR1, CXCR2 and CCR2, and migrate upon stimulation with CXCL8 but not CCL2.** *J Cell Biochem* 2007, **101**:135-146.
41. Honczarenko M, Le Y, Swierkowski M, Ghiran I, Glodek AM, Silberstein LE: **Human bone marrow stromal cells express a distinct set of biologically functional chemokine receptors.** *Stem Cells* 2006, **24**:1030-1041.

42. Von Luttichau I, Notohamiprodjo M, Wechselberger A, Peters C, Henger A, Seliger C, Djafarzadeh R, Huss R, Nelson PJ: **Human adult CD34-progenitor cells functionally express the chemokine receptors CCR1, CCR4, CCR7, CXCR5, and CCR10 but not CXCR4.** *Stem Cells Dev* 2005, **14**:329-336.
43. Sordi V, Malosio ML, Marchesi F, Mercalli A, Melzi R, Giordano T, Belmonte N, Ferrari G, Leone BE, Bertuzzi F, et al: **Bone marrow mesenchymal stem cells express a restricted set of functionally active chemokine receptors capable of promoting migration to pancreatic islets.** *Blood* 2005, **106**:419-427.
44. Chamberlain G, Wright K, Rot A, Ashton B, Middleton J: **Murine mesenchymal stem cells exhibit a restricted repertoire of functional chemokine receptors: comparison with human.** *PLoS One* 2008, **3**:e2934.
45. Shi M, Li J, Liao L, Chen B, Li B, Chen L, Jia H, Zhao RC: **Regulation of CXCR4 expression in human mesenchymal stem cells by cytokine treatment: role in homing efficiency in NOD/SCID mice.** *Haematologica* 2007, **92**:897-904.
46. Wynn RF, Hart CA, Corradi-Perini C, O'Neill L, Evans CA, Wraith JE, Fairbairn LJ, Bellantuono I: **A small proportion of mesenchymal stem cells strongly expresses functionally active CXCR4 receptor capable of promoting migration to bone marrow.** *Blood* 2004, **104**:2643-2645.
47. Potapova IA, Brink PR, Cohen IS, Doronin SV: **Culturing of human mesenchymal stem cells as three-dimensional aggregates induces functional expression of CXCR4 that regulates adhesion to endothelial cells.** *J Biol Chem* 2008, **283**:13100-13107.
48. Zielske SP, Livant DL, Lawrence TS: **Radiation increases invasion of gene-modified mesenchymal stem cells into tumors.** *Int J Radiat Oncol Biol Phys* 2009, **75**:843-853.
49. Klopp AH, Spaeth EL, Dembinski JL, Woodward WA, Munshi A, Meyn RE, Cox JD, Andreeff M, Marini FC: **Tumor irradiation increases the recruitment of circulating mesenchymal stem cells into the tumor microenvironment.** *Cancer research* 2007, **67**:11687-11695.
50. Coussens LM, Werb Z: **Inflammation and cancer.** *Nature* 2002, **420**:860-867.
51. Sarkar D, Vemula PK, Zhao W, Gupta A, Karnik R, Karp JM: **Engineered mesenchymal stem cells with self-assembled vesicles for systemic cell targeting.** *Biomaterials* 2010, **31**:5266-5274.
52. Sato H, Kuwashima N, Sakaida T, Hatano M, Dusak JE, Fellows-Mayle WK, Papworth GD, Watkins SC, Gambotto A, Pollack IF, Okada H: **Epidermal growth factor receptor-transfected bone marrow stromal cells exhibit enhanced migratory response and therapeutic potential against murine brain tumors.** *Cancer Gene Ther* 2005, **12**:757-768.
53. Sasportas LS, Kasmieh R, Wakimoto H, Hingtgen S, van de Water JA, Mohapatra G, Figueiredo JL, Martuza RL, Weissleder R, Shah K: **Assessment of therapeutic efficacy and fate of engineered human mesenchymal stem cells for cancer therapy.** *Proc Natl Acad Sci U S A* 2009, **106**:4822-4827.
