CELL & GENE THERAPY INSIGHTS

REVIEW

Mesenchymal stem cell homing and immunomodulatory properties in cancer therapies: searching for the perfect balance

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Mesenchymal stem cells are non-hematopoietic adult stem cells with multi-lineage potential. Their inherent tumor tropism and easy isolation, expansion and transduction, make them attractive vehicles for the delivery of anti-cancer agents. Mesenchymal stem cell tumor homing is still poorly understood and a wide variety of factors have been reported to affect this complex process, with some inconsistencies. Their immunomodulatory properties have led to some caution towards their use in cancer patients but this field remains controversial, as both immunosuppressive and immune-enhancing phenotypes have been described and appear reversible as well as highly sensitive to the local microenvironment. This review will focus on mesenchymal stem cell homing and immunobiology in the context of cancer and the translational potential of these cells.

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This review will discuss the recent developments in several important areas of mesenchymal stem cell (MSC) biology, although it is difficult to draw definitive conclusions; this is largely due to the plethora of cell sources and culture conditions used in this field and the subsequent heterogeneity in cell phenotype denoted by the term MSC. To address this, the International Society for Cellular Therapy have proposed minimum criteria for application of the term 'MSC':

- Adherence to tissue culture plastic;
- Absent expression of CD45, CD34, CD14, CD11b, CD19 and HLA-DR;
- Cell surface expression of CD105, CD73 and CD90; and finally
- Potential for *in vivo* differentiation to osteoblasts, adipocytes and chondroblasts under standard conditions [1].

However, a revision of these criteria may soon be required for example taking into account the distinction between high and low growth capacity MSCs [2].

HOMING TO TUMORS

Extravasation

MSC homing to tumors is thought to be due to inflammatory signalling in a tumor resembling that of an unresolved wound [3]. The mechanism and key players responsible for this tumor-targeted tropism remains to be fully elucidated. It is hypothesized that MSCs behave similarly to leukocytes and have chemotactic properties allowing response to a variety of secreted chemokines from tumor cells. Several studies have correlated an increase in circulating MSCs with increases in inflammatory cytokines [4–6]. Despite some key observations, the initial steps in MSC mobilization and intravasation into the blood stream remain unclarified.

To understand MSC homing, many investigators have studied well known factors involved in leukocyte homing as a starting point for investigation. Leukocyte chemotaxis is a multistep process involving capture, rolling, activation, arrest, adhesion, crawling, transendothelial migration and engraftment. Selectins, selectin ligands, integrins-immunoglobulin superfamily receptors (vascular cell adhesion molecule-1[V-CAM-1]), chemokines and their receptors, and a variety of other molecules play important roles in this process [7]. While there are as yet unresolved differences in studies it would appear MSC homing may use similar molecules and cell–chemokine interactions to leukocyte trafficking (reviewed in [8]).

The first step in recruitment and engraftment of MSCs is the exit from the vascular circulation (extravasation), which requires crossing the blood vessel endothelial cell (EC) barrier. For MSCs, this process of extravasation remains unclarified [9]. Leukocyte extravasation at sites of inflammation has been studied in depth and is characterized as a rapid multistep cascade. Within an inflammation context, the endothelium becomes activated by cytokines such as tumor necrosis factor (TNF)-α. Chemoattractants and surface proteins, including selectins and cell adhesion molecules (CAMs) are upregulated as a consequence, mediating rolling and adhesive interactions, respectively (Figure 1). Leukocytes use a wide range of various cytoskeletal protrusions to migrate laterally and cross the endothelium, including lamellipodia, pseudopods, and invadosomes. Cells may penetrate either through gaps in intercellular junctions (paracellular diapedesis) or directly through pores in individual ECs (transcellular diapedesis) [7,10]. In addition, ECs can further guide leukocyte transmigration by generating actin-dependent protrusions (transmigratory cups) [10]. Similarly to leukocytes, previous studies suggest that MSCs can be involved in selectin-mediated rolling [11] and integrin-mediated adhesion [11,12] in an inflammation context (Figure 1).

Very few studies have investigated MSC transmigration. The data generated from these studies led to the hypothesis that MSCs incorporate the endothelial monolayer following the retraction of ECs [13–15]. However, the molecular and cellular details of this process such as detailed 3D cellular architecture, distribution of adhesion, endothelial junction molecules, and interactions have not yet been investigated and remain unclarified. Teo *et al* elegantly used high-resolution confocal and dynamic live-cell imaging to show that MSCs can transmigrate in a leukocyte-like way by paracellular and transcellular diapedesis, in

