Contrasting views on the role of mesenchymal stromal/stem cells in tumour growth: a systematic review of experimental design

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LIST OF ABBREVIATIONS

MSC: Mesenchymal stromal/stem cell

SCF: Stem cell factor

c-Kit: Tyrosine-protein kinase Kit also known as mast/stem cell growth factor receptor (SCFR)

SDF-1: Stromal cell-derived factor 1

CXCR4: C-X-C Motif Chemokine Receptor 4

VEGF: Vascular endothelial growth factor

VEGFR: Vascular endothelial growth factor receptor

HGF: Hepatocyte growth factor

c-Met: Tyrosine-protein kinase Met or hepatocyte growth factor receptor

MCP-1: Monocyte chemotactic protein 1

CCR2: C-C Motif Chemokine Receptor 2

TGF-ß: Transforming growth factor-beta

IL-8: Interleukin 8

EGF: Epithelial growth factor

TNF-α: Tumour necrosis factor alpha

PDGF: Platelet-derived growth factor

IL-1β: Interleukin 1-beta

SC: Subcutaneous

IV: Intravenous

IP: Intraperitoneal

HNSCC: Head and neck squamous cell carcinoma

BM: Bone marrow

AD: Adipose tissue

UC: Umbilical cord

SCID: Severe combined immunodeficiency

CC: Co-culture

CM: Conditioned medium

ABSTRACT

The effect of mesenchymal stromal/stem cells (MSCs) on tumour growth remains controversial. Experimental evidence supports both an inhibitory and a stimulatory effect. We have assessed factors responsible for the contrasting effects of MSCs on tumour growth by doing a meta-analysis of existing literature between 2000 and May 2017. We assessed 183 original research articles comprising 338 experiments. We considered (a) *in vivo* and *in vitro* experiments; (b) whether *in vivo* studies were syngeneic or xenogeneic; and (c) if animals were immune competent or deficient. Furthermore, the sources and types of cancer cells and MSCs were considered together with modes of cancer induction and MSC administration. 56% of all 338 experiments reported that MSCs promote tumour growth. 78% and 79% of all experiments sourced human MSCs and cancer cells respectively. MSCs were used in their naïve and engineered form in 86% and 14% of experiments respectively, the latter to produce factors that could alter either their activity or that of the tumour. 53% of all experiments were conducted *in vitro* with 60% exposing cancer cells to MSCs via co-culture. Of all *in vivo* experiments, 79% were xenogeneic and 63% were conducted in immune competent animals. Tumour growth was inhibited in 80% of experiments that used umbilical cord-derived MSCs whereas tumour growth was promoted in 64% and 57% of experiments that used bone marrow- and adipose tissue-derived MSCs respectively. This contrasting effect of MSCs on tumour growth observed under different experimental conditions may reflect differences in experimental outcome. This analysis calls for careful consideration of experimental design. This is particularly important given the large number of MSC clinical trials currently underway.

Keywords: Mesenchymal stem cell; Tumour; Cancer; Xenogeneic; Syngeneic

1 INTRODUCTION

Interest in the effect of mesenchymal stromal/stem cells (MSCs) on tumour growth stems from two areas. The first relates to the fact that MSCs are being assessed in a growing number of clinical trials for a wide variety of diseases (Hong et al., 2014; Squillaro et al., 2016). The fear is that systemically-administered MSCs have the potential to activate dormant tumours through the production of paracrine growth stimulatory molecules (Lazennec and Lam, 2016).The second relates to the fact that in some experimental settings, MSCs have been shown to inhibit tumour growth, and this has sparked interest in the possible use of MSCs in the treatment of cancer.

Globally, cancer remains a leading cause of death. Cancer incidence and cancer-related mortality increased by approximately 11% and 17% respectively between 2008 and 2012. This trend is projected to increase by about 70% in the next two decades, with cancer incidence increasing from 14.1 million in 2012 to 22 million in 2030 while mortality will increase from 8.2 to 13 million (Ferlay et al., 2010). In 2008, about 169.3 million years of healthy life were lost due to cancer (Soerjomataram et al., 2012). While primary prevention of cancer includes raising public awareness and avoiding modifiable risk factors, there is a need for effective treatment for those already afflicted.

Several therapeutic measures exist for cancer, including chemotherapy, radiotherapy and immunotherapy. These therapies have their own side effects and limitations. Recently, the concept of cellular therapy for cancer was introduced, even though the effect of stem cell treatment on cancer is highly controversial (Hong et al., 2014). Mesenchymal stromal/stem cells (MSCs) contain cells with stem cell-like properties that are multipotent in nature and are able to self-renew (Bianco et al., 2013). It has also been reported that they have the ability to home to sites of injury and inflammation, and to tumours (Hong et al., 2014). The therapeutic potential of MSCs may lie in cellular rejuvenation or as a transport vehicle for other therapies (Serakinci et al., 2014). Hong, Lee and Kang provide a detailed explanation of the different interactions between MSCs and tumours (Hong et al., 2014). Here we have assessed whether there is a relationship between experimental design and observed results.

MSCs on their own are believed not to be tumourigenic, but several studies have reported both tumour promoting (Albarenque et al., 2011; De Boeck et al., 2013; Ljujic et al., 2013; Zhang et al., 2013) and inhibitory (Chao et al., 2012; Ganta et al., 2009; Maurya et al., 2010) effects. Experimental design is highly variable. *In vivo* experiments may be xenogeneic, syngeneic or isogeneic. The immune status of the animal may be immune competent, compromised or deficient. Outcomes of *in vitro* experiments could be influenced by whether MSCs and cancer cells were co-cultured or cancer cells were exposed to conditioned media from MSCs. The sources and types of cancer cells and MSCs may influence the outcome of the experiments. MSCs can be sourced from different animals including rabbits, mice and humans, and can be found in various tissues including bone marrow, umbilical cord blood, peripheral blood, placenta and adipose tissue. Experimental design may therefore have an important influence on the outcomes of experiments that assess the tumourigenic action of MSCs.

Understanding how MSCs interact with cancer cells and the experimental factors that influence the results may direct future research and the ultimate use of MSCs to treat cancer. Likewise, the incidental tumour promoting effects of MSCs on latent/dormant tumours in patients being treated for other conditions needs to be avoided. This is because tumour microenvironment continuously produces and releases various cytokines and mediators that establish a state of inflammation which has the capacity to attract MSCs. This tumor-directed migratory potential of MSCs has been observed in almost all cancer types tested so far which includes breast (Patel et al., 2010), lung (Loebinger et al., 2009), ovarian (Kidd et al., 2009), pancreatic (Zischek et al., 2009), colon (Menon et al., 2007), skin (Studeny et al., 2002) and brain cancer (Sasportas et al., 2009), even though the underlying mechanism of this MSCs tropism remains unknown. Stem cell factor (SCF)/c-Kit, SDF-1/CXCR4, VEGF/VEGFR, HGF/c-Met and MCP-1/CCR2 are some of the chemokine/receptor pairs reported to be associated with homing of MSCs to disease sites. In addition, TGF-ß, IL-8, EGF, neurotrophin-3, TNF-α, PDGF, and IL-1ß are other growth factors, angiogenic factors and inflammatory cytokines known to stimulate MSC migration. Most of these chemokines and cytokines are produce and release by tumours (Motaln et al., 2010; Nakamizo et al., 2005), which may serve as chemoattractants (ligands) for receptors on MSCs. This chemokine/receptor axis between tumours and MSCs may lead MSCs that are administered

to patients for the treatment of other diseases migrating and homing to sites of latent/dormant tumours, thereby stimulating their growth.

