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Genetic engineering of mesenchymal stromal cells for cancer therapy: turning partners in crime into Trojan horses

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Abstract: Mesenchymal stromal cells (MSCs) are adult progenitor cells with a high migratory and differentiation potential, which influence a broad range of biological functions in almost every tissue of the body. Among other mechanisms, MSCs do so by the secretion of molecular cues, differentiation toward more specialized cell types, or influence on the immune system. Expanding tumors also depend on the contribution of MSCs to building a supporting stroma, but the effects of MSCs appear to go beyond the mere supply of connective tissues. MSCs show targeted "homing" toward growing tumors, which is then followed by exerting direct and indirect effects on cancer cells. Several research groups have developed novel strategies that make use of the tumor tropism of MSCs by engineering them to express a transgene that enables an attack on cancer growth. This review aims to familiarize the reader with the current knowledge about MSC biology, the existing evidence for MSC contribution to tumor growth with its underlying mechanisms, and the strategies that have been developed using MSCs to deploy an anticancer therapy.

Keywords: HSV-Tk; mesenchymal stromal cells; MSC; suicide gene therapy; tumor stroma.

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Introduction

Mesenchymal stem/stromal cells (MSCs) represent a population of precursor cells with high differentiation potential, which can be isolated and expanded easily in large amounts from adult mammals without ethical conflicts. The main source of these cells is the bone marrow, but their presence has been demonstrated in several other tissues. Recent studies, which underline the immune privilege, plasticity, and migratory potential of these cells, have made them ideal candidate cells for applications touching the clinical field of surgical oncology. The plasticity of MSCs toward specialized cells of mesenchymal tissue (i.e. chondrocytes, adipocytes, and osteoblasts) strongly suggests the utilization of these cells in regenerative medicine. This continues to be one of the main fields of MSC research and potential clinical application of these cells but goes beyond the scope of this review. Yet, the aspects of high differentiation and migration capacity also made these cells noteworthy for cancer research. There is now a large body of evidence supporting the concept of MSCs representing a major precursor population for cells contributing to tumor-associated stromal components. Within the tumor microenvironment, MSCs have been shown to give rise to tumor-associated fibroblasts (TAF), pericytes, endothelial cells, and fibrovascular stroma. The role of the tumor-supporting stroma concerning different important aspects of malignant tumor growth (i.e. invasion, metastasization, chemoresistance, and radioresistance) remains controversially discussed, just as the contribution of MSCs to these processes is. However, irrespective of the potential protumorigenic or antitumorigenic effects that the natural biology of these cells may convey, the utilization of MSCs as delivery vehicles for anticancer therapies of different kinds has been an emerging concept pursued by several research groups, including ours. The aim of this present review is to familiarize the reader with the current knowledge about general MSC biology (focusing

on aspects important for the clinical application of these cells), their involvement in tumor growth, and their use as delivery vehicles for cancer therapy.

Biology of MSCs

Characterization and sources of MSCs

MSCs were first described by Friedenstein et al. as non-hematopoietic stromal cells of the bone marrow capable of bone formation and termed as colony-forming-units-fibroblasts (CFU-Fs) [1]. Caplan used the term "mesenchymal stem cell" after his group discovered that CFU-Fs are possibly capable of self-renewal and differentiation [2]. The report from Pittenger et al. on the multilineage differentiation potential of MSCs prompted further interest of the research community in these cells [3]. In the last two decades, the number of published studies dealing with MSCs has increased exponentially.

At the same time, protocols for the isolation and expansion of MSCs, and along with this the cell types described within these studies, began to vary markedly. Thus, the term "MSCs" in research articles may subsume several different types of more or less specialized stem or even progenitor cells. Currently, not a single cellular marker or receptor has been described to be unique to MSCs. Thus, to harmonize reports on MSC studies, the International Society for Cellular Therapy issued a position statement with the minimum requirements for isolated cell lines to classify as MSCs [4]. The prerequisites for such a cell line are as follows: (1) it must grow adherent to plastic in a cell culture flask; (2) it must express CD105, CD73, and CD90 and lack the expression of CD45, CD34, CD14 or CD11b, CD79α, or CD19 and HLA-DR; and (3) it must be capable to give rise to adipocytes, chondrocytes, and osteoblasts when put under specific culture conditions *in vitro*. However, the plasticity of MSCs does not seem to end with these three cell types. There have been reports showing the differentiation of MSCs to epithelial cells and neurons (i.e. cells of ectodermal origin) and to muscle cells, gut epithelial cells, and lung cells (i.e. cells of endodermal origin) [5].

Researchers have been able to isolate cells with MSC characteristics from a variety of tissues, including bone marrow, adipose tissue [6], umbilical cord blood [7], Wharton's jelly [8], skeletal muscle [9], periosteum [10], liver, brain, spleen, kidney, lung, thymus, and pancreas [11]. The distribution pattern of MSCs in virtually all postnatal organs have led to the theory that MSCs reside in the perivascular niche and should thus be termed "multipotent perivascular-derived cells" [12]. In fact, research results indicate that perivascular cells may account for a majority of tissue-resident MSCs. Pericytes isolated from several adult organs by combination of the surface markers NG2, CD146, and PDGFRβ showed the MSC characteristics of trilineage differentiation *in vitro* and osteoblast differentiation *in vivo* [13]. However, not the entire population of pericytes shows MSC characteristics and MSCs have been shown to be the progeny of cells other than perivascular (such as glial cells, for example) [14, 15].

Physiological functions of MSCs

The physiological function of endogenous MSCs has been studied most intensely in the bone marrow, which represents the main source of MSCs. Here, MSCs are important for the construction and maintenance of the hematopoietic stem cell (HSC) niche [16]. MSCs do so by the expression of the "HSC maintenance factors" CXCL-12, c-kit ligand, angiopoietin-1, interleukin (IL)-7, vascular cell adhesion molecule-1, and osteopontin [17]. An important function of MSCs in this context seems to be the inhibition of inappropriate HSC differentiation, which is achieved by an immunosuppressive phenotype of MSCs [18]. The perivascular localization of MSCs is believed to enable these cells to detect local or distant tissue damage and respond to such by directed migration and participation in the healing process. This hypothesis has been supported by studies from Seppanen et al. in a fetal microchimerism model in mice. Here, labeled fetal MSCs colonized the bone marrow of the mother. After the infliction of skin wounds to the mother postpartum, these cells showed migration toward these wounds and differentiated into a collagen-producing fibroblast-like cell [19]. Furthermore, the homing of MSCs to sites of inflammation is not only a trait observed in endogenous MSCs after being released from the bone marrow but can also be seen after the systemic injection of exogenous MSCs. In a skin wound model in an immunocompromised mouse, human MSCs showed targeted tropism to the wound 3 days after injection and remained there for the duration of the experiment [20].