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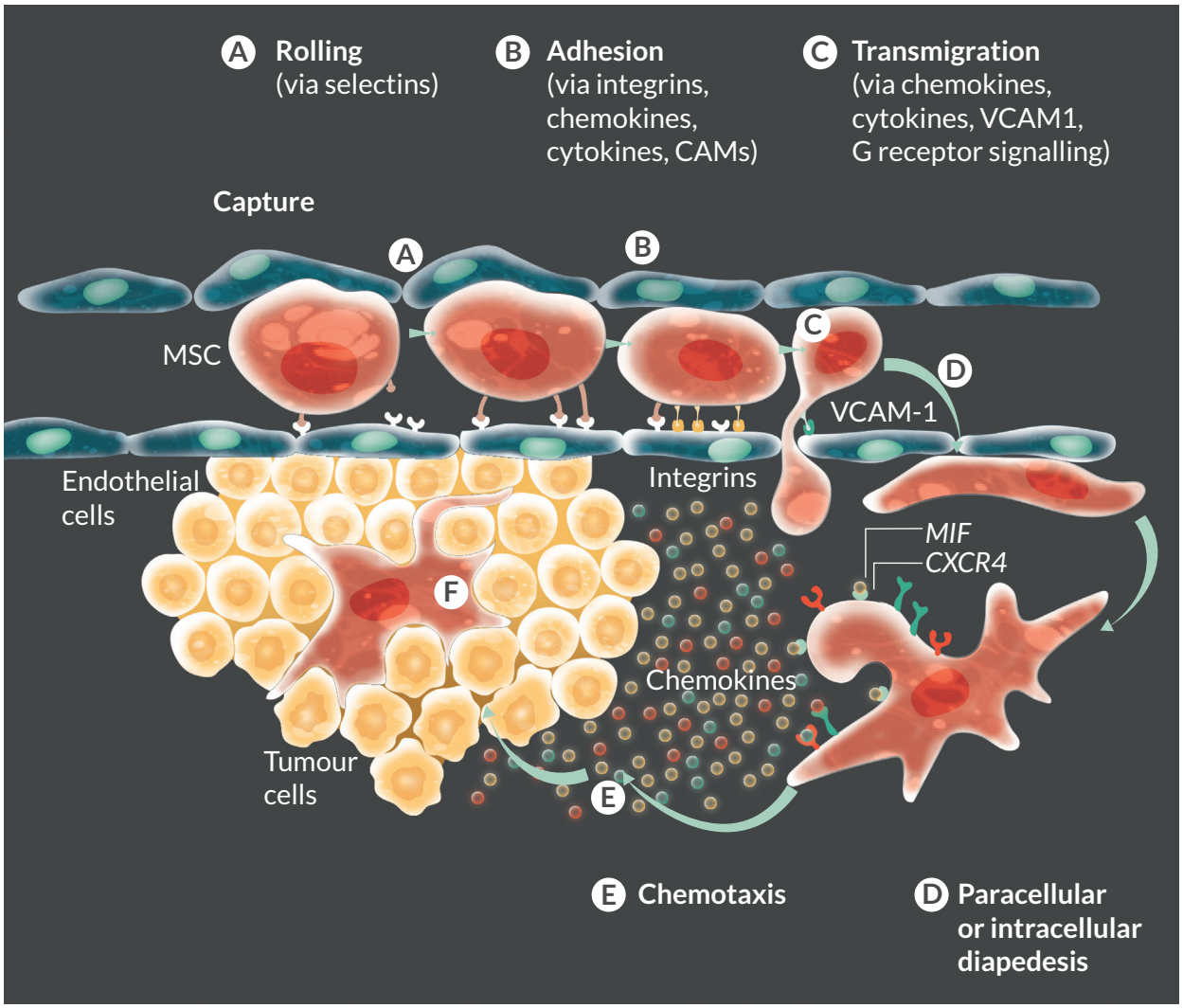
FIGURE 1

MSC homing model.



The early steps of homing for MSCs appear similar to leukocyte trafficking: **A)** rolling via selectins, **B)** adhesion via integrins and CAMs, and **C)** transmigration via chemokines, VCAM-1 and G receptor signalling. The transmigration *per se* occurs via paracellular or intracellular diapedesis **D)**. Tumors secrete high levels of different chemokines, including MIF, establishing a chemotactic gradient attracting MSCs **E)**. MSCs sense the chemokine gradient established, migrate towards the tumor and engraft **F)**.
CAM: cellular adhesion molecules; MSC: mesenchymal stem cell; MIF: macrophage migration inhibitory factor; VCAM-1: vascular cell adhesion molecule-1.

association with endothelial transmigratory cups [9]. However, they did not observe MSC transmigration by the formation of lamellipodia or invadosomes – instead, MSCs formed membrane blebs allowing endothelial crossing. The process was also significantly slower than that seen in leukocytes and was suggested to occur via VCAM-1 and G receptor signalling-dependent mechanisms [9]. Several studies have suggested that MSC homing to tumors occurs by a combination of active recruitment by chemokines and inflammatory processes as well as passive entrapment in the vasculature due to their size (Figure 1).

Chemotaxis

Multiple adhesion molecules, integrins and chemoattractants play established roles in leukocyte trafficking and may have significant overlap

TABLE 1

Cell surface markers expressed on MSCs associated with cell migration/homing and their respective ligands.