Here we have reviewed available published literature over the last 16 years which has assessed the effects of MSCs on tumour growth. We (a) looked at which experimental factors were associated with specific outcomes and (b) how these factors might have influenced experimental outcomes.

2 METHODS

We conducted a systematic review and a meta-analysis of the available literature from January 2000 to May 2017. We used the search terms MSC, cancer and tumour growth on Google Scholar and PubMed search engines. A total of 1586 articles were generated from which we selected 183 after applying our exclusion criteria. These 183 articles comprised 338 experiments that assessed the effects of MSCs on tumourigenesis**.**

2.1 Inclusion criteria

We included original research articles published in or with an expanded abstract in English between January 2000 and May 2017. The earliest article testing the effect of MSCs on tumour progression was published in 2003 (Djouad et al., 2003). All included articles have a definite end-point regarding the effect of MSCs on tumour growth or metastasis.

2.2 Exclusion Criteria

Duplicate and non-original research articles, such as review articles, were excluded. Articles that studied the effect of MSCs on pathologies other than cancer/tumours were excluded. Articles that studied the effect of other substances besides MSCs on cancer were excluded. We excluded studies where no definite effects of MSCs on tumour progression were reported. Studies where MSCs were derived from tumours or other pathological tissues were also excluded (Figure 1).

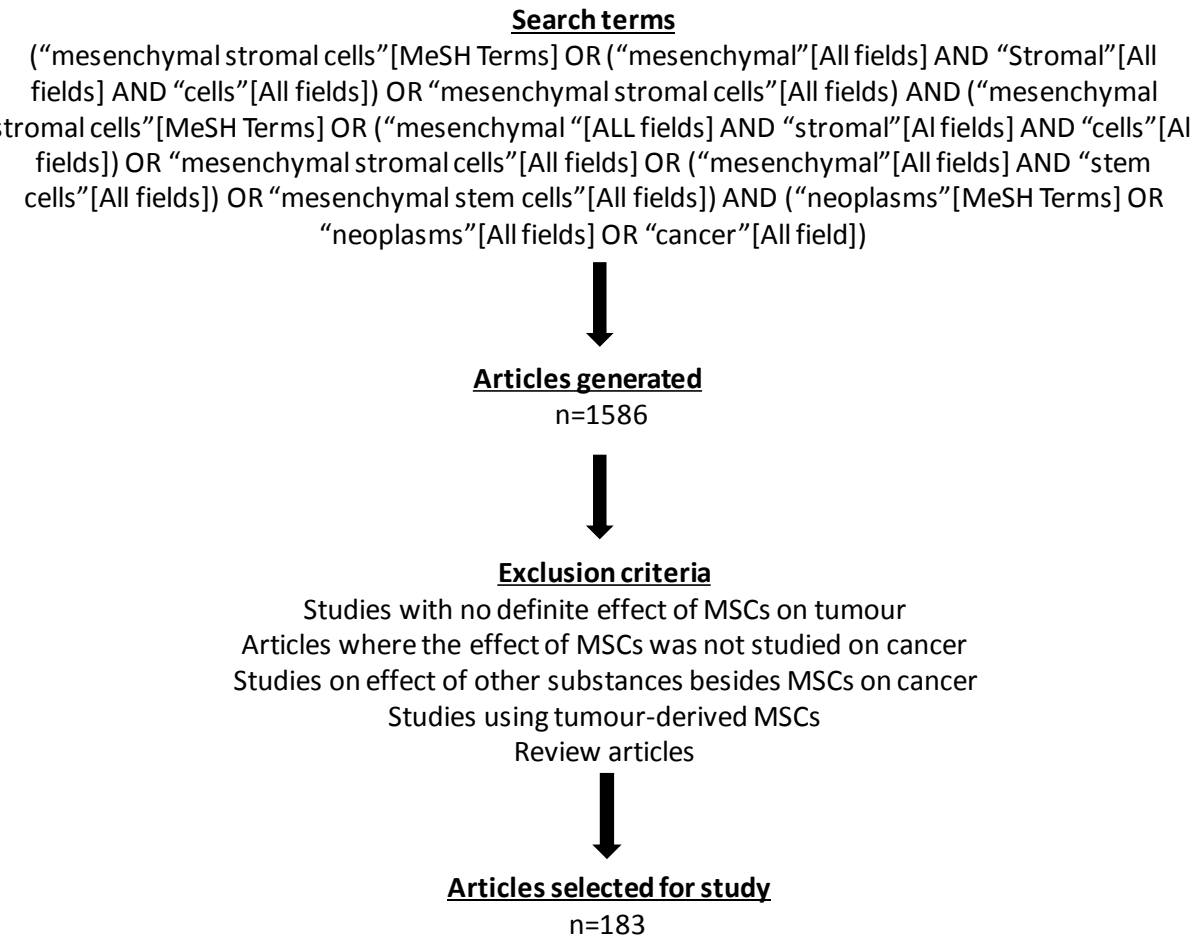

Figure 1: Method of searching the literature for the effect of MSCs on tumour growth

3 RESULTS AND DISCUSSION

3.1 Effects of MSCs on tumour growth (inhibition versus stimulation)

Our review revealed that MSCs had a stimulatory effect on tumour growth in 56% (90 *in vivo* and 100 *in vitro* experiments) and an inhibitory effect in 44% (69 *in vivo* and 79 *in vitro* experiments) of all studies assessed (Figure 2). The response of tumours to MSCs was not evenly distributed per experimental type, exposure type, experimental animals used, MSCs or cancer cell types.

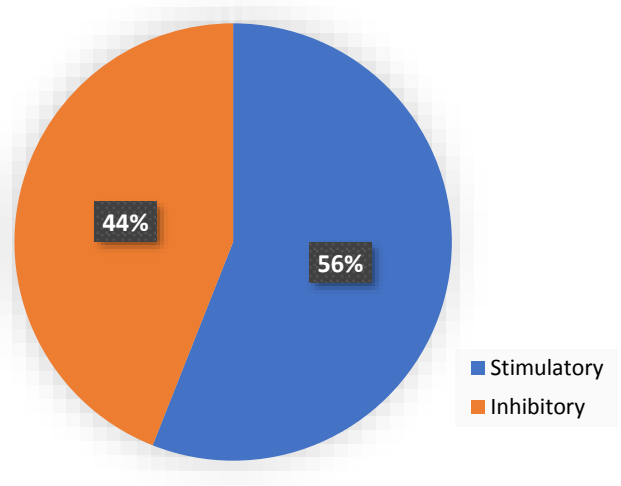

Figure 2: The effect of MSCs on tumour growth

The effects (stimulatory or inhibitory) of different MSC factors/parameters considered in this review on tumour growth *in vivo* or *in vitro* are summarized in Table 1.