Immunological properties of MSCs

MSCs are hypothesized to be poorly immunogenic due to their low expression level of major histocompatibility

complex (MHC) I, lack of MHC II expression, and lack of expression of the costimulatory ligands CD40, CD80, and CD86 [21]. By this, MSCs have shown to avoid the recognition of circulating T cells [22]. Thus, allogeneic transplantation of MSCs is believed to involve a reduced risk of transplant rejection, giving rise to the idea of an allogeneic MSC preparation to be a "one-size-fits all, off-the-shelf" therapy [12]. However, data from experimental and clinical studies prompted evidence that the transplantation of allogeneic MSCs may nonetheless provoke an immune response that leads to MSC rejection. Zangi et al. examined the persistence of labeled MSCs and fibroblasts in a host mouse after injection and compared an allogeneic to a syngeneic transplantation setting [23]. The authors reported that syngeneic MSCs as well as fibroblasts survived in the host for the duration of the experiment (40 days). In the allogeneic setting, however, fibroblasts and MSCs vanished by days 10 and 20 after injection, respectively. Although the prolonged survival of MSCs versus fibroblasts observed in the latter experiment indicate the immunoevasiveness of MSCs, the fact that they did not survive as long as syngeneic MSCs supports the hypothesis of a rejection after transplantation. This is further supported by the findings that, in mice previously injected with allo-MSCs, the rejection of fibroblasts from the same donor was accelerated to day 2 after injection and these mice harbored an elevated memory T-cell count. The immunogenic properties of allogeneic MSCs after transplantation can be explained by the findings that the low to nonexistent MHC expression levels in naïve MSCs become elevated after exposure to interferon (IFN)-γ or differentiation into mature cells [24]. Data from a clinical study also point toward allogeneic MSCs being immunogenic with a high potential of being rejected after transplantation. An autopsy study on patients who died within a year after transplantation of MHC-mismatched or haploidentical MSCs revealed that donor DNA was only observable in peripheral tissues of only 1 of the 18 patients studied. This single patient received the allo-MSC infusion just 7 days before his death and while being severely immunocompromised due to severe sepsis [25]. In all the other 17 patients, the transplanted allo-MSCs appear to have fallen victim to rejection.

On the contrary, MSCs have a high capacity of modulating the response of both innate and adaptive immune systems by differentially influencing the proliferation of immune cells. The main mechanism for this is believed to lie in the capacity of MSCs to secrete anti-inflammatory mediators and inhibitory molecules such as transforming growth factor-β (TGF-β), hepatocyte growth factor (HGF), prostaglandin E2 (PGE2), PD-L1, and FasL [26–29]. MSCs inhibit the proliferation and maturation of B cells and natural killer (NK) cells while showing protective activities toward neutrophils [30, 31]. Furthermore, MSCs have been shown to inhibit the proliferation of $CD4^+$ and $CD8^+$ T cells [32] and may also promote the induction of regulatory T and B cells as well as anti-inflammatory macrophages [33, 34]. The clinical effect of this MSC-mediated immunosuppression is observable in the prolonged survival of MHC-mismatched skin grafts in baboons achieved by an intravenous injection of MSCs immediately before transplantation [35]. The immunosuppressive effects of MSCs are so profound that they have been successfully used in patients to ameliorate graft-versus-host disease (GVHD) [36].

These properties of MSCs as well as others have yielded a high interest of the scientific community in a potential clinical use of these cells.

MSCs in cancer biology

Tumor stroma as a hallmark of cancer

In an attempt to condense the complexity of cancer biology, Hanahan and Weinberg identified several traits that appear mandatory for cancer cells to develop the full clinical picture of the disease and that describe the general phenomenon shared by several cancer entities. These "hallmarks of cancer" include cellular self-sufficiency in growth signals, insensitivity to antigrowth signals, evasion of cell death programs, and limitless replicative potential as well as tumor angiogenesis, tissue invasion and metastasization, metabolic deregulation, evasion of the immune system, and inflammation [37, 38].

It is by now a well-established concept that cancer cells not only possess cell autonomous mechanisms acquired by genetic mutations that convey some of these traits, but that their interaction with the local environment and even the whole organism may also play an important role in this. The tumor microenvironment that surrounds expanding cancer cells with immediate contact – also termed "stroma" – is believed to mainly function as a pool of resident and recruited "normal" cells that then become conscripted and corrupted by cancer cells to aid in the formation of an organ-like macroscopic tumor [39]. These cells can be categorized as infiltrating immune cells, cancer-associated fibroblasts (CAFs), and angiogenic vascular cells (AVCs; endothelial cells and pericytes) [40]. Although some aspects of the tumor-cell-to-stroma interaction touching the hallmarks of cancer

(i.e. tumor angiogenesis and remodeling of the extracellular matrix) have been thoroughly studied in the last decades [41, 42], the constituents of the stromal compartment appear to elicit a vast but yet to be fully understood impact on cancer cells that may both permit and inhibit cancer growth. Although recent studies [43, 44] raised doubt (at least in the case of pancreatic carcinoma) about the prevailing conception that the tumor stroma elicits mainly tumor-supporting effects, a tumor-promoting influence of stromal cells has been demonstrated for all hallmarks of cancer, except for the capability of limitless replication of cancer cells [39]. Although the contribution of some stromal cell types to certain hallmarks may be self-evident (i.e. for endothelial cells to contribute to angiogenesis), the influence of stromal cells on cancer hallmarks appears to be much broader, mainly through paracrine and juxtacrine mitogens produced by these stromal cells [39].

MSCs contribute to the stroma

Both experimental and clinical studies have supplied convincing evidence supporting the theory that MSCs contribute to the cancer stroma. Expanding tumors constantly alter their surrounding tissue causing an inflammatory response, which resembles that of a chronic wound [45]. Although MSCs physiologically have the capacity to become recruited to the sites of injury, they show a similar behavior (potentially by the same mechanisms) in the setting of a growing tumor [46]. In an animal model of melanoma, Studeny et al. were the first to describe the homing of labeled MSCs within the tumor stroma both after subcutaneous coinjection with tumor cells and after intravenous administration [47]. Bioluminescence imaging allows for the longitudinal monitoring of systemically injected, labeled MSCs. Using this technique, Kidd et al. delivered a detailed information about the spatial distribution of MSCs after tail vein injection in mice with different conditions [20]. In mice bearing subcutaneous breast cancers, MSCs were detectable in the lung capillaries during the first 6 days after injection but then colocalized with the growing tumors followed by cell proliferation in the subsequent 6 days. The homing of MSCs to the tumor stroma has been demonstrated for several other tumor entities besides breast cancer and melanoma including pancreatic [48], ovarian [49], prostate [50], gastric [51], and hepatocellular carcinoma (HCC) [52] as well as glioma [53].

Although not fully understood, the process of MSC homing to tumors is believed to be a multistep process with similarities to that observed in leukocyte trafficking. In the first step, in the case of endogenous MSCs, these are mobilized from the bone marrow and enter the circulation after chemotactic stimuli [54]. MSCs must then interact with the endothelium of the target tissue and adhere, extravasate, and engraft at sites of tumor. Several of these steps appear to be orchestrated by cytokines and chemokines. The adhesion of MSC to the endothelium, for example, is enhanced by the activation of the latter with the proinflammatory cytokine tumor necrosis factor-α (TNF-α) [55]. The chemokine (C-X-C motif) ligand 12 (CXCL12) is expressed in tissues after injury and hypoxia in a hypoxia-inducible factor-1 (HIF-1)-dependent manner [56]. The chemokine axis of CXCL12/stromal cell-derived factor-1 (SDF-1) and CXCR4 plays a pivotal role in both HSC homing and tumor cell metastasization to the bone marrow [57, 58]. Recent findings also indicate the significance of CXCR4 signaling through both CXCL12 and macrophage migration inhibitory factor (MIF) signaling for MSC homing to tumors [59, 60]. Although the reports in the literature differ in the expression levels of CXCR4 on MSCs, it appears that CXCR4 in nonactivated MSCs is expressed at a low level on the cell surface and at a high level in the intracellular compartment. It has been hypothesized, however, that MSCs – upon activation – are capable of quickly translocating CXCR4 molecules to the cell surface, which then enables MSCs to follow CXCR4-mediated migration cues toward tumors [51, 61].