	Cell surface receptors found on MSCs	Ligands
Growth factor receptors	EGFR	EGF
	HGFR	HGF
	IGF1R	IGF1
	PDGFR	PDGF
	VEGFR1	VEGF
	VEGFR2	VEGF
	FGFR2	FGF2
	Tie-2	Ang-1
	TGFβRI	TGFβ1/2
	TGFβRII	TGFβ1/2
Chemokine receptors	CCR1	CCL3, CCL5, CCL7, CCL13, CCL14, CCL15, CCL16, CCL23
	CCR2	CCL2, CCL7, CCL8, CCL13, CCL16
	CCR3	CCL5, CCL7, CCL8, CCL11, CCL13, CCL15, CCL16, CCL24, CCL26, CCL28
	CCR4	CCL17, CCL22
	CCR5	CCL3, CCL4, CCL5, CCL8, CCL11, CCL14, CCL16
	CCR6	CCL20
	CCR7	CCL19, CCL21
	CCR8	CCL1
	CCR9	CCL25
	CCR10	CCL27, CCL28
	CXCR1	CXCL6, CXCL7, CXCL8
	CXCR2	CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, CXCL8, MIF
	CXCR3 A/B	CXCL4, CXCL9, CXCL10, CXCL11
	CXCR4	CXCL12, MIF
	CXCR5	CXCL13
	CXCR6	CXCL16
	CX3CR1	CX3CL1
	XCR1	XCL1, XCL2
Cytokine receptors	IL1R	IL1α, IL1β IL1RA
	IL3R	IL3
	IL4R	IL4, IL13
	IL6R	IL6
	IL7R	IL7
	IFNγR	IFNγ
	TNFRI and II	TNFα, TRAF2, TRAAD

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Cell–matrix receptors	CD44	Hyaluronan
	α1β1 (VLA-1)	Collagens, laminins
	α2β1 (VLA-2)	Collagens, laminins
	α3β1 (VLA-3)	Laminin-5
	α5β1 (VLA-5)	Fibronectin, proteases
	α6β1 (VLA-6)	Laminins
	αvβ1	Vitronectin, fibrinogen
	αvβ3 (vitronectin receptor)	Vitronectin, fibronectin, fibrinogen, osteopontin, Cyr61
	α1β5	Vitronectin
Cell–cell receptors	VCAM-1	β1 integrin / α4 integrin
	ICAM-1/3	LFA-1
	ALCAM	CD6
	CD105	TGFβ1/3
Immuno-modulating receptors	TLR1	Lipopeptides, peptidoglycan
	TLR2	Peptidoglycans, lipopeptides
	TLR3	dsRNA
	TLR4	LPS
	TLR5	Flagellin
	TLR6	Peptidoglycans, lipopeptides
	TLR9	Unmethylated CpG DNA

Adapted from Spaeth *et al* [3]; dsRNA: double-stranded RNA; EGF: epidermal growth factor; EGFR: epidermal growth factor receptor; FGF: fibroblast growth factor; FGFR: fibroblast growth factor receptor; HGF: hepatocyte growth factor; HGFR: hepatocyte growth factor receptor; IGF: insulin-like growth factor; IGFR: insulin-like growth factor receptor; IL: interleukin; LFA: lymphocyte function-associated antigen; PDGF: platelet-derived growth factor; PDGFR: platelet-derived growth factor receptor; LPS: lipopolysaccharide; TGFβRI: transforming growth factor beta receptor I; IFNγ: interferon gamma; TNFα: tumor necrosis factor alpha; TRAF: TNF receptor-associated factor; TLR: toll-like receptor; VEGF: vascular endothelial growth factor; VEGFR: vascular endothelial growth factor receptor.

with MSC homing mechanisms [16]. A variety of different ligand/receptor pairs have been identified as players in the process, as shown by Table 1 listing markers and receptors typically associated with cell migration and known to be expressed in MSCs (reviewed in [3])

The most extensively studied MSC chemotactic axis is CXCR4/stromal cell-derived factor (SDF)-1. High levels of SDF-1 have been shown to be important in recruiting and retaining hematopoietic stem cells (HSCs) within bone marrow [17], and there is increasing evidence that cancer cells home to bone marrow following similar CXCR4 expression [18–21]. Soluble chemoattractants secreted by tumor cells have been suggested to activate MSC migration by triggering them to secrete SDF-1. Recently, investigators found that soluble factors secreted from tumor cells can trigger SDF-1 secretion from MSCs, activating their migration [22]. The role of SDF-1 is disputed, however, as several studies have shown that tumors generally do not produce SDF-1 [23].

We recently identified macrophage migration inhibitory factor (MIF) as a key factor through screening soluble factors secreted from tumor cell lines [24]. Other studies have shown MSC migration to be regulated by numerous other

factors including tumor cell-specific receptors, extracellular matrix and soluble tumor-derived factors such as SDF-1, TNF-α and interleukins (ILs) [22,25]. MSCs are undoubtedly responsive to other chemokines. In our studies, we also see stimulation of MSC migration towards gradients of IL1β, IL6, IL8 and CCL2. Roles for IL6, IL8 and CCL2 have been delineated by several other groups [26–29]. Similar to the work on SDF-1, we see up-regulation of IL1β, IL6, IL8 and CCL2 in MSCs treated with tumor-conditioned medium. Interestingly we observe chemotactic effects with these four cytokines, suggesting a positive feedback loop possibly leading to amplification of chemoattraction to tumor cells, likely triggered by MIF. This MIF-dependent amplification is seen in other studies showing MIF-dependent up-regulation of these cytokines in an inflammatory context [30–33].