Table 1: The effect of MSCs on tumour growth

Experimental type (n=338)	*In vivo* (n=159; 47%)		*In vitro* (n=179; 53%)	
Effect on tumour growth	**Stimulatory**	**Inhibitory**	**Stimulatory**	**Inhibitory**
	n=90 (57%)	n=69 (43%)	n=100 (56%)	n=79 (44%)
Experimental model/design	Syngeneic (n=22) Xenogeneic (n=68)	Syngeneic (n=15) Xenogeneic (n=54)	n/a	n/a
Animal model	Mouse (n=87) Rat (n=2) Other (n=1)	Mouse (n=61) Rat (n=7) Other (n=1)	n/a	n/a
Animal immune status	Competent (n=62) Deficient and compromised (n=28)	Competent (n=39) Deficient and compromised (n=30)	n/a	n/a
Species from which MSCs were derived	Human (n=65) Mouse (n=22) Rat (n=3)	Human (n=49) Mouse (n=11) Rat (n=8) Hamster (n=1)	Human (n=80) Mouse (n=15) Rat (n=5)	Human (n=68) Mouse (n=5) Rat (n=6)
Source of MSCs	BM (n=67) AD (n=10) UC (n=6) Others (n=7)	BM (n=40) AD (n=9) UC (n=15) Others (n=4)	BM (n=65) AD (n=23) UC (n=4) Others (n=8)	BM (n=34) AD (n=16) UC (n=25) Others (n=4)
Sources of cancer cells	Human (n=65) Mouse (n=22) Rat (n=2) Chemical (n=1)	Human (n=47) Mouse (n=13) Rat (n=6) Chemical (n=3)	Human (n=88) Mouse (n=11) Rat (n=1)	Human (n=66) Mouse (n=8) Rat (n=4) Chemical (n=1)

Types of cancer	Breast (n=22) Lung (n=7) Colorectal (n=14) Prostate (n=7) Glioma (n=3) HNSCC (n=2) Hepatic (n=1) Gastric (n=9) Sarcoma (n=9) Others (n=16)	Breast (n=14) Lung (n=8) Colorectal (n=2) Prostate (n=9) Glioma (n=10) HNSCC (n=1) Hepatic (n=7) Gastric (n=1) Sarcoma (n=4) Others (n=13)	Breast (n=36) Lung (n=5) Colorectal (n=5) Prostate (n=11) Glioma (n=3) HNSCC (n=6) Hepatic (n=5) Gastric (n=7) Sarcoma (n=7) Others (n=15)	Breast (n=24) Lung (n=8) Colorectal (n=4) Prostate (n=4) Glioma (n=9) HNSCC (n=3) Hepatic (n=7) Gastric (n=2) Sarcoma (n=4) Others (n=14)
Methods of cancer induction	SC (n=58) IV (n=4) IP (n=4) Ortho (n=19) Others (n=5)	SC (n=27) IV (n=11) IP (n=6) Ortho (n=17) Others (n=8)	Coculture (n=59) Conditioned medium (n=41)	Coculture (n=46) Conditioned medium (n=33)
Mode of administration of MSCs	SC (n=54) IV (n=14) IP (n=4) Intra-tumoural (n=13) Others (n=5)	SC (n=17) IV (n=25) IP (n=9) Intra-tumoural (n=11) Others (n=7)		
MSCs status	Naïve (n=84) Engineered (n=6)	Naïve (n=44) Engineered (n=25)	Naïve (n=95) Engineered (n=5)	Naïve (n=66) Engineered (n=13)

n, number of studies; SC, subcutaneous; IV, intravenous; IP, intraperitoneal; Ortho, orthotopically; HNSCC, head and neck squamous cell carcinoma; n/a, not applicable

3.2 Types of experiment (*in vivo* versus *in vitro*)

179 (53%) of the 338 experiments reviewed were conducted *in vitro*, of which 100 (56%) reported a stimulatory effect on tumour growth (Figure 3). Forty-seven percent (159) of experiments were conducted *in vivo* (Figure 3), of which 90 (57%) revealed that MSCs promote tumour growth (Figure 3). The secretome of transplanted MSCs is known to be largely determined by their microenvironment, and the same MSCs will have a different profile *in vitro* to that in *in vivo* when they are transplanted (Dittmer and Leyh, 2014). The lack of differentiation between tumour response and experimental type indicates a need to conduct simultaneous *in vivo* and *in vitro* experiments and to interpret the latter with particular caution.

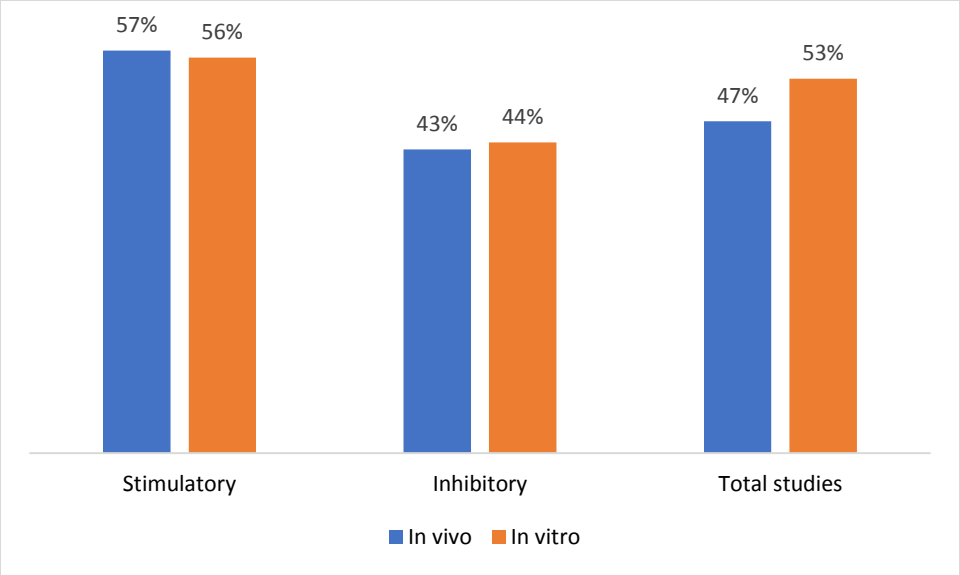

Figure 3: Experimental type (*in vivo* and *in vitro*) used to assess the effect of MSCs on tumour growth. Virtually equal numbers of studies showed stimulatory or inhibitory effects although the number of studies conducted *in vitro* was slightly higher.

3.3 Effect of MSCs on tumour growth – the role of *in vivo*-specific factors

The effect of the immune status of the animal and the nature of the animal model and experimental design (syngeneic or xenogeneic) are some of the *in vivo* parameters/factors which are likely to affect the outcome of studies on the effect of MSCs on tumour growth.

3.3.1 Immune status of experimental animals

101 (64%) of the 159 *in vivo* experiments used immune competent animals while 58 (36%) used immune deficient or compromised animals. Of the 159 *in vivo* experiments reviewed, 37 used severe combined immunodeficiency (SCID) or athymic mice in a xenogeneic experimental design. Quante et al. (2011) is the only syngeneic experimental study in SCID mice that assessed the effect of murine BM-MSCs on mouse lung cancer, and this revealed a stimulatory effect (Quante et al., 2011). Conducting xenogeneic experiments using immune deficient animals may reduce the immune response of the host to both the cancer and MSCs from other species. Immune competent animals with intact immunosurveillance systems should have a natural resistance to and reject either or both cancer cells and MSCs from another species.

MSCs stimulated tumour growth in 61% (n=62) of experiments that used immune competent animals, suggesting an interaction with the host immune system. MSCs inhibited tumour growth in 52% (n=30) of experiments that used immune deficient or compromised animals (Figure 4). Immune deficient animals such as athymic mice have been used to validate human MSCs prior to Phase II clinical trials. Even though human cells are successfully transplanted into these animals and subsequently survive and thrive in them, the lack of a competent immune system can mask natural responses to MSCs (Tholpady et al., 2003) and tumour cells. Athymic animals are also prone to developing subclinical infections and systemic illness (Lopez and Spencer, 2011), which may mask the effect of MSCs. The immune status of animals used for *in vivo* experiments is therefore likely to play an important role in determining the effect of MSCs on tumour growth.

3.3.2 Species in experimental animal models

148 (93%) of the *in vivo* experiments used mice while 9 used rats (6%) and other models including hamster and rabbit (1%; n=2). MSCs stimulated tumour growth in 59% (n=87) of *in vivo* experiments using mice, whereas tumour growth was inhibited in 78% (n=7) of studies using rats (Figure 4), although the number of experiments using rats (n=9) was very small compared to mice (n=148).