54. Sonabend AM, Ulasov IV, Tyler MA, Rivera AA, Mathis JM, Lesniak MS: **Mesenchymal stem cells effectively deliver an oncolytic adenovirus to intracranial glioma.** *Stem cells (Dayton, Ohio)* 2008, **26**:831-841.

55. Stoff-Khalili MA, Rivera AA, Mathis JM, Banerjee NS, Moon AS, Hess A, Rocconi RP, Numnum TM, Everts M, Chow LT, et al: **Mesenchymal stem cells as a vehicle for targeted delivery of CRAds to lung metastases of breast carcinoma.** *Breast cancer research and treatment* 2007, **105**:157-167.
56. Pereboeva L, Komarova S, Mikheeva G, Krasnykh V, Curiel DT: **Approaches to utilize mesenchymal progenitor cells as cellular vehicles.** *Stem cells (Dayton, Ohio)* 2003, **21**:389-404.
57. Rubio D, Garcia-Castro J, Martin MC, de la Fuente R, Cigudosa JC, Lloyd AC, Bernad A: **Spontaneous human adult stem cell transformation.** *Cancer research* 2005, **65**:3035-3039.
58. Wang Y, Huso DL, Harrington J, Kellner J, Jeong DK, Turney J, McNiece IK: **Outgrowth of a transformed cell population derived from normal human BM mesenchymal stem cell culture.** *Cytotherapy* 2005, **7**:509-519.
59. Bernardo ME, Zaffaroni N, Novara F, Cometa AM, Avanzini MA, Moretta A, Montagna D, Maccario R, Villa R, Daidone MG, et al: **Human bone marrow derived mesenchymal stem cells do not undergo transformation after long-term in vitro culture and do not exhibit telomere maintenance mechanisms.** *Cancer research* 2007, **67**:9142-9149.
60. Zhou YF, Bosch-Marce M, Okuyama H, Krishnamachary B, Kimura H, Zhang L, Huso DL, Semenza GL: **Spontaneous transformation of cultured mouse bone marrow-derived stromal cells.** *Cancer research* 2006, **66**:10849-10854.
61. Miura M, Miura Y, Padilla-Nash HM, Molinolo AA, Fu B, Patel V, Seo BM, Sonoyama W, Zheng JJ, Baker CC, et al: **Accumulated chromosomal instability in murine bone marrow mesenchymal stem cells leads to malignant transformation.** *Stem cells (Dayton, Ohio)* 2006, **24**:1095-1103.
62. Tolar J, Nauta AJ, Osborn MJ, Panoskaltsis Mortari A, McElmurry RT, Bell S, Xia L, Zhou N, Riddle M, Schroeder TM, et al: **Sarcoma derived from cultured mesenchymal stem cells.** *Stem cells (Dayton, Ohio)* 2007, **25**:371-379.
63. Li H, Fan X, Kovi RC, Jo Y, Moquin B, Konz R, Stoicov C, Kurt-Jones E, Grossman SR, Lyle S, et al: **Spontaneous expression of embryonic factors and p53 point mutations in aged mesenchymal stem cells: a model of age-related tumorigenesis in mice.** *Cancer research* 2007, **67**:10889-10898.
64. Karnoub AE, Dash AB, Vo AP, Sullivan A, Brooks MW, Bell GW, Richardson AL, Polyak K, Tubo R, Weinberg RA: **Mesenchymal stem cells within tumour stroma promote breast cancer metastasis.** *Nature* 2007, **449**:557-563.
65. Muehlberg FL, Song YH, Krohn A, Pinilla SP, Droll LH, Leng X, Seidensticker M, Ricke J, Altman AM, Devarajan E, et al: **Tissue-resident stem cells promote breast cancer growth and metastasis.** *Carcinogenesis* 2009, **30**:589-597.