We have confirmed recombinant SDF-1 as a chemoattractant for MSCs. However we do not believe it has a role in the *in vivo* tumor context after failing to detect significant levels secreted by several cancer cell lines (A549, MDAMB231, H376, A431 or Jurkat cells) and a failure to block migration of MSCs to these tumor cells with an SDF-1 neutralizing antibody. Of note, MSCs did show reduced migration with SDF-1 blockade towards the U87MG cell line (described to secrete SDF-1 [34,35]); however even in this cell line, migration was more severely diminished by CXCR4 or MIF antagonists [24].

These results reinforce our hypothesis that MIF is the dominant chemoattractant in the recruitment of MSCs to tumors, even in the presence of SDF-1 (Figure 1). These findings are consistent with other reports showing high secretion levels of MIF and rare secretion of SDF-1 in the majority of tumors [23,36–42]. MIF–CXCR4 has been described as important in a variety of other contexts: regulation of endothelial progenitor cell migration, cancer metastasis and cancer proliferation/growth [43].

MSC IMMUNOGENICITY

MSCs have long been described as hypoimmunogenic or immune privileged, and this property has been widely explored for the creation of 'off-the-shelf' MSC therapies as a means of circumventing major histocompatibility barriers. However, several preclinical and clinical observations have led to controversy as to the true presence and extent of MSC immune privilege and their subsequent potential for universal donor therapies (reviewed in [44]). Questioning the immune privileged status of MSCs, an elegant study by Zangi *et al* compares the persistence of syngeneic and allogeneic MSCs *in vivo* [45]. The authors show a significant decline in detectable MSCs in an allogeneic setting. Others have also shown innate immune responses to allogeneic MSCs [46,47]. Disparities between reported results may be due to discrepancies between MSC microenvironments. Culture-expanded MSCs express low levels of major histocompatibility complex (MHC) class I antigens, and are negative for MHC class II; however, following treatment with IFN-γ, MSCs have been shown to up-regulate these markers [48]. The timing and severity of MSC rejection appears to be strongly dependent on context and dictated by a balance between the expression of immunogenic

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and immunosuppressive factors. Intriguingly, when the immunosuppressive phenotype is not activated, MSCs may acquire an antigen presenting-like phenotype, able to promote an immune response *in vivo* [49,50]. Harnessing this response may improve anti-cancer cellular therapies.

MSCS IN THE TUMOR MICROENVIRONMENT

The interaction of MSCs in the tumor microenvironment is complex and driven by the interplay with numerous types including:

i) Blood and lymphatic endothelial cells, which induce angiogenesis and recruitment of immune-suppressive cells, appearing as a crucial regulator of the host immune response to cancer [51,52].

ii) Cancer-associated fibroblasts (CAFs), which represent the predominant non-hematopoietic stromal cell type and are correlated with poor prognosis in many tumors [53,54]. CAFs have been shown to support tumor growth by providing growth factors [55] and hinder anti-tumor immune responses [56]. MSCs may contribute to this population.

iii) Pericytes, which differentiate from mesenchymal precursors and are recruited to tumors by platelet-derived growth factor-β (PDGF-β) [57], where they populate the luminal side of blood vessels. It is suggested that pericytes may prevent lymphocyte extravasation and activation at tumor sites [58–60].

iv) Tumor infiltrating leukocytes such as regulatory T cells (Treg), myeloid-derived suppressor cells (MDSC) and alternatively activated type 2 macrophages (M2). These cells create a highly immunosuppressive microenvironment with the secretion of cytokines including IL10 and TGF-β, vascular endothelial growth factor (VEGF), galectins, and expression of inhibitory receptors such as CTLA4 and PD-L1, and secretion of amino acid depleting enzymes such as arginase and IDO, PGE2. The combination of all these processes inhibits and inactivates key players of both the innate and adaptive immune system (inhibition of CD8 T cells, dendritic cells, NK cells (reviewed in [61]).

v) The recruitment of MSCs to the tumor stroma has both the potential to amplify the immunosuppressive tumor microenvironment and promote tumor growth, or enhance immune properties tilting the balance towards an anti-tumor and pro-inflammatory microenvironment. Defining which molecular cues lead to each MSC-induced environment might be important to their use in clinical settings and is discussed in the following sections.

MSC IMMUNOSUPPRESSIVE PROPERTIES

Immune suppression by MSCs is multifactorial, occurring both by soluble signals and direct cell contact (reviewed in [62]). *In vivo*, MSCs produce basal levels of cytokines, adhesion molecules and inflammatory mediators, increasing their secretion of immuneosuppressive factors as well as several chemoattractants, leading to recruitment of immune cells, in response to inflammatory cytokines (IFN-γ and TNF-α). Induction of lymphocyte-specific cytokines such as CXCL9, CXCL10 and CXCL11 is dependent on the combined action of IFN-γ and

TNF-α [63,64]. MSCs also express other molecules involved in feto–maternal tolerance including leukemia inhibitory factor (LIF) [65], HLA-G [66,67], and galectins 1, 3 and 8 [68,69] all of which are upregulated by exposure to IFN-γ, inhibiting T and NK cell activities. Adhesion molecules (ICAM-1 and VCAM-1) are also upregulated, though the significance of this for MSC–immune cell interactions is debated [63,70]. TSG-6 is another example of an immunoesuppressive factor upregulated in MSCs following exposure to TNF-α [71,72].