3.3.3 Experimental model/design

The source of MSCs and cancer cell lines used for *in vivo* studies was mainly human. Most of the *in vivo* experiments - 122 (77%) - were xenogeneic while the remaining 37 (23%) were syngeneic. MSCs promoted tumour growth in 56% (n=68) and 59% (n=22) of xenogeneic and syngeneic studies respectively (Figure 4). The origin of MSCs and cancer cells may affect the immune response in the experimental animals employed, and differences have been reported between allogenic and xenogeneic experiments in several animal models (Revell and Athanasiou, 2009; Sigrist et al., 2005).

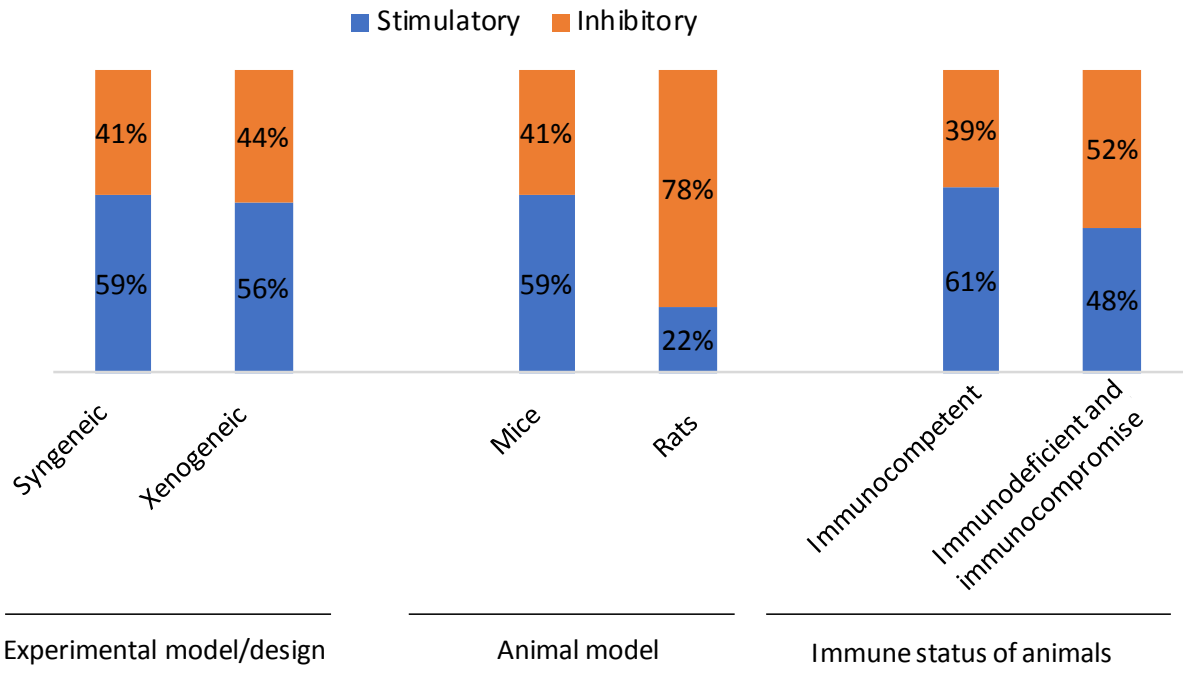

Figure 4: Effect of *in vivo*-specific factors on tumour growth in response to administered MSCs. A greater percentage of studies showed that MSCs promote tumour growth *in vivo* in mice and immune competent animals whereas they inhibit tumour growth in rats and immune deficient animals.

3.4 MSC sources and types used in *in vivo* and *in vitro* experiments

MSCs used were from humans (78%; n=262), mice (16%; n=53), rats (6%; n=22) and hamsters (n=1). Tumour growth was stimulated in 55% (n=145) and 70% (n=37) of experiments that used MSCs from humans and mice respectively, whereas, growth was inhibited in 64% (n=14) of experiments that used rat MSCs (Figure 5). Sources of MSCs may influence the immune response of the animals used in *in vivo* experiments. Using xenogeneic or syngeneic cells in *in vivo* experiments may affect the immune system (Figure 4) and thus influence the effect of MSCs on tumour growth.

MSCs were derived from BM, umbilical cord (UC), adipose tissue (AD) and a few studies used MSCs derived from peripheral blood mononuclear cells, foetal dermis, liver and umbilical cord blood, amongst others. 61% (n=206) of experiments used BM-MSCs, 15% (n=50) used UC-MSCs, 17% (n=58) used AD-MSCs while the remaining 7% (n=23) were sourced from other tissues like dermis, decidua, liver, umbilical cord blood and peripheral blood. BM-MSCs

stimulated tumour growth in 64% (n=132) of experiments (Figure 5), regardless of whether the experiment was conducted *in vivo* or *in vitro*. BM-MSCs stimulated tumour growth in 66% (n=65) of *in vitro* experiments and 63% (n=67) of *in vivo* experiments. The stimulatory effect of BM-MSCs was primarily associated with breast cancer cells (Supplementary Table S1 a, b and S2 a, b). Studies assessing the action of BM-MSCs on breast cancer cells were highly prevalent amongst those reviewed. UC-MSCs inhibited tumour growth in 80% (n=40) of experiments where they were used (Figure 5) regardless of the experimental type. Experiments were conducted both *in vitro* (n=29) and *in vivo* (n=21). UC-MSCs inhibited tumour growth in 86% (n=25) of *in vitro* experiments and in 71% (n=15) of *in vivo* experiments (Supplementary Table S3 a, b and S4 a, b). Tumour growth was promoted in 57% (n=33) of studies where AD-MSCs were used irrespective of the experimental type (Figure 5). Experiments were conducted both *in vitro* (n=39) and *in vivo* (n=19). AD-MSCs promoted tumour growth in 53% (n=10) of *in vivo* and in 59% (n=23) of *in vitro* experiments (Supplementary Table S5 and S6). MSCs derived from other tissue sources such as dermis, peripheral blood and umbilical cord blood had a stimulatory (65%; n=15) or inhibitory (35%; n=8) effect on tumour growth.

Even though MSCs isolated from distinct tissue sources display some characteristics that are similar, certain inherent genetic or cellular variations exist between tissues (Wagner et al., 2005; Zhou et al., 2013). For example, breast cancer may be stimulated by BM-MSCs and inhibited by UC-MSCs, or AD-MSCs may inhibit prostate cancer but promote melanomas (Supplementary Table S5 a, b and S6 a, b). It thus appears that the type of MSCs used is an important factor that influences tumour growth *in vivo* and *in vitro*.