Function of MSCs in tumors

Although there are some reports on the tumor-suppressing effects of native MSCs (e.g. [62]), the overwhelming majority of articles on this topic report the enhancing effects on nearly all aspects of malignant tumor growth [63]. Cuiffo and Karnoub described four general functional categories by which MSCs within the tumor stroma may influence tumor growth [63]: (1) through direct actions on tumor cells, (2) through indirect effects; such as the enhancement of angiogenesis; (3) through their immunosuppressive properties; and (4) as progenitors for tumor stromal cells.

First, MSCs seem to be capable of exerting a direct paracrine effect on cancer cells, resulting in the promotion of tumor proliferation, invasion, and metastasis. Specifically, this has been shown for chemokines, cytokines, and growth factors released by MSCs. For example, MSC-derived CXCL1/2 and CXCL12/SDF-1 enhance cancer cell proliferation through signaling on

their respective CXCR2 and CXCR4 receptors expressed by cancer cells [64, 65], IL-6 and IL-8 released by MSCs enhance malignancy in breast and colorectal cancer models [66, 67], and epidermal growth factor (EGF) secreted by MSCs enhances tumorigenesis in a breast cancer model [68]. The secretion of bioactive molecules by MSCs appears to happen as a context-dependent response upon interaction with certain cancer microenvironments. Whereas, for example, breast and pancreatic cancer cells were capable of inducing high levels of CCL5 secretion from MSCs, which in turn led to enhanced tumor growth and metastasization, other cancer cell lines did not provoke CCL5 secretion to increase [48, 69]. Furthermore, MSCs appear to be capable of regulating and supporting cancer stem cells (CSC) in a similar fashion as within their physiological function with HSCs in the bone marrow. In the tumor stroma of breast cancer, MSCs augment the population of cancer cells that show CSC characteristics by paracrine secretion of IL-6 and CXCL7 after homing to tumor sites [67]. Similar results were observed in a model of ovarian cancer, where MSCs raised the CSC count through BMP2 signaling [70]. To add to the tumor beneficial effects elicited by MSCs directly on cancer cells, MSCs have been shown to convey chemoresistance by releasing chemoprotective polyunsaturated fatty acids upon treatment with a platinum analog [71].

Second, MSCs within the tumor stroma may indirectly promote tumor growth by aiding in the process of tumor angiogenesis. Specifically, MSCs may do so by recruitment of endothelial progenitor cells and by enabling the formation and maturation of tumor vasculature [72, 73]. Their capacity to enhance angiogenesis was demonstrated in both wound healing and tumor models and conveyed by the secretion of proangiogenic factors such as vascular endothelial growth factor (VEGF), angiopoietins, and EGF [74, 75].

Third, MSCs within the tumor stroma may exercise direct or indirect immunomodulatory effects that defend tumor cells from both adaptive and innate immune systems, as described earlier in this review. Although not necessarily based on cancer models, it has been proven that MSCs are capable of influencing the vast majority of key immune activities involved in the process of tumor formation. The immunosuppressive effects of MSCs are so profound that it appears that they are unmatched by other cell types of the tumor stroma, thus making MSCs a central element in the immunoevasiveness of tumors [63].

Fourth, MSCs may participate in the tumor microenvironment by providing a cell pool that forms the basis for several specialized stromal cells. Within the tumor stroma, MSCs not only influence cancer cells through paracrine signaling but also are themselves subjected to an array of signaling molecules, which in turn may result in the differentiation of MSCs toward a more specialized cell phenotype. The transdifferentiation of MSCs to CAFs and myofibroblasts, which in turn promote tumor growth, was demonstrated after signaling from tumor-conditioned medium [76], tumor-derived exosomes [77], and xenografts from breast, pancreatic, and ovarian cancers [78] as well as prostate cancer [50]. Interestingly, using an inflammation-induced gastric cancer model, Quante et al. were able to demonstrate that the process of MSC homing and differentiation toward CAFs is a process occurring early in the phase of dysplasia and tumor niche formation and is of utter importance to drive carcinogenesis [51]. In a prostate cancer model, the signaling of CXCL16 from the tumor microenvironment on the CXCR6 receptor of MSCs seems to be of great importance for the differentiation of MSCs toward CAFs to occur [50].

Engineered MSCs for cancer therapy

The MSC traits of tumor tropism, deep migration into the tumor microenvironment, immune evasion, and wide availability as well as expandability have evoked a substantial interest in their use as tumor-specific vehicles for the delivery of therapeutic agents. Figure 1 provides an overview of the most commonly used strategies to genetically engineer MSCs to target tumors, which are discussed in this section.

Studeny et al. used engineered MSCs with forced expression of IFN-β, a cytokine that conveys strong antiproliferative effects on several cell types, including tumor cells, and demonstrated the inhibited growth of metastatic melanoma. Here, the MSC-based delivery of IFN-β proved more capable of tumor inhibition compared to systemic IFN-β therapy [47]. Considering the existing evidence of the tumor-promoting properties of MSCs, however, an ideal setup would require MSCs containing antitumor therapeutics to stay viable until the delivery of the therapeutic and then die immediately after the therapeutic effect has worn off to avoid tumor beneficial effects [79]. Whereas some MSC-based therapies, such as those using “suicide gene” strategies, bear this problem in mind, others do not and thus may potentially be too harmful to ever be used in a trial in human.