MSCs constitutively express COX-2 and low levels of PGE2, well known T-cell inhibitors, which are increased following exposure to IFN-γ or TNF-α. PGE2 is known to have an inhibitory action on T cells, and MSC-derived PGE2 has been heavily investigated and found to reduce T cell proliferation [68,73] and induce an IL10-secreting macrophage phenotype in an *in vivo* model of sepsis [74]. In a recent clinical trial involving lupus patients, MSC injections were suggested to inhibit Th17 polarization with the induction of a shift into IL10-producing cells and an increase in Tregs [75,76]. In another study, PGE2-secreting MSCs inhibited NK cell cytotoxicity [77]; however, other groups have shown PGE2 inhibition to have only a marginal effect on the suppression of proliferating T cells activated by MSC-secreted PGE2 [63,64,78].

Other pathways have been defined by clear cut results. Inhibition of nitric oxide (NO) (in mouse MSCs) or indoleamine 2,3-dioxygenase (IDO) (in human MSCs) totally abrogates the suppression of T-cell proliferation [63,64,79,80]. At high concentrations NO also appears to inhibit T cell activation and leads to IL10 production by macrophages. Meanwhile IDO catabolites supress NK and T cell proliferation and induce T cell apoptosis and Treg differentiation.

Other factors have also been implicated in MSC immunosuppression. MSC-derived TGF-β inhibits NK and T cells *in vivo* [81,82] and induces Tregs *in vivo* [83].

MSC IMMUNE-ENHANCING PROPERTIES

The first of over 250 clinical trials using MSCs were performed to treat graft-versus-host disease (GvHD) patients [84]. However, therapeutic effects were not borne out and in some cases MSC administration resulted in accelerated graft rejection [85,86]. Further *in vivo* studies showed low dose concanavalin A or the addition of IL10, abrogated the immunosuppressive effect of MSCs [87]. Exposure of MSCs to insufficient inflammatory cytokines also results in low MSC NO secretion [88] (reviewed in [89]).

Galipeau *et al* showed that MSCs can act as antigen presenting cells (APCs) by engineering MSCs to stably express the kinase-inactive rat ERBB2/HER2/neu (MSC/Neu). They observed that subcutaneous injection of naïve non-activated syngeneic and allogeneic mouse MSC/Neu could induce Her-2/neu-specific T cells and antibodies, leading to the rejection of transplanted neu-expressing tumors [90].

In a different study, APC properties increased when MSCs were pre-treated with IFN-γ. However,

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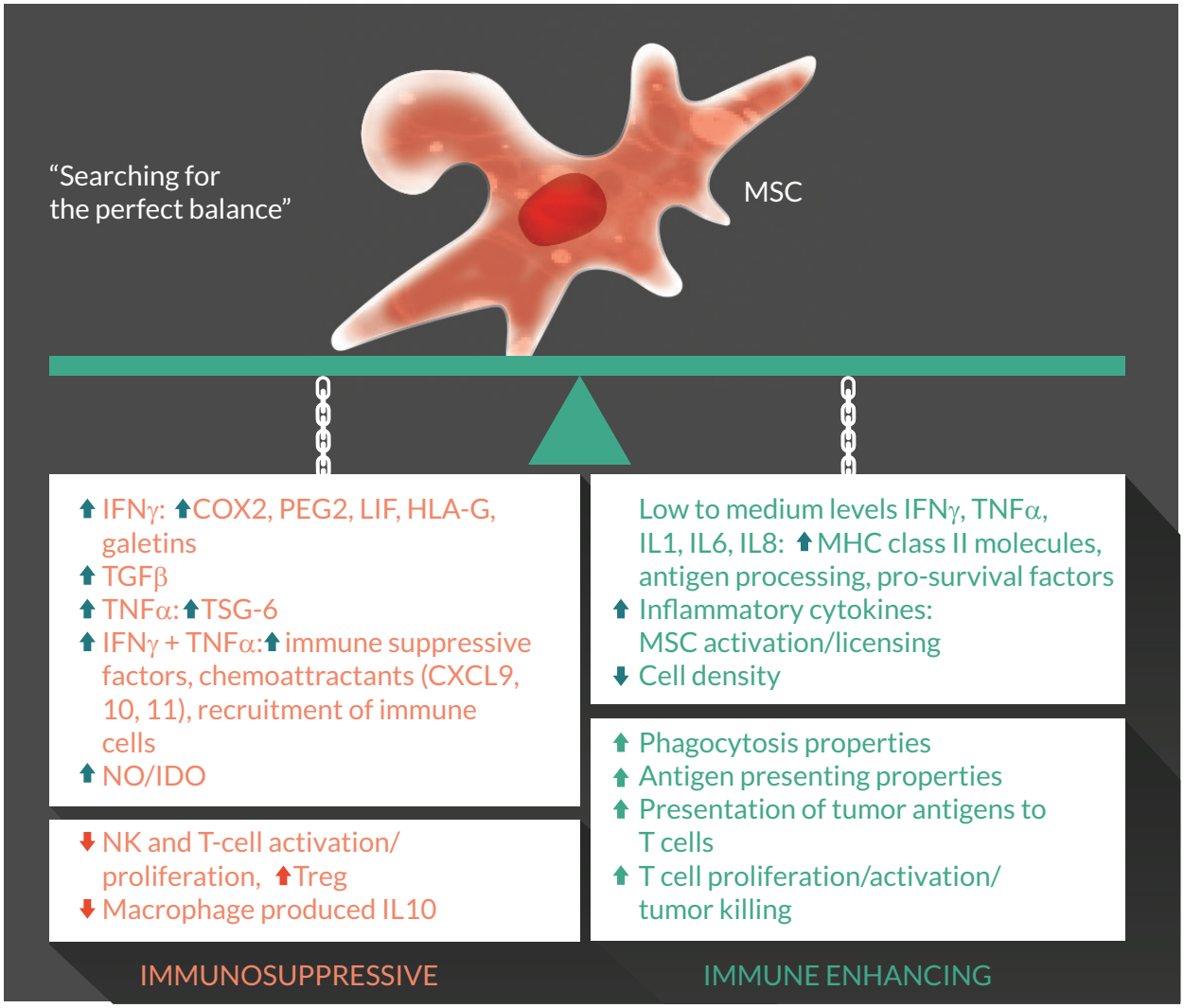
this would only happen at low doses of IFN-γ, with higher doses pushing the MSCs towards an immunosuppressive phenotype [91]. In light of these studies, it is hypothesized that priming with IFN-γ not only increased antigen processing and presentation in MSCs but also activated them for the production of immunosuppressive factors. Importantly, these conclusions highlight the potential consequences of using MSCs as APCs in cancer immunotherapy in the presence of concomitant inflammatory response, which triggers increased antigen processing but also immune suppression by MSCs.