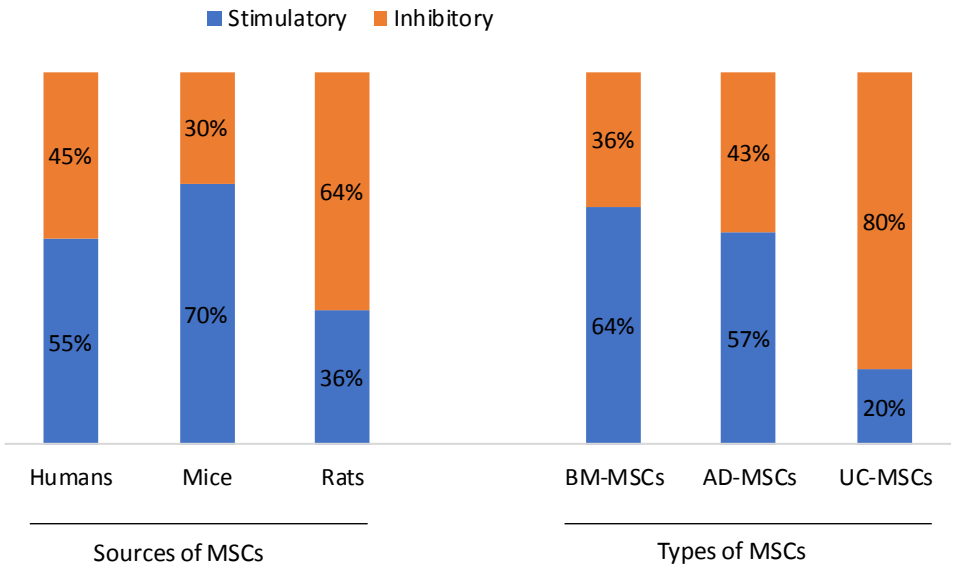

Figure 5: Effect of sources and types of MSCs on tumour growth. A greater proportion of studies analysed showed that MSCs from humans and mice promote tumour growth while rat MSCs showed an inhibitory effect regardless of experimental type. BM- and AD-MSCs promote tumour growth in most of the studies where they were used unlike UC-MSCs which inhibited tumour growth irrespective of the experimental type.

3.5 Status of MSCs used in experimental studies

MSCs were used either in their natural form after expansion or they were modified or genetically altered to produce a particular cytokine or chemokine. MSCs used in their native form after expansion are referred to as naïve MSCs and modified/altered MSCs that produce tailor-made effects are referred to as engineered MSCs. In this review, 289 (85%) of studies used naïve MSCs (Table 1). 179 (62%) studies reported a stimulatory effect on tumour growth by naïve MSCs. Tumour growth was inhibited in 38 (78%) experiments where engineered MSCs were used (Table 1). The inhibitory effect of engineered MSCs on tumour growth is not surprising, given that these MSCs were engineered to produce substances that are known to possess tumouricidal or tumour growth inhibitory properties (Li et al., 2014; Nakamura et al., 2004).

3.6 Cancer sources and types used to evaluate the effect of MSCs on tumour growth

266 (79%) of the 338 experiments analysed used human cancer cells, 16% (n=54) used murine cancer cells, 4% (n=13) used rat cancer cells and 1% (n=5) of the experiments induced cancer

using chemical methods. MSCs promoted growth of human and mouse cancer cells in 153 (57%) and 33 (61%) of studies respectively, whereas MSCs inhibited growth of rat cancer cells in 10 (77%) studies (Figure 6). Tumour growth was inhibited in both experiments in which cancer was induced by chemical means (Chen et al., 2014b; Paris et al., 2016).

The effects of MSCs on breast cancer were studied in 96 (29%) of the experiments included in this review. The effects of MSCs on lung cancer (8%; n=28), prostate cancer (9%; n=31), glioma (7%; n=25), colorectal carcinoma (7%; n=25), HNSCC (4%; n=12), hepatic cancer (6%; n=20), gastric cancer (6%; n=19), sarcoma (7%; n=24) and others (17%; n=58) were studied in experiments included in this review. Cancer types classified as other include melanoma, myeloma, pancreatic cancer, cancer of the bladder, lymphoma, and ovarian cancer amongst others. Different types of cancer displayed different susceptibility to MSCs *in vivo* and *in vitro*. For instance, MSCs stimulated the growth of breast cancer in 60% (n=58), colorectal cancer in 76% (n=19), prostate cancer in 58% (n=18), gastric cancer in 84% (n=16), sarcoma in 67% (n=16) and HNSCC in 67% (n=8) of experiments in which they were used. Conversely, MSCs inhibited lung cancer in 57% (n=16), hepatic cancer in 60% (n=14) and glioma in 76% (n=19) of experiments in which they were studied (Figure 6). Studies carried out on breast cancer used BM-MSCs (47%; n=45), UC-MSCs (22%; n=21) and AT-MSCs (19%; n=18).

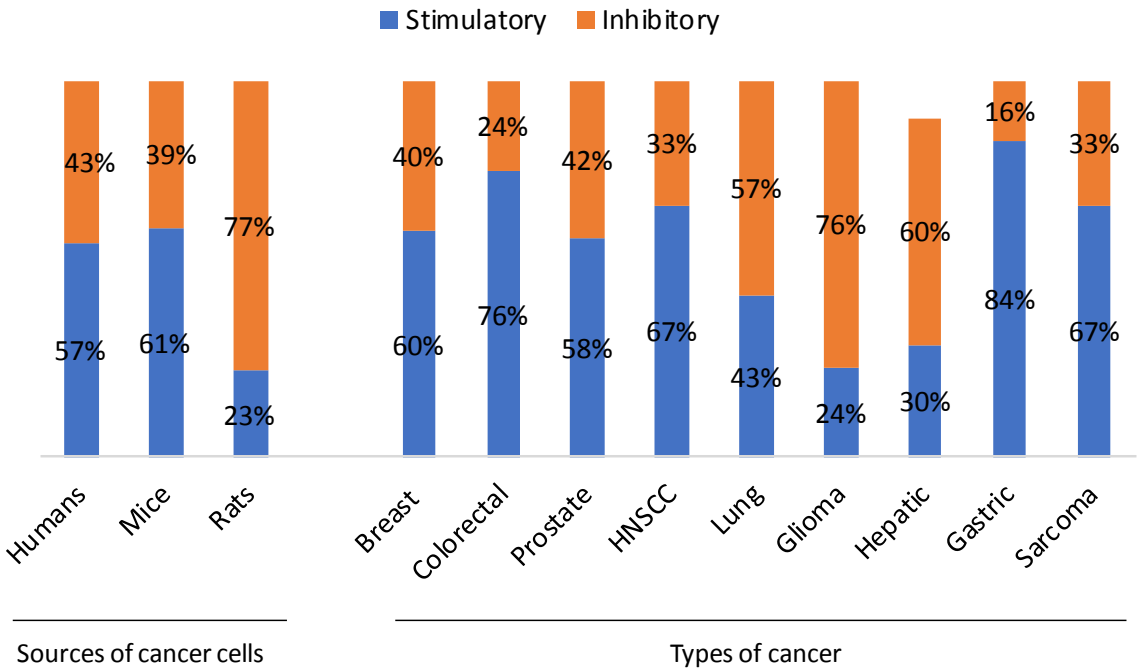

Figure 6: Effect of MSCs on the sources and types of cancer cells studied *in vivo* and *in vitro*. The majority of the studies using cancer cells from humans and mice revealed that growth was promoted by MSCs while growth of cancer cells from rats was inhibited by MSCs in the majority of studies. MSCs promoted growth of breast, colorectal, prostate and gastric cancers, HNSCC, and sarcoma in the majority of the studies in which they were used, whereas an inhibitory effect of MSCs on lung, hepatic and glioma tumour growth was observed in the majority of the studies in which they were used.

3.7 Methods of inducing cancer and administering MSCs *in vivo*

Most of the *in vivo* experiments included in this review used a first-generation mouse model for human cancer involving xenogeneic or syngeneic transplants (Bock et al., 2014). Tumour cells were implanted subcutaneously or orthotopically into the experimental animal. In 85 (53%) of the *in vivo* experiments, cancer cells were injected subcutaneously. Cancer cells were injected orthotopically (23%; n=36), intravenously (9%; n=15), intraperitoneally (6%; n=10) or via other routes (8%; n=13) in the remaining *in vivo* experiments.