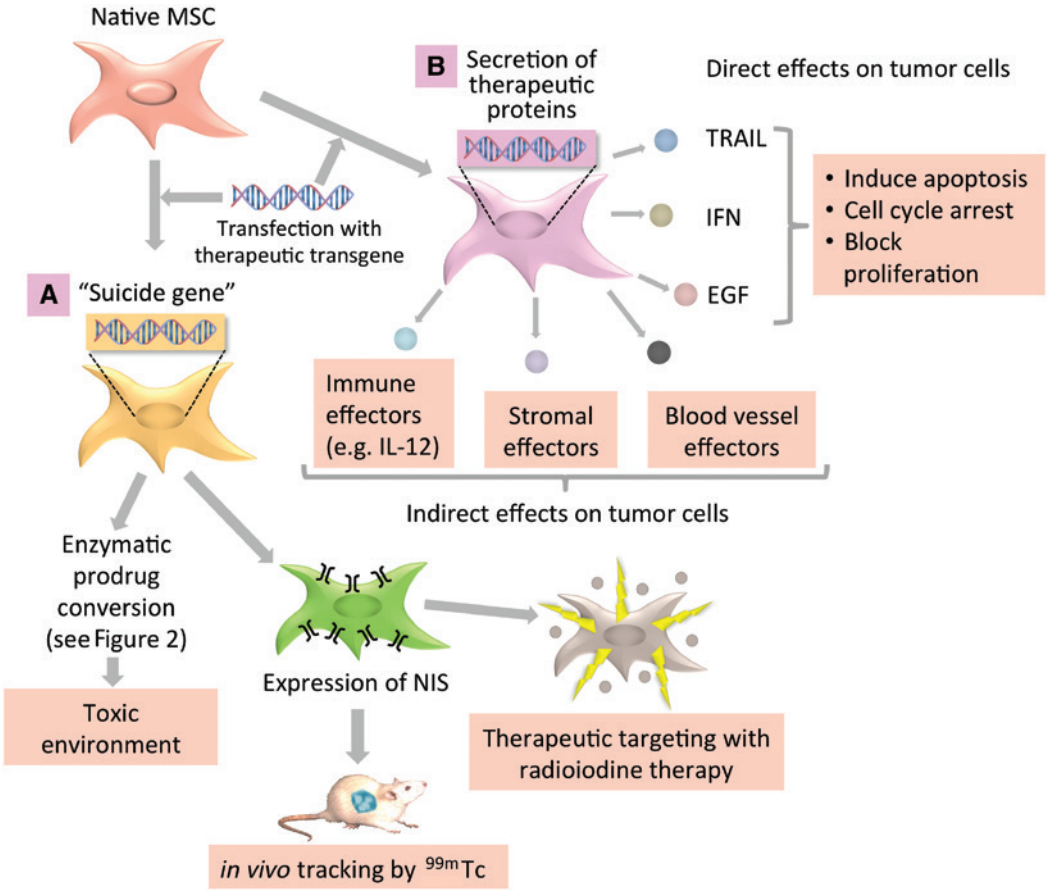

Figure 1: Strategies for targeting MSCs against cancer.
Native MSCs, which are isolated and expanded from autologous or allogeneic donors, are consequently transfected with a therapeutic transgene. The most commonly used transgenes encode for either a "suicide gene" (A) or allow MSCs to secrete therapeutic proteins that either directly or indirectly affect tumor growth (B). (A) The "suicide gene" strategy foresees the insertion of a gene that enables the researcher to selectively target the transfected cells with a subsequently administered and otherwise nontoxic drug. When this drug is applied after homing of MSCs into the tumors, this strategy resembles that of a Trojan horse because, after the conversion/uptake of the then toxic drug, not only MSCs but also the surrounding tumor and stromal cells are killed (see Figure 2). These suicide genes can encode either for an enzyme (GDEPT; Figure 2) or for the NIS. (B) A wide range of genes encoding for therapeutic proteins have been transfected into MSCs and used to target different aspects of tumors. These can be divided into proteins that act directly on tumor cells and their receptors, such as TRAIL, IFN, and EGF, and proteins that indirectly affect tumor growth.

Engineered MSCs to modulate immune response

MSCs have been engineered to express and secrete high levels of cytokines with known antitumor activity into the microenvironment, with the aim to boost endogenous immunity against cancer [47, 80]. A series of studies show that the tumor-promoting effects of MSCs through the suppression of the immune system can be overcome through genetic engineering of the cells to achieve the opposite. Both adaptive and innate immune systems have successfully been stimulated by genetically engineered MSCs to inhibit tumor growth. The former, for example, has been achieved by MSCs expressing high levels of IL-2. This approach has yielded a prolonged survival of rats suffering from invasive glioma [81]. MSC-based overexpression of IL-12, a cytokine with known effects on both T-cell and NK cell function, has been shown to inhibit the formation of lung metastasis and prolong the survival in several cancer models including renal cell carcinoma and glioma [82, 83]. These studies specifically emphasize the influence of MSC-derived IL-12 on the innate immune system, specifically on NK cells, as the former study was conducted in an animal model lacking an adaptive immune system and thus only influencing the innate immune system [83] and the latter study showing increased intratumoral NK cell count compared to T cells [82].

Furthermore, MSCs overexpressing the chemokine CX3CL1 were shown to inhibit the formation of lung metastasis and prolong animal survival [84]. CX3CL1 is an immunostimulatory molecule that is expressed on the cell surface but can also be shed into the extracellular compartment. In its soluble form, it acts as a chemoattractant for T cells and monocytes, whereas its surface-bound version aids in the adhesion of leukocytes to activated endothelium [85]. However, it has also been shown that CX3CL1 enhances the attraction of CX3CR1-expressing tumor cells to the bone with a concomitant formation of bone metastasis [85]. Thus, the expression of CX3CL1 appears to have site-dependent and tumor type-dependent effects on cancer cell behavior and thus may not be regarded as the "golden bullet" against all types of cancer.

Delivery of cytotoxic agents and inhibitory molecules

MSCs have been genetically engineered to express molecules with known apoptotic effects on tumor cells. Among these molecules, the cytokine TNF-α-related apoptosis-inducing ligand (TRAIL) has shown very promising results. Although TRAIL induces apoptosis in tumor cells of numerous entities through signaling on the TRAIL receptors DR4 and DR5, these receptors are not expressed in most normal tissues [86]. The systemic administration of recombinant TRAIL protein or agonistic antibodies to the TRAIL receptor has shown only moderate therapeutic efficacy in clinical trials [87, 88]. This is mainly due to the insufficient bioavailability of these proteins at the tumor site [89]. To overcome this, MSCs have been engineered to constitutively express TRAIL and its soluble variant sTRAIL. This approach resulted in significantly inhibited tumor growth in models of colorectal [90], pancreatic [91], and hepatocellular [92] carcinoma as well as mesothelioma [93].

Another example of the efficacy of inhibitory molecules expressed by engineered MSCs to inhibit cancer growth is that of NK4, which acts as an antagonist of HGF. In an animal model of gastric cancer, MSC-derived NK4 expression within the tumor leads to both increased tumor cell apoptosis and lower microvessel density, indicative of the inhibition of tumor angiogenesis [94].

"Suicide gene" therapy and prodrugs

Gene-directed enzyme-producing therapy (GDEPT) makes use of enzymes that are capable of metabolizing an otherwise harmless prodrug into a toxic metabolite and that are normally not present at a relevant concentration in healthy human cells. Gene vectors encoding for such foreign, prodrug-converting enzymes are termed "suicide genes" and can be stably transfected into MSCs and thereby be targeted into tumors (Figure 2). After the expression of the GDEPT enzymes by MSCs inside the tumor, the systemic delivery of the nontoxic prodrug can result in a localized antitumor effect after the conversion of the prodrug. Toxic metabolites can then cause the death of the delivering MSC, which prevents any concomitant tumor-promoting effects of the MSC [95]. Furthermore, due to the prodrug's increased bioavailability, permeability, and increased half-life compared to conventional chemotherapy, these metabolites can diffuse, be actively transported to surrounding cells through gap junctions, or be taken up by phagocytosis [96]. This "bystander effect" results in the death of neighboring tumor and stromal cells.

The two GDEPT strategies that have been most frequently deployed to MSCs include herpes simplex virus-thymidine kinase (HSV-Tk) with ganciclovir (GCV) as the prodrug and cytosine deaminase (CD) with 5-fluorocytosine (5-FC) as the prodrug (reviewed in [97]).

In cells expressing HSV-Tk, the prodrug GCV is phosphorylated by the enzyme into a monophosphate form, which then is converted to GCV biphosphate and triphosphate by endogenous kinases. GCV triphosphate effectively inhibits DNA synthesis and consequently leads to cell death after cell cycle arrest [97]. In this model of GDEPT, the bystander killing effect is highly dependent on gap junctions and phagocytosis of the active metabolite after cell death, as GCV triphosphate is unable to passively permeate cell membranes [96, 98]. The tumor-killing effects of HSV-Tk-transfected MSCs in combination with GCV has been shown in tumor models of glioma. Miletic et al. showed that the injection of HSV-Tk-expressing MSCs in the vicinity or inside the tumor followed by the systemic administration of GCV inhibited tumor growth [99]. To enhance the efficacy of GDEPT, MSCs coexpressing HSV-Tk and TRAIL were tested in tumor models. The application of these cells resulted in beneficial effects in animal models of glioblastoma and renal cell carcinoma [95, 100].