Inflammatory diseases play a significant role in the etiology of cancer. A variety of inflammatory cytokines and chemokines are produced within the tumor such as TNF-α, IL1, IL6 and IL8. This microenvironment will define MSC phenotype switch between immune suppressive and immune

FIGURE 2

The balance between immunosuppressive and immune-enhancing properties in MSCs.



Depending on the surrounding microenvironment, MSCs can either acquire immunosuppressive properties (left panel of the "balance") or when the levels of inflammatory cytokines are not high enough, MSCs can acquire antigen-presenting properties and boost T cell response against tumor antigens (right panel of the "balance"). IFN: interferon; LIF: leukemia inhibitory factor; MSC: mesenchymal stem cell; NK: natural killer cell; TNF: tumor necrosis factor; Treg: regulatory T cell.

enhancing. It appears clear that MSCs switch between immunosuppressive and immune-enhancing phenotypes in response to surrounding environmental cues, and therefore a better knowledge of different tumor microenvironments and their effects on MSC phenotype will be critical for successful clinical application.

The few factors that have been elucidated thus far for the switch between immunosuppressive and immune-enhancing properties in MSCs are depicted in Figure 2.

TRANSLATION INSIGHT

Modulation of cancer by MSCs

Depending on the site and specific cancer behavior, tumors can display unique microenvironmental cues for MSCs. Both the tumor cells and the surrounding stroma release chemotactic and cytokine signals, and glucose and oxygen metabolism are often altered. MSCs respond to these cues and change their behavior accordingly.

It is highly controversial as to whether MSCs have pro- or anti-tumorigenic effects. Indeed, several conflicting *in vivo* and *in vivo* studies show MSC to either stimulate tumor cell proliferation or induce their apoptosis (reviewed in [92]).

For example, under low oxygen conditions, MSCs show increased migration ability, the capacity to form capillary-like structures [93] and secretion of high levels of VEGF in HIF-1α-dependent fashion [93]. This suggests that MSCs can be affected by the microenvironment created within hypoxic solid tumors and gain an active role in their growth.

On the one hand MSCs can potentially promote tumor growth by secreting several factors such as bFGF, VEGF, platelet-derived growth factor (PDGF), HGF, EGF receptor-ligand, insulin-like growth factor 1 (IGF-1), SDF-1 and TGF. On the other hand, MSCs have been shown to induce apoptosis in tumor cells and/or induce their growth arrest at the G1 phase of the cell cycle. This effect has been related to MSC secretion of DKK-1 [94,95], a negative regulator of Wnt/β-catenin pathway and its secretion from MSCs appears dependent on cell density [96]. *In vivo* models have also demonstrated discrepancies between MSC pro- and anti-tumorigenic effects. Several mouse tumor models showed an anti-tumorigenic effect from intravenously-injected MSCs (reviewed in [92]). In contrast, in other models where cells were injected subcutaneously or intraperitoneally, pro-tumorigenic effects appear more common.

Clinically only one study showed a pro-tumorigenic effect when MSCs were injected at the same time as HSC transplantation in leukemic patients [97]. It is however encouraging to note that only one of the diverse and numerous clinical trials using MSCs (45 clinical trials: https://clinicaltrials.gov; reviewed in [98]) reported tumor-promoting effects, in different settings such as HSC-transplanted patients or in patients treated for inflammatory or degenerative diseases. Over 300 clinical trials are registered using MSCs for different applications (reviewed in [99]) and 20 are registered for cancer patients (https://clinicaltrials.gov), with an emerging trend for the use of MSCs as vehicles for oncolytic viruses.