MSCs exhibited a stimulatory effect on tumour growth in 68% (n=58) and 53% (n=19) of *in vivo* experiments where cancer cells were transplanted subcutaneously or orthotopically respectively. Conversely, tumour growth was inhibited by MSCs in experiments where cancer cells were

transplanted intravenously (73%; n=11), intraperitoneally (60%; n=6) or via other routes (61%; n=8) (Figure 7).

The ability of MSCs to migrate to tumour sites (tumour tropism) is one of the features purportedly associated with MSCs therapy. MSCs are known to reach tumours via the vascular system. In *in vivo* experiments, MSCs were administered subcutaneously (45%; n=71), intravenously (24%; n=39), intraperitoneally (8%; n=13) and via intra-tumoural injection (15%; n=24). Other studies (8%; n=12) administered MSCs via intramuscular and intra-arterial routes. Tumour growth was promoted in 54 (76%) and in 13 (54%) experiments where MSCs were administered subcutaneously and intra-tumourally respectively. Conversely, tumour growth was inhibited in 25 (64%), 13 (69%) and 7 (58%) of experiments that administered MSCs intravenously, intraperitoneally or via other routes respectively (Figure 7). The route of MSC administration appears to determine access to the tumour which is likely to determine if MSCs will be able to interact directly with the tumour.

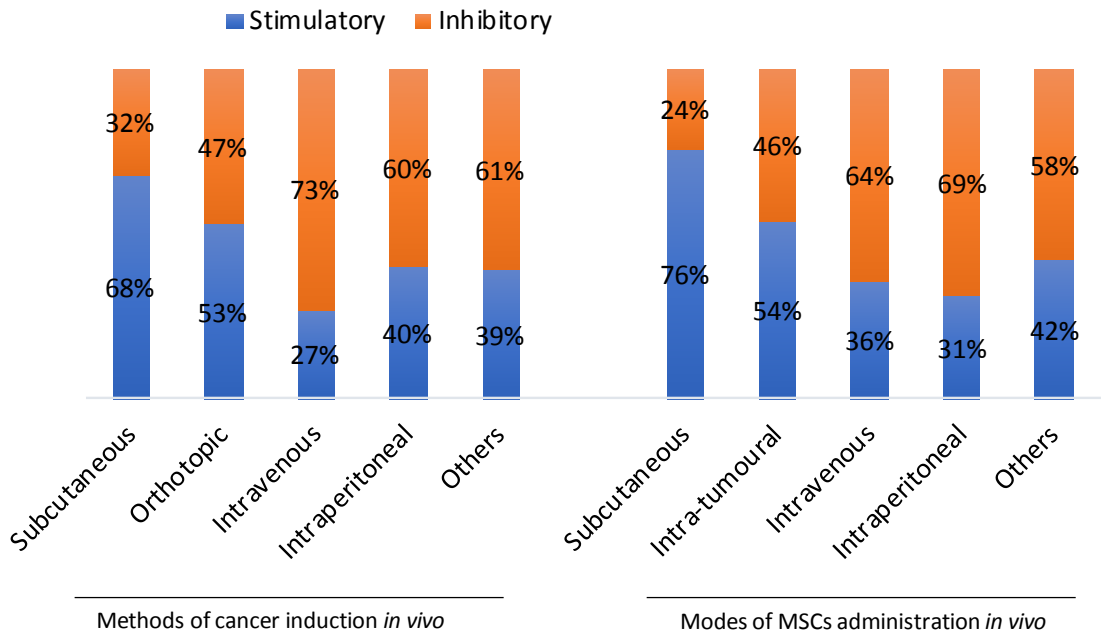

Figure 7: Effect of the methods of cancer induction and MSC administration *in vivo* on tumour growth. The majority of experiments showed a stimulatory effect on tumour growth by MSCs either when the tumour was induced or the MSCs were administered subcutaneously or orthotopically, whereas tumour growth was inhibited in the majority of studies in which the cancer was induced or MSCs were administered intravenously, intraperitoneally or via other methods.

3.8 Methods of exposure of cancer to MSCs *in vitro*

To assess the effect of MSCs on tumour growth in *in vitro* experiments, cancer cells were either co-cultured with MSCs or they were exposed to MSC conditioned medium. Cancer cells and MSCs were co-cultured in 105 (59%) of the *in vitro* experiments while cancer cells were exposed to MSC conditioned media in 74 (41%) of the *in vitro* experiments. Cancer growth was stimulated by MSCs in *in vitro* experiments either when they were co-cultured with MSCs (56%; n=59) or when the cancer cells were exposed to MSC conditioned medium (55%; n=41). Exposure of MSCs to cancer cells via co-culture experiments or conditioned medium may affect the growth of tumour cells differently in *in vitro* experiments. In co-culture experiments, cytokines and/or chemokines from MSCs diffuse towards and influence the activities of cancer cells, while secretions from cancer cells also diffuse towards and influence the activity of MSCs. Conversely, in experiments where cancer cells are exposed to MSC conditioned media, only secretions (cytokines and/or chemokines) from MSCs in the conditioned media will influence the activity of cancer cells and not vice versa.

4 CONCLUSIONS

Our review of original research articles assessing the effect of MSCs on tumour growth has revealed the existence of varied responses to MSCs, which may be due to several experimental factors such as the origin of the MSCs and cancer cells, the route of administration of MSCs, methods of inducing cancer and the immune status of the experimental animals as well as the experimental animal model used. The diversity of experimental factors greatly limits the interpretation and comparison of different studies even when performed under similar conditions. However, we have attempted to summarize our assessment of the above factors in the 338 experimental studies reviewed, and have only considered those experimental factors for which the number of *in vivo* and *in vitro* experiments is ≥ 10 and the difference in the effect on tumour growth by MSCs is $\geq 10\%$. This analysis is shown in Table 2.

Table 2: Summary of some of the experimental factors which are likely to have affected the outcome of the studies assessed. Only factors with ≥10 experimental studies in both *in vivo* and *in vitro* settings and for which the difference in experimental outcome was ≥10%, were selected.

Effect on tumor growth	**Stimulatory**		**Inhibitory**	
Experimental condition	*In vivo*	*In vitro*	*In vivo*	*In vitro*
Mode of MSC administration	SC (n=71; 76%)	CC (n=105; 56%) and CM (n=74; 55%)	IP (n=13; 69%) and IV (n=39; 64%)	n/a
Method of cancer induction	SC (n=85; 68%)		IP (n=10; 60%) and IV (n=15; 73%)	
Source of MSCs	Mouse (n=33; 67%) and human (n=114; 57%)	Mouse (n=20; 75%)	Rat (n=11; 73%)	Rat (n=11; 55%)
Source of cancer cells	Human (n=112; 58%) and mouse (n=35; 63%)	Human (n=154; 57%) and mouse (n=19; 58%)		
Origin of MSCs	BM (n=107; 63%)	BM (n=99; 66%) and AD (n=39; 59%)	UC (n=21; 71%)	UC (n=29; 86%)
Immune status of animal	Immune competent (n=101; 61%)	n/a		n/a
Animal model	Mouse (n=148; 59%)			
Experimental design	Syngeneic (n=37; 59%) and xenogeneic (n=112; 56%)			
Type of cancer	Breast (n=36; 61%), sarcoma (n=13; 69%) colorectal (n=16; 87%) and gastric (n=10; 90%)	Breast (n=60; 60%), sarcoma (n=11; 64%) and prostate (n=15; 73%)	Prostate (n=16; 56%) and glioma (n=13; 77%)	Glioma (n=12; 75%), lung (n=13; 62%) and hepatic (n=12; 58%)

SC, subcutaneous; IV, intravenous; IP, intraperitoneal; CC, co-culture; CM, conditioned medium; BM, bone marrow; AD, adipose-derived; UC, umbilical cord; NA, not applicable; n, number of experiments; n/a, not applicable.