Similar effects were achieved using MSCs that were transfected with CD as suicide gene, which is capable of metabolizing 5-FC to its toxic form 5-fluorouracil (5-FU). 5-FU is a commonly used chemotherapeutic agent for the treatment of several tumor entities, but chemoresistance against 5-FU is frequently observed. However, using the prodrug/suicide gene strategy,

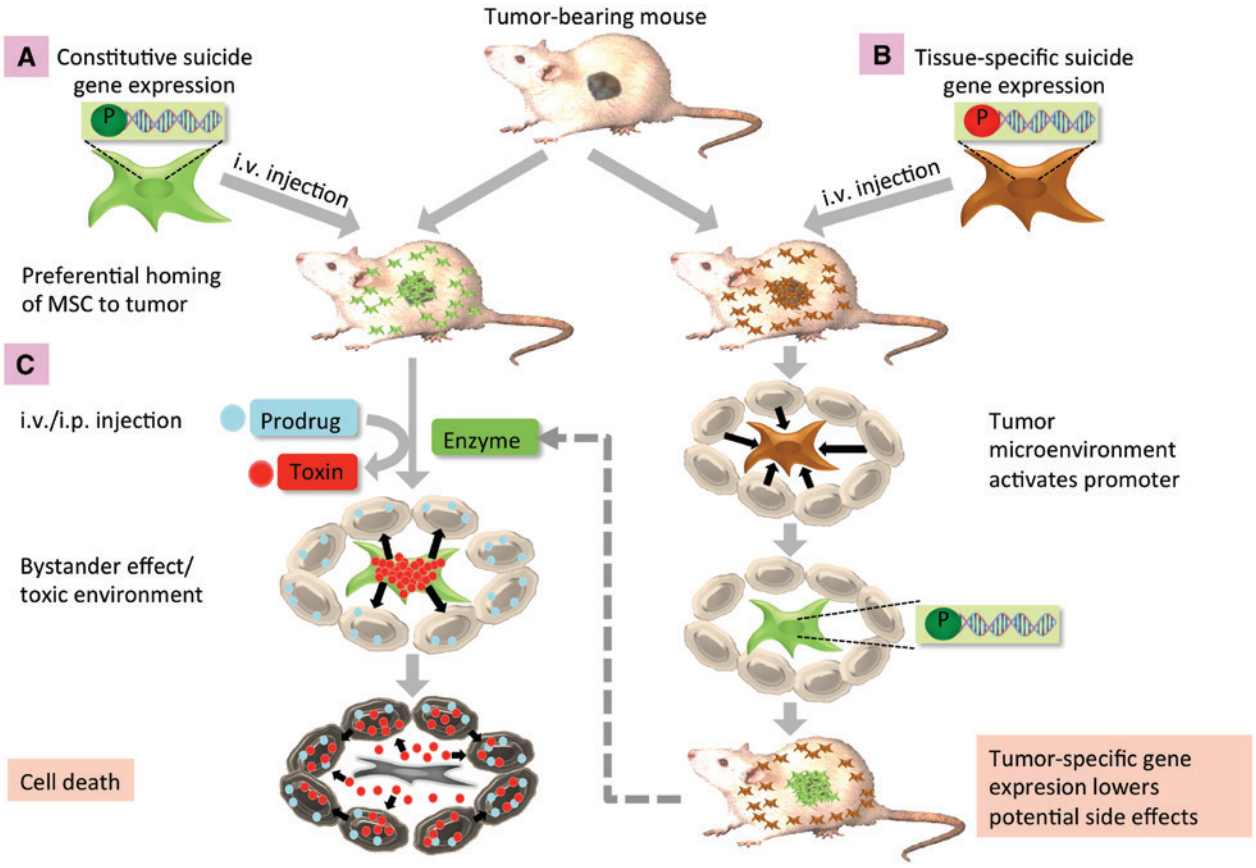

Figure 2: Constitutive vs. tumor-specific expression of suicide genes.
(A) When using a gene vector, in which suicide gene expression is driven by a constitutively activated promoter (e.g. the CMV promoter), all MSCs express this gene regardless of their surrounding environment. When systemically injected into tumor-bearing mice, the tumor tropism of MSCs will lead to the preferential homing of MSCs into the tumor in which they can be targeted by prodrug administration (C). However, not all MSCs home into the tumor. A majority of suicide gene-expressing cells will reside in healthy organs, such as the lungs and spleen, thereby potentially causing off-target toxicity. (B) In an attempt to further enhance the tumor specificity of therapy, the expression of suicide genes has been put under the control of promoters that are suspected to become activated specifically after the homing of MSCs to tumors. These promoters include the Tie-2 promoter and the CCL5/RANTES promoter. In this setting, MSCs only express the suicide gene following the respective stimuli from the tumor microenvironment and thereby MSCs that did not home into the tumor but into healthy organs do not express the suicide gene. This approach is believed to raise the amount of GDEPT enzymes within the tumor and limit off-target toxicity. (C) The mechanism of cell killing is the same for both strategies. After the systemic injection of an otherwise nontoxic prodrug, it becomes converted into the active drug only in cells that express the respective enzyme. Via the bystander effect, the toxin is distributed to the surrounding cells, which leads to subsequent cell killing.

much higher levels of 5-FU can be achieved within the tumor compared to the systemic infusion of 5-FU while off-target concentration and thus toxicity are limited [101]. Furthermore, the easy diffusion of 5-FU through cell membranes independently of gap junctions yields a greater bystander effect of the CD/5-FU system compared to other GDEPT strategies [97]. The therapeutic efficacy of MSCs transfected with CD followed by 5-FC application has been shown in animal models of colon cancer [101], osteosarcoma [102], glioma [103], and prostate cancer [104].

Tumor-specific transgene expression

The lack of tumor specificity of systemically applied conventional cancer treatment agents – which results in insufficient concentration of the agent in tumor cells and toxic effects in normal cells – is one of the major obstacles faced in cancer treatment (see Figure 2). Although all therapeutic strategies outlined above make use of the innate capability of MSCs to home to growing tumors and by this comprise a tactic to increase the tumor specificity of systemically applied therapy, this homing process

is not exclusive to tumors. After injection, MSCs can also be found in large number in otherwise healthy tissues, presumably in the context of normal tissue homeostasis [105]. Whereas in all studies mentioned above the therapeutic transgene is expressed constitutively, thus possibly still causing off-target toxicity, several groups have developed gene vectors where the therapeutic transgene is driven under the control of gene promoters that are activated after the homing of MSCs to tumors, with the aim to further increase tumor specificity. The promoters controlling suicide gene expression were selected according to the known functions of MSCs after homing to the tumor and that are outlined above [106]. Because these signaling processes occur after MSC homing and within the tumor microenvironment, they can be regarded as tumor-specific triggers for gene expression.

Tie-2, a cell surface receptor for the proangiogenic factor angiopoietin-1, is expressed mainly on endothelial cells and pericytes, in which MSCs can act as a precursor cell for both [74, 107–109]. MSCs transfected with HSV-Tk as a suicide gene, whose expression is put under the control of the Tie-2 promoter/enhancer, express this therapeutic gene after homing to pancreatic, breast, and liver cancers in respective animal models [52, 110]. The treatment of these animals with GCV injections resulted in reduced tumor growth and prolonged survival with little off-target gene expression.

Karnoub et al. previously demonstrated the strong expression of CCL5/RANTES after homing to breast cancer xenografts, a process believed to occur as part of the differentiation process of MSCs to CAFs [69]. This process requires the immediacy of MSCs to tumor cells, which in turn is a prerequisite for achieving tumor-specific transgene expression. Using the CCL5/RANTES promoter to control HSV-Tk expression in MSCs resulted in strong expression of the suicide gene in orthotopic animal models for pancreatic and liver cancers [48, 52]. The treatment with GCV significantly reduced tumor growth in both models as well as reduced peritoneal and liver metastasis in the latter model.