66. Rhodes LV, Muir SE, Elliott S, Guillot LM, Antoon JW, Penfornis P, Tilghman SL, Salvo VA, Fonseca JP, Lacey MR, et al: **Adult human mesenchymal stem cells enhance breast tumorigenesis and promote hormone independence.** *Breast cancer research and treatment* 2010, **121**:293-300.
67. Galie M, Konstantinidou G, Peroni D, Scambi I, Marchini C, Lisi V, Krampera M, Magnani P, Merigo F, Montani M, et al: **Mesenchymal stem**



- cells share molecular signature with mesenchymal tumor cells and favor early tumor growth in syngeneic mice. *Oncogene* 2008, **27**:2542-2551.
68. Spaeth EL, Dembinski JL, Sasser AK, Watson K, Klopp A, Hall B, Andreeff M, Marini F: **Mesenchymal stem cell transition to tumor-associated fibroblasts contributes to fibrovascular network expansion and tumor progression.** *PloS one* 2009, **4**:e4992.
  69. Bexell D, Gunnarsson S, Tormin A, Darabi A, Gisselsson D, Roybon L, Scheding S, Bengzon J: **Bone marrow multipotent mesenchymal stroma cells act as pericyte-like migratory vehicles in experimental gliomas.** *Mol Ther* 2009, **17**:183-190.
  70. Yu JM, Jun ES, Bae YC, Jung JS: **Mesenchymal stem cells derived from human adipose tissues favor tumor cell growth in vivo.** *Stem Cells Dev* 2008, **17**:463-473.
  71. Shinagawa K, Kitadai Y, Tanaka M, Sumida T, Kodama M, Higashi Y, Tanaka S, Yasui W, Chayama K: **Mesenchymal stem cells enhance growth and metastasis of colon cancer.** *International journal of cancer* 2010, [Epub ahead of print].
  72. Zhu W, Xu W, Jiang R, Qian H, Chen M, Hu J, Cao W, Han C, Chen Y: **Mesenchymal stem cells derived from bone marrow favor tumor cell growth in vivo.** *Exp Mol Pathol* 2006, **80**:267-274.
  73. Wu Y, Wang J, Scott PG, Tredget EE: **Bone marrow-derived stem cells in wound healing: a review.** *Wound Repair Regen* 2007, **15 Suppl 1**:S18-26.
  74. Wu Y, Chen L, Scott PG, Tredget EE: **Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis.** *Stem Cells* 2007, **25**:2648-2659.
  75. Mishra PJ, Mishra PJ, Humeniuk R, Medina DJ, Alexe G, Mesirov JP, Ganesan S, Glod JW, Banerjee D: **Carcinoma-associated fibroblast-like differentiation of human mesenchymal stem cells.** *Cancer research* 2008, **68**:4331-4339.
  76. Bagley RG, Weber W, Rouleau C, Yao M, Honma N, Kataoka S, Ishida I, Roberts BL, Teicher BA: **Human mesenchymal stem cells from bone marrow express tumor endothelial and stromal markers.** *Int J Oncol* 2009, **34**:619-627.
  77. Qiao L, Xu Z, Zhao T, Zhao Z, Shi M, Zhao RC, Ye L, Zhang X: **Suppression of tumorigenesis by human mesenchymal stem cells in a hepatoma model.** *Cell research* 2008, **18**:500-507.
  78. Maestroni GJ, Hertens E, Galli P: **Factor(s) from nonmacrophage bone marrow stromal cells inhibit Lewis lung carcinoma and B16 melanoma growth in mice.** *Cell Mol Life Sci* 1999, **55**:663-667.
  79. Khakoo AY, Pati S, Anderson SA, Reid W, Elshal MF, Rovira, II, Nguyen AT, Malide D, Combs CA, Hall G, et al: **Human mesenchymal stem cells exert potent antitumorigenic effects in a model of Kaposi's sarcoma.** *J Exp Med* 2006, **203**:1235-1247.