In summary, the administration of MSCs or induction of cancer in *in vivo* experiments via subcutaneous injection stimulated tumour growth whereas tumour growth was inhibited when these procedures were done intraperitoneally or intravenously. Both co-culture and exposure of tumour cells to MSC condition medium *in vitro*, stimulated tumour growth.

When MSCs or cancer cells from mouse were used, this resulted in an overall stimulatory effect on mouse and human tumour cell growth in both *in vivo* and *in vitro* experiments. MSCs or cancer cells from human showed an overall stimulatory effect on tumour growth *in vivo* whereas in *in vitro* experiments a stimulatory effect was observed only when cancer cells from human were used. MSCs from rat showed an overall inhibitory effect on tumour growth in both *in vivo* and *in vitro* experiments.

In both *in vivo* and *in vitro* experiments, BM-MSCs showed a stimulatory effect on tumour growth while an inhibitory effect was seen in response to UC-MSCs. AD-MSCs showed a stimulatory effect on tumour growth only in *in vitro* experiments. In cases where immune competent animals were used and when the experimental animal was mouse, irrespective of whether the model was syngeneic or xenogeneic in design, there was an overall stimulatory effectofy MSCs on tumour growth *in vivo*.

MSCs stimulated tumour growth in both *in vivo* and *in vitro* experiments in which breast cancer and sarcoma were used, whereas a stimulatory effect of MSCs on colorectal and gastric cancer was only observed in *in vivo* experiments. An overall inhibitory effect on tumour growth by MSCs was observed in glioma whereas growth of lung and hepatic cancer was inhibited by MSCs in *in vitro* experiments only. Experiments on prostate cancer showed the opposite effect *in vivo* and *in vitro* as an overall stimulatory and inhibitory effect was observed in the former and the latter respectively.

It is believed that MSCs have the ability to migrate and engraft at tumour sites where they either exert a stimulatory or inhibitory effect on tumour growth (Hong et al., 2014; Kidd et al., 2009; Lazennec and Lam, 2016; Ridge et al., 2017). How the tumour cells and MSCs interact or cross-talk with each other (directly or indirectly) will determine if MSCs will either stimulate or inhibit tumour growth. MSCs are known to exhibit their pro-tumorigenic effects by regulating immune surveillance (immune suppression), differentiating into stromal cells (thereby contributing to the tumour microenvironment), promoting angiogenesis and stimulating an epithelial–mesenchymal transition, whereas inhibition of tumour growth by MSCs is reported to be through the inhibition of survival signaling pathways such as Akt and Wnt/β-catenin. The ability of MSCs to engraft and secrete cytokines at tumour sites has made them an attractive candidate to be engineered and used for delivery of anti-tumour agents. However, how tumour cells and MSCs cross-talk with

each other is largely dependent on experimental factors as assessed in this review. Understanding these interactions through carefully designed experiments performed under controlled conditions which eliminate the variables alluded to above, will help to understand the molecular basis of the effect of naïve MSCs on tumour growth. Furthermore, alternative strategies involving the modification of MSCs through genetic engineering with exogenous anticancer genes for the expression and/or secretion of a desired inhibitory factor could be exploited as a tool for developing a safer and more effective anticancer therapy.

COMPETING INTERESTS

No conflicts of interest, financial or otherwise, are declared by the authors

AUTHORS' CONTRIBUTION

MSP conceptualized the idea of the review, AKO and MAA did the literature search, AKO and MAA analysed the data, AKO and MAA prepared the manuscript, MSP edited and reviewed the drafted manuscript, AKO, MAA and MSP approved of the final version of the manuscript.

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SUPPLEMENTARY DATA

Table S1a: *In vitro* experiments that reported a stimulatory effect of BM-MSCs on breast cancer cells.

Source of BM-MSCs	Type of experiment	Source of cancer cells	MSC status	References
Mouse	*In vitro*	Mouse	Naïve	(Halpern et al., 2011)
Human	*In vitro*	Human	Naïve	(Patel et al., 2010)
Human	*In vitro*	Human	Naïve	(Sasser et al., 2007)
Mouse	*In vitro*	Mouse	Naïve	(Zhang et al., 2013)
Human	*In vitro*	Human	Naïve	(Hung et al., 2013)
Human	*In vitro*	Human	Naïve	(De Luca et al., 2012)
Human	*In vitro*	Human	Naïve	(Molloy et al., 2009)
Human	*In vitro*	Human	Naïve	(Klopp et al., 2010)
Human	*In vitro*	Human	Naïve	(Zhao et al., 2015)
Human	*In vitro*	Human	Naïve	(Cuiffo et al., 2014)
Human	*In vitro*	Human	Engineered to produce TGFBR2	(Shin et al., 2010)
Human	*In vitro*	Human	Naïve	(Tobar et al., 2014)

Table S1b: *In vivo* experiments that reported a stimulatory effect of BM-MSCs on breast cancer cells.

Source of BM-MSCs	Type of experiment	Source of cancer cells	MSC status	References
Human	*In vivo*	Human	Naïve	(Albarenque et al., 2011)
Human	*In vivo*	Human	Naïve	(Rhodes et al., 2010)
Mouse	*In vivo*	Mouse	Naïve	(Ke et al., 2013)
Human	*In vivo*	Human	Naïve	(Cuiffo et al., 2014)
Mouse	*In vivo*	Mouse	Naïve	(Yu et al., 2017)

Table S2a: *In vitro* experiments reporting an inhibitory effect of BM-MSCs on breast cancer cells.

Source of BM-MSCs	Type of experiment	Source of cancer cells	MSC status	References
Human	*In vitro*	Mouse	Naïve	(Kéramidas et al., 2013)
Human	*In vitro*	Human	Naïve	(Clarke et al., 2015)
Human	*In vitro*	Human	Naïve	(Ono et al., 2014)
Mouse	*In vitro*	Mouse	Naïve	(Lee et al., 2013)
Mouse	*In vitro*	Human	Naïve	(Usha et al., 2013)
Human	*In vitro*	Human	Naïve	(Lee et al., 2012)

Table S2b: *In vivo* experiments reporting an inhibitory effect of BM-MSCs on breast cancer cells.

Source of BM-MSCs	Type of experiment	Source of cancer cells	MSC status	References
Human	*In vivo*	Human	Engineered to produce BMP9	(Wan et al., 2014)
Mouse	*In vivo*	Mouse	Naïve	(Lee et al., 2013)
Mouse	*In vivo*	Human	Naïve	(Usha et al., 2013)
Human	*In vivo*	Human	Naïve	(Lee et al., 2012)

Table S3a: *In vitro* experiments reporting a stimulatory effect of UC-MSCs on tumour growth.

Source of UC-MSCs	Type of experiment	Source of cancer cells	Type of cancer cell	MSC status	References
Human	*In vitro*	Human	Oesophageal	Naïve	(Yang et al., 2014a)
Human	*In vitro*	Human	Breast	Naïve	(Di et al., 2014)
Human	*In vitro*	Human	Breast	Naïve	(Ma et al., 2015)

Table S3b: *In vivo* experiments reporting a stimulatory effect of UC-MSCs on tumour growth.