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Improving MSC tumor homing for clinical applications

As discussed above, multiple different mechanisms have been implicated in MSC tumor homing, and data pertaining to these are often conflicting. Various experimental factors may account for these discrepancies, such as:

- Cell density;
- Cell expansion and the use of low versus high MSC passages;
- Differing MSC culture methods;
- Cytokines present in the model microenvironment;
- *In vivo* delivery route, and
- Cell dose.

Indeed, extensive passages of MSCs affected their activation and protection in an ischemia model, due to reduced secretion of growth factors [100]. Additionally, high passage MSCs tend to lose surface receptors, affecting their chemotaxis ability [101,102]. In support of this, CXCR4 has notably been shown to decrease at the cell surface after extensive passages (up to 10 in our laboratory). However, hypoxic conditions appear to enhance CXCR4 expression and increase MSC engraftment [103]. Different cocktails of cytokines can promote this effect and we have shown that tumor conditioned medium treatment can increase CXCR4 surface expression in MSC [24]. Others have also shown that pre-treatment with TNF-α, TGF-β and IL1b can stimulate MSCs to secrete high levels of matrix metalloproteinases, facilitating migration through the extracellular matrix in response to chemokines [104].

Various routes of injection have been tried, including intravenous, intraperitoneal, intra-arterial, *in situ* and pleural, each affecting the efficiency of homing to target organs or tumors, and therefore impacting their clinical benefit for example as anti-cancer molecule vehicles [101,105]. In general, intravenous delivery appears to be the most convenient and successful in treating certain types of diseases, although superior cell engraftment has been observed following intra-arterial and *in situ* injections for myocardial infarction, kidney transplantation and brain injury. We recently demonstrated in a mouse model that the delivery of the anti-cancer molecule TRAIL by MSCs reduced lung metastasis following intravenous injection, but that the same cells had no effect when administered pleurally [105].

Administration of MSCs *in situ*, appears less clinically attractive, as it is invasive and is the least efficient in maintaining the viability of the injected cells [106]. Other factors should not be ignored such as the stage of disease, timing of MSC delivery and number of MSCs injected, as more MSC administration does not always produce a better therapeutic effect, for example in brain injury animal models [107].

A wide range of novel techniques may be required to further elucidate MSC homing mechanisms (rolling, adhesion and transmigration), cell survival and interaction and integration within tumors and normal organs. For example, defining whether selective interactions of MSCs with tumor blood vessels or other blood vessels have a role in homing, which could be assessed with the use of intravital microscopy [108]. The use of this technique coupled to spatiotemporal FRET-based aptamer microenvironment sensors will allow the

study of spatiotemporal localization of MSCs and the measure of critical signalling molecules within the tumor microenvironment [109]. The development of this wide range of novel techniques will render it possible to visualize and assess directly the interactions between MSCs and tumors *in vivo*.

MSCs express surface receptors capable of sensing signals released in sites of injury, inflammation or tumors. Many groups have attempted to modify MSCs to enhance surface marker expression and thereby enhance MSC migration and homing. In particular, several studies have focused on enhancing MSC expression of CXCR4. MSC transduction with CXCR4 retroviral constructs, mRNA transfection of CXCR4–GFP [110], and cytokine pre-treatment particularly with TNF-α were successful in enhancing MSC migration toward a SDF-1α gradient *in vivo* [111,112]. The homing receptor CCR2, highly expressed at sites of inflammation, has also been studied as a candidate for receptor enhancement. GFP-labelled CCR2-expressing MSCs were infused into transgenic mice expressing CCR2 in the myocardium. A higher number of GFP-positive cells were present in the myocardium of the transgenic mice compared to control mice [26].

Maijenburg *et al* used gene expression profiling to identify 12 genes differentially expressed in migratory MSCs. Within this group, the nuclear receptors Nur77 and Nurr1 were those most expressed, and pre-treatment with SDF-1α and PDGF-BB was shown to upregulate their expression. In addition, MSCs engineered to overexpress Nur77 showed increased migration in response to SDF-1α. [113].

Kumar *et al* transduced mouse MSCs with an adenovirus construct to upregulate the expression of the α4 subunit of VLA-4-integrin. They showed that the dimerization of this with β1-integrin improved MSC homing to bone marrow by over 10-fold in syngeneic female mice [114].

A separate, equally promising strategy for enhancing MSC homing would be to use lipid vesicles to load MSCs with surface receptors or other molecules such as SLex or P/L-selectin targeting aptamer. This circumvents difficulties seen with viral vectors in achieving effective receptor conformations and cell surface recruitment. Furthermore, the absence of genetic manipulation of the MSC product is clinically more attractive in terms of patient safety. This approach showed promising results, successfully increasing homing to inflamed endothelium, both *in vivo* and *in vitro* [109,115–117].

MSC-based therapies in clinical practice – the universal donor paradigm

It is becoming apparent that MSCs are not immune privileged, as claimed in the literature; however their use in clinical trials is escalating (https://clinicaltrials.gov/). The scientific community appears reluctant to abandon the immune privileged paradigm supporting the notion of a "universal donor" but it is clear that immunogenicity needs to be recognized as an important characteristic of MSCs.