Yan et al. evaluated the promoter region of α-fetoprotein (AFP) as a regulator for tumor-specific sTRAIL expression by MSCs in an animal model of HCC [111]. After the injection of MSCs transfected with a gene vector, in which sTRAIL is expressed under the control of the AFP promoter, merely the hepatic tumor environment appears to elicit the activation stimuli of the AFP promoter in MSCs, as the authors were able to find sTRAIL expression only within the tumors. This therapy was potent enough to significantly inhibit tumor growth.

"Theranostic" sodium/iodide symporter (NIS) for tumor-specific MSC therapy

NIS is normally expressed on the surface of thyroid follicular cells and facilitates the transport of iodide into the cell [112]. Its physiological occurrence is highly specific for the thyroid gland and its expression is mandatory for radioiodine therapy to be effective but can also serve diagnostic purposes using ^{99m}Tc- or ^{123}I-scintigraphy/single-photon emission computed tomography (SPECT) as well as ^{124}I- or ^{18}F-TFB-PET imaging. Engineering otherwise NIS-negative cells to express NIS is thus an elegant method to both make these cells susceptible to targeted therapy and track them *in vivo* by aforementioned imaging strategies. Radioiodine therapy with ^{131}I is a well-established and very effective therapy for thyroid cancer and involves a profound bystander effect, as the path length of this β-emitter is 2.4 mm, thus also subjecting immediately surrounding tumor and stromal cells to radiation [113].

MSCs engineered for constitutive NIS expression were tracked over 2 weeks in an animal model of breast cancer using ^{99m}Tc SPECT imaging. On day 14 after injection, NIS expression within the tumor had visibly increased, whereas in nontarget tissues NIS expression had significantly decreased compared to the early phase after injection [114]. The MSC-based mediation of NIS expression within the tumors resulted in their susceptibility to radioiodine therapy, which caused a significant inhibition of tumor growth. Similar results were obtained in an animal model of HCC, where NIS-transfected MSCs triggered the proneness of HCC cells to ^{131}I therapy both *in vitro* and *in vivo* [115].

Using the same animal model, the tumor microenvironment-dependent expression of NIS was successfully achieved using the CCL5/RANTES promoter to trigger NIS expression in MSCs. After the injection of cells, the group observed a tumor-selective radionuclide accumulation in scintigraphy and high efficacy of the concomitant radioiodine therapy [116]. Furthermore, by means of the same RANTES/NIS-transfected MSC cell line, the same group was even capable of targeting liver metastases from colon cancer in a respective animal model [117]. This intriguing study (1) demonstrates that MSC-based gene therapy may be an effective method not only for primary tumors but also for metastatic disease and (2) opens up the prospect of using genetically engineered MSCs as a diagnostic tool to render micrometastasis, which are otherwise invisible to conventional imaging, visible, for example, to SPECT.

Clinical studies using MSC for cancer therapy

We initiated a phase I/II clinical study in which autologous MSCs from patients suffering from advanced, recurrent, or metastatic adenocarcinoma of the gastrointestinal or hepatopancreatobiliary system are used to deliver a GDEPT [118]. After isolation from a bone marrow aspirate from each study participant, MSCs were transfected with a gene vector that contained the CCL5/RANTES promoter to control HSV-Tk expression. Patients received three treatment cycles, with each consisting of one injection of MSCs followed by 3 days of GCV injection starting 48 h after MSC infusion. The aims of the study were to determine the safety and feasibility of the therapy in humans, to detect possible side effects, and to find the optimal dosage of cells.

Another phase I study on genetically engineered MSCs for cancer therapy is currently being prepared at the MD Anderson Cancer Center. The published study protocol (ClinicalTrials.gov number NCT02530047) foresees the use of MSCs isolated from healthy donors and genetically modified to express IFN-β. These cells are then administered intraperitoneally in patients suffering from advanced ovarian cancer. The study is scheduled to start recruiting in August 2016.

In an attempt to deliver oncolytic viruses to recurrent ovarian cancers, a research group from the Mayo Clinic has initiated a phase I/II clinical trial in which adipose tissue-derived MSCs are used as cellular vehicles for the delivery of NIS-expressing measles viruses (ClinicalTrials.gov number NCT02068794). The success rate of virus spread to the tumor can then be monitored using the NIS system in SPECT imaging.

Conclusion and perspective

MSCs are multipotent adult cells with diverse biological functions. Among other functions, MSCs contribute to tissue regeneration; thus, MSCs have become an intensely studied cell population for strategies to improve this process. However, MSCs are also encroached by growing tumors to aid in the formation of tumor stroma, thus exerting a multitude of effects on cancer growth. Our growing knowledge about the mechanisms of this phenomenon allows us to use MSCs as delivery vehicles for cancer therapeutics. Genetic engineering with tissue-dependent expression of therapeutic transgenes further increases the tumor specificity of this therapy. Although the first clinical studies have been initiated just recently, there is still a wide range of unanswered questions but also possibilities for the optimization of this therapeutic approach. The most pressing issues in this regard are the following: Which tissue source should be used for MSC isolation? Which therapeutic construct is most effective for which tumor type? Which administration route is ideal? Which strategy of genetic engineering yields the most stable transgene expression but is still safe to use in humans? Will the best results be achieved with MSCs from young, healthy donors, which may have the benefits of being more easily expandable and transfectable in culture and possibly also show more profound tumor homing and transdifferentiation, or does the problem of rejection that possibly occurs during an allogeneic MSC infusion outweigh these benefits? By which strategy can the tumor beneficial effects of exogenously applied MSCs be counteracted? How can we enhance the number of MSCs actually homing to the tumor? Furthermore, a more profound knowledge about the molecular mechanisms of endogenous MSC mobilization, homing, and differentiation and how to abrogate each one of these steps could potentially yield an effective anticancer therapy in itself.

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Author Contributions

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References

[1] Friedenstein AJ, Piatetzky S, II, Petrakova KV. Osteogenesis in transplants of bone marrow cells. J Embryol Exp Morphol 1966;16:381–390.
[2] Caplan AI. Mesenchymal stem cells. J Orthop Res 1991;9:641–650.
[3] Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. Science 1999;284:143–147.
[4] Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The