Source of UC-MSCs	Type of experiment	Source of cancer cells	Type of cancer cell	MSC status	References
Human	*In vivo*	Human	Oesophageal	Naïve	(Yang et al., 2014c)
Human	*In vivo*	Human	Gastric	*Engineered	(Yang et al., 2014b)
Human	*In vivo*	Human	Breast	Naïve	(Di et al., 2014)
Human	*In vivo*	Human	Breast	Naïve	(Ma et al., 2015)
Human	*In vivo*	Mouse	Breast	Naive	(Yu et al., 2017)

*Engineered here refers to UC-MSC activated by macrophages to produce inflammatory cytokines

Table S4a: *In vitro* experiments reporting an inhibitory effect of UC-MSCs on tumour growth.

Source of UC-MSCs	Type of experiment	Source of cancer cells	Type of cancer cell	MSC status	References
Human	*In vitro*	Human	Breast	Naïve	(Fong et al., 2011)
Human	*In vitro*	Human	Colorectal	Naïve	(Fong et al., 2011)
Human	*In vitro*	Human	Hepatic	Naïve	(Fong et al., 2011)
Rat	*In vitro*	Rat	Breast	Naïve	(Kawabata et al., 2013)
Human	*In vitro*	Human	Bladder	Naïve	(Wu et al., 2013)
Rat	*In vitro*	Mouse	Lung	Naïve	(Maurya et al., 2010)
Rat	*In vitro*	Rat	Breast	Naïve	(Ganta et al., 2009)
Human	*In vitro*	Human	Breast	Engineered to express IFN-β	(Rachakatla et al., 2008)
Human	*In vitro*	Human	Glioma	Naïve	(Yang et al., 2014a)
Human	*In vitro*	Human	Breast	Naïve	(Chao et al., 2012)
Human	*In vitro*	Human	Myeloma	Naïve	(Ciavarella et al., 2015)
Human	*In vitro*	Human	Prostate	Naïve	(Han et al., 2014)

Table S4b: *In vivo* experiments reporting an inhibitory effect of UC-MSCs on tumour growth.

Source of UC-MSCs	Type of experiment	Source of cancer cells	Type of cancer cell	MSC status	References
Rat	*In vivo*	Rat	Breast	Naïve	(Kawabata et al., 2013)
Human	*In vivo*	Human	Bladder	Naïve	(Wu et al., 2013)
Rat	*In vivo*	Mouse	Lung	Naïve	(Maurya et al., 2010)
Rat	*In vivo*	Rat	Breast	Naïve	(Ganta et al., 2009)
Human	*In vivo*	Human	Breast	Engineered to express IFN-β	(Rachakatla et al., 2008)
Human	*In vivo*	Human	Lung	Naïve	(Rachakatla et al., 2007)
Human	*In vivo*	Human	Lung	Engineered to express human IFN-β	(Rachakatla et al., 2007)
Human	*In vivo*	Human	Breast	Naïve	(Chao et al., 2012)
Human	in vivo	Human	Myeloma	Naïve	(Ciavarella et al., 2015)
Human	in vivo	Human	Prostate	Naïve	(Han et al., 2014)

Table S5a: *In vitro* experiments reporting a stimulatory effect of AD-MSC on tumour growth.

Source of AD-MSCs	Type of experiment	Source of cancer cells	Type of cancer cell	MSC status	References
Human	*In vitro*	Human	Melanoma	Naïve	(Kucerova et al., 2010)
Human	*In vitro*	Human	Glioma	Naïve	(Kucerova et al., 2010)
Human	*In vitro*	Human	Glioma	Naïve	(Yu et al., 2008)
Human	*In vitro*	Human	Lung	Naïve	(Park et al., 2013)
Human	*In vitro*	Human	Breast	Naïve	(Kamat et al., 2015)
human	*In vitro*	Human	Head and neck	Naïve	(Scherzed et al., 2013)
Human	*In vitro*	Human	Gastric	Naïve	(Nomoto-Kojima et al., 2011)
Rat	*In vitro*	Human	Gastric	Naïve	(Nomoto-Kojima et al., 2011)
Human	*In vitro*	Human	Breast	Naïve	(Chen et al., 2014a)
Human	*In vitro*	Human	Breast	Naïve	(Lin et al., 2013)
Human	*In vitro*	Human	Breast	Naïve	(Zhao et al., 2012)

Human	*In vitro*	Human	Melanoma	Naïve	(Kucerova et al., 2014)
Human	*In vitro*	Human	Sarcoma	Naïve	(Bonuccelli et al., 2014)
Human	*In vitro*	Human	Breast	Naïve	(Senst et al., 2013)
Human	*In vitro*	Human	Breast	Naïve	(Xu et al., 2012)
Human	*In vitro*	Human	Ovarian	Naïve	(Zhang et al., 2017)

Table S5b: *In vivo* experiments reporting a stimulatory effect of AD-MSC on tumour growth.

Source of AD-MSCs	Type of experiment	Source of cancer cells	Type of cancer cell	MSC status	References
Human	*In vivo*	Human	Melanoma	Naïve	(Kucerova et al., 2010)
Human	*In vivo*	Human	Glioma	Naïve	(Kucerova et al., 2010)
Human	*In vivo*	Human	Lung	Naïve	(Yu et al., 2008)
Human	*In vivo*	Human	Glioma	Naïve	(Yu et al., 2008)
Human	*In vivo*	Human	Melanoma	Naïve	(Kucerova et al., 2014)
Human	*In vivo*	Human	Breast	Naïve	(Yu et al., 2017)

Table S6a: *In vitro* experiments reporting an inhibitory effect of AD-MSCs on tumour growth.

Source of AD-MSCs	Type of experiment	Source of cancer cells	Type of cancer cell	MSC status	References
Human	*In vitro*	Human	Glioma	Naïve	(Yang et al., 2014c)
Human	*In vivo*	Human	Melanoma	Engineered (CD-MSC/5FC)	(Kucerova et al., 2014)
Human	*In vitro*	Human	Melanoma	Engineered (CD-MSC/5FC)	(Kucerova et al., 2014)
Human	*In vitro*	Human	Hepatic	Naïve	(Zhao et al., 2012)
Human	*In vitro*	Human	Lymphoma	Naïve	(Ahn et al., 2014)
Human	*In vitro*	Human	Bladder	Naïve	(Yu et al., 2016)
Human	*In vitro*	Human	Breast	Naïve	(Zhao et al., 2013)
Human	*In vitro*	Human	Glioma	Engineered to secrete BMP4	(Li et al., 2014)
Human	*In vitro*	Human	Glioma	Naïve	(Li et al., 2014)
Human	*In vitro*	Human	Breast	Naïve	(Yu et al., 2017)

CD-MSC/5FC represents MSC express fusion yeast cytosine deaminase::uracil phosphoribosyltransferase (CD-MSC) in combination with 5-fluorocytosine (5FC)

Table S6b: *In vivo* experiments reporting an inhibitory effect of AD-MSCs on tumour growth.

Source of AD-MSCs	Type of experiment	Source of cancer cells	Type of cancer cell	MSC status	References
Human	*In vivo*	Human	Melanoma	Engineered (CD-MSC/5FC)	(Kucerova et al., 2014)
Mouse	*In vivo*	Mouse	Prostate	Engineered (CD-MSC)	(Abrate et al., 2014)
Human	*In vivo*	Mouse	Prostate	Engineered (CD-MSC)	(Abrate et al., 2014)
Human	*In vivo*	Human	Lymphoma	Naïve	(Ahn et al., 2014)
Human	*In vivo*	Human	Glioma	Engineered to secrete BMP4	(Li et al., 2014)
Human	*In vivo*	Human	Glioma	Naïve	(Li et al., 2014)

CD-MSC/5FC represents MSC express fusion yeast cytosine deaminase::uracil phosphoribosyltransferase (CD-MSC) in combination with 5-fluorocytosine (5FC)