Both syngeneic and allogeneic MSCs are currently being used in clinical trials; however very few studies focused on direct comparisons between the two. Despite the fact that both sources were deemed safe in several trials with no major adverse effects reported, two clinical

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trials (POSEIDON and a phase 2 mesoblast trial) observed an anti-donor response in patients treated with allogeneic MSCs [118].

The secretion of trophic and immunomodulatory factors, or the production of exosomes, immediately following MSC injection may account for their therapeutic effect by a so-called 'hit-and-run' mechanism [119,120]. However, this concept is challenged by the idea that the main therapeutic benefit of MSCs is achieved through reprogramming of the immune system through apoptotic bodies [121]. Nevertheless, both concepts support the consensus that a benefit of MSCs can be boosted by extending their persistence after injection [122].

A better understanding of the particular MSC mechanisms contributing to therapeutic effect in each disease setting is required in order to clarify whether allogeneic or syngeneic MSCs are more appropriate. If allogeneic MSCs were shown to possess more potent 'hit-and-run' effects, then one could envision their dominance in clinical settings where MSC persistence is not necessarily required. The notion that extended persistence of MSCs will result in a sustained therapeutic effect and improved clinical outcome has yet to be tested and proven clinically, depending, as discussed above, on the different disease settings and need for persistence. Nevertheless, the use of allogeneic MSC therapy and the concept of "off the shelf", "one fits all", needs revision and further investigation. Encouragingly, allogeneic MSC therapies have been consistently shown to be safe, allowing future trials to be conducted with improved design and standardized protocols, using refined MSC-based approaches [123]. As allogeneic MSCs appear to be cleared only marginally faster than syngeneic MSCs, combination approaches to avoid rejection and mitigate transplantation shock could be explored to extend persistence. Next-generation trials for MSC therapies should aim for in-depth characterization and fine tuning of MSC engraftment, immunogenicity, survival, potency and disease specific mechanisms of action.

FUTURE OF MSC-BASED THERAPIES FOR CANCER: WHERE ARE WE HEADING?

Oncolytic viruses, exosomes, iPS-derived MSCs

This review summarized the current challenges we face regarding the use of MSCs in a clinical setting. Our group has recently started a phase I/II clinical trial based on *in vivo* studies showing inhibition of tumor growth in a lung cancer model using MSCs carrying TRAIL [105].

A key area for the future will be the use of oncolytic viruses. These viruses specifically target and infect cancer cells, triggering immune activation upon cancer cell lysis. Some of the oncolytic viruses (for example vaccinia virus and vesicular stomatitis virus) are shown to inhibit the tumor immunosuppressive microenvironment, switching to an anti-tumor and pro-inflammatory microenvironment by reducing the secretion of immune-suppressive cytokines, reducing the recruitment of immune suppressive cells and inducing a vasculature shutdown leading to cancer cell necrosis (reviewed in [124,125]). A main challenge for

this strategy is the clearance of the virus before it reaches the tumor bed by the host defence mechanisms. In an attempt to circumvent this issue, one strategy is to use cells as carriers, with MSCs appearing to be a good candidate.

In addition to the capacity to carry the virus to the tumors using their homing capacities, MSCs have been shown to successfully protect different oncolytic viruses from neutralizing antibodies and other host antiviral mechanisms, successfully delivering the viruses to the tumor site [126–129]. An example of one such clinical trial is the treatment of recurrent ovarian cancer using MSCs loaded with measles virus (NCT02068794).

Emerging strategies are focusing on the use of cell-free products, such as exosomes. Exosomes exert their effects via the transfer of a variety of different biomolecules (endosome-associated proteins, membrane proteins, lipid raft proteins and RNA including small interfering RNAs [siRNAs]) [130]. Exosomes isolated from MSCs have shown promising results in various animal models (reviewed in [131]). Customized production of MSC-derived exosomes is achievable. These biological nanocarriers can be loaded with drugs and siRNAs either by electroporation or chemical disruption or they can be incorporated during their biogenesis using genetically modified MSCs. The potential advantages of this strategy compared with whole MSC-based therapies include: i) higher safety profile, ii) inherent anti-inflammatory and pro-regenerative effects (which are still not fully clarified), and iii) a lower chance of rejection in allogeneic settings.

Another interesting strategy to circumvent potential safety issues following the injection of MSCs is to derive them from induced pluripotent stem cells (iPSCs). MSCs derived from iPSCs have been shown to maintain their regenerative and immunomodulatory properties [132,133]. Identification and utilization of genetically modified MSCs, having a "safe harbor" integration, is limited because of the short lifespan of primary MSCs *in vivo*. Using iPSCs can generate indefinite fresh MSCs. Additionally, the use of lentiviruses to modify MSCs can lead to unwanted integration and present a safety issue. Taking this issue into consideration, genetically engineered MSC clones could be generated from iPSCs after accurate screening for a vector integration site and cells with "safe harbor" integrations could potentially be expanded indefinitely, allowing the establishment of a bank of cells and an 'off the shelf' therapeutic product (reviewed in [134,135]).

FINANCIAL & COMPETING INTERESTS DISCLOSURE

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