International Society for Cellular Therapy position statement. Cytotherapy 2006;8:315–317.
[5] Jiang Y, Jahagirdar BN, Reinhardt RL, et al. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 2002;418:41–49.
[6] Zuk PA, Zhu M, Mizuno H, et al. Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng 2001;7:211–228.
[7] Erices A, Conget P, Minguell JJ. Mesenchymal progenitor cells in human umbilical cord blood. Br J Haematol 2000;109:235–242.
[8] Wang HS, Hung SC, Peng ST, et al. Mesenchymal stem cells in the Wharton's jelly of the human umbilical cord. Stem Cells 2004;22:1330–1337.
[9] Sinanan AC, Hunt NP, Lewis MP. Human adult craniofacial muscle-derived cells: neural-cell adhesion-molecule (NCAM; CD56)-expressing cells appear to contain multipotential stem cells. Biotechnol Appl Biochem 2004;40:25–34.
[10] Ringe J, Leinhase I, Stich S, et al. Human mastoid periosteum-derived stem cells: promising candidates for skeletal tissue engineering. J Tissue Eng Regen Med 2008;2:136–146.
[11] da Silva Meirelles L, Chagastelles PC, Nardi NB. Mesenchymal stem cells reside in virtually all post-natal organs and tissues. J Cell Sci 2006;119:2204–2213.
[12] Ankrum JA, Ong JF, Karp JM. Mesenchymal stem cells: immune evasive, not immune privileged. Nat Biotechnol 2014;32:252–260.
[13] Crisan M, Yap S, Casteilla L, et al. A perivascular origin for mesenchymal stem cells in multiple human organs. Cell Stem Cell 2008;3:301–313.
[14] Feng J, Mantesso A, De Bari C, Nishiyama A, Sharpe PT. Dual origin of mesenchymal stem cells contributing to organ growth and repair. Proc Natl Acad Sci USA 2011;108:6503–6508.
[15] Kaukua N, Shahidi MK, Konstantinidou C, et al. Glial origin of mesenchymal stem cells in a tooth model system. Nature 2014;513:551–554.
[16] Sacchetti B, Funari A, Michienzi S, et al. Self-renewing osteoprogenitors in bone marrow sinusoids can organize a hematopoietic microenvironment. Cell 2007;131:324–336.
[17] Mendez-Ferrer S, Michurina TV, Ferraro F, et al. Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. Nature 2010;466:829–834.
[18] Uccelli A, Moretta L, Pistoia V. Immunoregulatory function of mesenchymal stem cells. Eur J Immunol 2006;36:2566–2573.
[19] Seppanen E, Roy E, Ellis R, Bou-Gharios G, Fisk NM, Khosrotehrani K. Distant mesenchymal progenitors contribute to skin wound healing and produce collagen: evidence from a murine fetal microchimerism model. PLoS One 2013;8:e62662.
[20] Kidd S, Spaeth E, Dembinski JL, et al. Direct evidence of mesenchymal stem cell tropism for tumor and wounding microenvironments using *in vivo* bioluminescent imaging. Stem Cells 2009;27:2614–2623.
[21] Rastegar F, Shenaq D, Huang J, et al. Mesenchymal stem cells: molecular characteristics and clinical applications. World J Stem Cells 2010;2:67–80.
[22] Yagi H, Soto-Gutierrez A, Parekkadan B, et al. Mesenchymal stem cells: mechanisms of immunomodulation and homing. Cell Transplant 2010;19:667–679.
[23] Zangi L, Margalit R, Reich-Zeliger S, et al. Direct imaging of immune rejection and memory induction by allogeneic mesenchymal stromal cells. Stem Cells 2009;27:2865–2874.
[24] Le Blanc K, Tammik C, Rosendahl K, Zetterberg E, Ringden O. HLA expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells. Exp Hematol 2003;31:890–896.
[25] von Bahr L, Batsis I, Moll G, et al. Analysis of tissues following mesenchymal stromal cell therapy in humans indicates limited long-term engraftment and no ectopic tissue formation. Stem Cells 2012;30:1575–1578.
[26] Di Nicola M, Carlo-Stella C, Magni M, et al. Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. Blood 2002;99:3838–3843.
[27] Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood 2005;105:1815–1822.
[28] Augello A, Tasso R, Negrini SM, et al. Bone marrow mesenchymal progenitor cells inhibit lymphocyte proliferation by activation of the programmed death 1 pathway. Eur J Immunol 2005;35:1482–1490.
[29] Gu YZ, Xue Q, Chen YJ, et al. Different roles of PD-L1 and FasL in immunomodulation mediated by human placenta-derived mesenchymal stem cells. Hum Immunol 2013;74:267–276.
[30] Krampera M, Cosmi L, Angeli R, et al. Role for interferon-gamma in the immunomodulatory activity of human bone marrow mesenchymal stem cells. Stem Cells 2006;24:386–398.
[31] Spaggiari GM, Capobianco A, Abdelrazik H, Becchetti F, Mingari MC, Moretta L. Mesenchymal stem cells inhibit natural killer-cell proliferation, cytotoxicity, and cytokine production: role of indoleamine 2,3-dioxygenase and prostaglandin E2. Blood 2008;111:1327–1333.
[32] Glennie S, Soeiro I, Dyson PJ, Lam EW, Dazzi F. Bone marrow mesenchymal stem cells induce division arrest anergy of activated T cells. Blood 2005;105:2821–2827.
[33] Engela AU, Hoogduijn MJ, Boer K, et al. Human adipose-tissue derived mesenchymal stem cells induce functional de-novo regulatory T cells with methylated FOXP3 gene DNA. Clin Exp Immunol 2013;173:343–354.
[34] Maggini J, Mirkin G, Bognanni I, et al. Mouse bone marrow-derived mesenchymal stromal cells turn activated macrophages into a regulatory-like profile. PLoS ONE 2010;5:e9252.
[35] Bartholomew A, Sturgeon C, Siatskas M, et al. Mesenchymal stem cells suppress lymphocyte proliferation *in vitro* and prolong skin graft survival *in vivo*. Exp Hematol 2002;30:42–48.
[36] Le Blanc K, Rasmusson I, Sundberg B, et al. Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. Lancet 2004;363:1439–1441.
[37] Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000;100:57–70.
[38] Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144:646–674.
[39] Hanahan D, Coussens LM. Accessories to the crime: functions of cells recruited to the tumor microenvironment. Cancer Cell 2012;21:309–322.
[40] Tlsty TD, Coussens LM. Tumor stroma and regulation of cancer development. Annu Rev Pathol 2006;1:119–150.
[41] Folkman J. Tumor angiogenesis. Adv Cancer Res 1974;19:331–358.
[42] Bissell MJ, Hall HG, Parry G. How does the extracellular matrix direct gene expression? J Theor Biol 1982;99:31–68.

[43] Rhim AD, Oberstein PE, Thomas DH, et al. Stromal elements act to restrain, rather than support, pancreatic ductal adenocarcinoma. Cancer Cell 2014;25:735–747.
[44] Ozdemir BC, Pentcheva-Hoang T, Carstens JL, et al. Depletion of carcinoma-associated fibroblasts and fibrosis induces immunosuppression and accelerates pancreas cancer with reduced survival. Cancer Cell 2014;25:719–734.
[45] Dvorak HF. Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. N Engl J Med 1986;315:1650–1659.
[46] Spaeth E, Klopp A, Dembinski J, Andreeff M, Marini F. Inflammation and tumor microenvironments: defining the migratory itinerary of mesenchymal stem cells. Gene Ther 2008;15:730–738.
[47] Studeny M, Marini FC, Champlin RE, Zompetta C, Fidler IJ, Andreeff M. Bone marrow-derived mesenchymal stem cells as vehicles for interferon-β delivery into tumors. Cancer Res 2002;62:3603–3608.
[48] Zischek C, Niess H, Ischenko I, et al. Targeting tumor stroma using engineered mesenchymal stem cells reduces the growth of pancreatic carcinoma. Ann Surg 2009;250:747–753.
[49] Komarova S, Kawakami Y, Stoff-Khalili MA, Curiel DT, Pereboeva L. Mesenchymal progenitor cells as cellular vehicles for delivery of oncolytic adenoviruses. Mol Cancer Ther 2006;5:755–766.
[50] Jung Y, Kim JK, Shiozawa Y, et al. Recruitment of mesenchymal stem cells into prostate tumours promotes metastasis. Nat Commun 2013;4:1795.
[51] Quante M, Tu SP, Tomita H, et al. Bone marrow-derived myofibroblasts contribute to the mesenchymal stem cell niche and promote tumor growth. Cancer Cell 2011;19:257–272.
[52] Niess H, Bao Q, Conrad C, et al. Selective targeting of genetically engineered mesenchymal stem cells to tumor stroma microenvironments using tissue-specific suicide gene expression suppresses growth of hepatocellular carcinoma. Ann Surg 2011;254:767–774; discussion 74–75.
[53] Nakamizo A, Marini F, Amano T, et al. Human bone marrow-derived mesenchymal stem cells in the treatment of gliomas. Cancer Res 2005;65:3307–3318.
[54] Hong HS, Lee J, Lee E, et al. A new role of substance P as an injury-inducible messenger for mobilization of CD29(+) stromal-like cells. Nat Med 2009;15:425–435.
[55] Segers VF, Van Riet I, Andries LJ, et al. Mesenchymal stem cell adhesion to cardiac microvascular endothelium: activators and mechanisms. Am J Physiol Heart Circ Physiol 2006;290:H1370–H1377.
[56] Ceradini DJ, Kulkarni AR, Callaghan MJ, et al. Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. Nat Med 2004;10:858–864.
[57] Muller A, Homey B, Soto H, et al. Involvement of chemokine receptors in breast cancer metastasis. Nature 2001;410:50–56.
[58] Peled A, Petit I, Kollet O, et al. Dependence of human stem cell engraftment and repopulation of NOD/SCID mice on CXCR4. Science 1999;283:845–848.
[59] Kucia M, Reca R, Miekus K, et al. Trafficking of normal stem cells and metastasis of cancer stem cells involve similar mechanisms: pivotal role of the SDF-1-CXCR4 axis. Stem Cells 2005;23:879–894.
[60] Lourenco S, Teixeira VH, Kalber T, Jose RJ, Floto RA, Janes SM. Macrophage migration inhibitory factor-CXCR4 is the dominant chemotactic axis in human mesenchymal stem cell recruitment to tumors. J Immunol 2015;194:3463–3474.
[61] Wynn RF, Hart CA, Corradi-Perini C, et al. 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.
[62] Khakoo AY, Pati S, Anderson SA, et al. Human mesenchymal stem cells exert potent antitumorigenic effects in a model of Kaposi's sarcoma. J Exp Med 2006;203:1235–1247.
[63] Cuiffo BG, Karnoub AE. Mesenchymal stem cells in tumor development: emerging roles and concepts. Cell Adhes Migr 2012;6:220–230.
[64] Rhodes LV, Antoon JW, Muir SE, Elliott S, Beckman BS, Burow ME. Effects of human mesenchymal stem cells on ER-positive human breast carcinoma cells mediated through ER-SDF-1/CXCR4 crosstalk. Mol Cancer 2010;9:295.
[65] Halpern JL, Kilbarger A, Lynch CC. Mesenchymal stem cells promote mammary cancer cell migration *in vitro* via the CXCR2 receptor. Cancer Lett 2011;308:91–99.
[66] Tsai KS, Yang SH, Lei YP, et al. Mesenchymal stem cells promote formation of colorectal tumors in mice. Gastroenterology 2011;141:1046–1056.
[67] Liu S, Ginestier C, Ou SJ, et al. Breast cancer stem cells are regulated by mesenchymal stem cells through cytokine networks. Cancer Res 2011;71:614–624.
[68] Yan XL, Fu CJ, Chen L, et al. Mesenchymal stem cells from primary breast cancer tissue promote cancer proliferation and enhance mammosphere formation partially via EGF/EGFR/Akt pathway. Breast Cancer Res Treat 2012;132:153–164.
[69] Karnoub AE, Dash AB, Vo AP, et al. Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. Nature 2007;449:557–563.
[70] McLean K, Gong Y, Choi Y, et al. Human ovarian carcinoma-associated mesenchymal stem cells regulate cancer stem cells and tumorigenesis via altered BMP production. J Clin Invest 2011;121:3206–3219.
[71] Roodhart JM, Daenen LG, Stigter EC, et al. Mesenchymal stem cells induce resistance to chemotherapy through the release of platinum-induced fatty acids. Cancer Cell 2011;20:370–383.
[72] Koike N, Fukumura D, Gralla O, Au P, Schechner JS, Jain RK. Tissue engineering: creation of long-lasting blood vessels. Nature 2004;428:138–139.
[73] Au P, Tam J, Fukumura D, Jain RK. Bone marrow-derived mesenchymal stem cells facilitate engineering of long-lasting functional vasculature. Blood 2008;111:4551–4558.
[74] Beckermann BM, Kallifatidis G, Groth A, et al. VEGF expression by mesenchymal stem cells contributes to angiogenesis in pancreatic carcinoma. Br J Cancer 2008;99:622–631.
[75] Chen L, Tredget EE, Wu PY, Wu Y. Paracrine factors of mesenchymal stem cells recruit macrophages and endothelial lineage cells and enhance wound healing. PLoS ONE 2008;3:e1886.
[76] Mishra PJ, Mishra PJ, Humeniuk R, et al. Carcinoma-associated fibroblast-like differentiation of human mesenchymal stem cells. Cancer Res 2008; 8:4331–4339.
[77] Cho JA, Park H, Lim EH, Lee KW. Exosomes from breast cancer cells can convert adipose tissue-derived mesenchymal stem cells into myofibroblast-like cells. Int J Oncol 2012;40:130–138.
[78] Spaeth EL, Dembinski JL, Sasser AK, et al. Mesenchymal stem cell transition to tumor-associated fibroblasts contributes to

fibrovascular network expansion and tumor progression. PLoS One 2009;4:e4992.
[79] Droujinine IA, Eckert MA, Zhao W. To grab the stroma by the horns: from biology to cancer therapy with mesenchymal stem cells. Oncotarget 2013;4:651–664.
[80] Chen X, Lin X, Zhao J, et al. A tumor-selective biotherapy with prolonged impact on established metastases based on cytokine gene-engineered MSCs. Mol Ther 2008;16:749–756.
[81] Nakamura K, Ito Y, Kawano Y, et al. Antitumor effect of genetically engineered mesenchymal stem cells in a rat glioma model. Gene Ther 2004;11:1155–1164.
[82] Hong X, Miller C, Savant-Bhonsale S, Kalkanis SN. Antitumor treatment using interleukin-12-secreting marrow stromal cells in an invasive glioma model. Neurosurgery 2009;64:1139–1146; discussion 46–47.
[83] Gao P, Ding Q, Wu Z, Jiang H, Fang Z. Therapeutic potential of human mesenchymal stem cells producing IL-12 in a mouse xenograft model of renal cell carcinoma. Cancer Lett 2010;290:157–166.
[84] Xin H, Kanehira M, Mizuguchi H, et al. Targeted delivery of CX3CL1 to multiple lung tumors by mesenchymal stem cells. Stem Cells 2007;25:1618–1626.
[85] Borsig L, Wolf MJ, Roblek M, Lorentzen A, Heikenwalder M. Inflammatory chemokines and metastasis – tracing the accessory. Oncogene 2014;33:3217–3224.
[86] LeBlanc HN, Ashkenazi A. Apo2L/TRAIL and its death and decoy receptors. Cell Death Differ 2003;10:66–75.
[87] Ashkenazi A, Holland P, Eckhardt SG. Ligand-based targeting of apoptosis in cancer: the potential of recombinant human apoptosis ligand 2/tumor necrosis factor-related apoptosis-inducing ligand (rhApo2L/TRAIL). J Clin Oncol 2008;26:3621–3630.
[88] Trarbach T, Moehler M, Heinemann V, et al. Phase II trial of mapatumumab, a fully human agonistic monoclonal antibody that targets and activates the tumour necrosis factor apoptosis-inducing ligand receptor-1 (TRAIL-R1), in patients with refractory colorectal cancer. Br J Cancer 2010;102:506–512.
[89] Ashkenazi A, Pai RC, Fong S, et al. Safety and antitumor activity of recombinant soluble Apo2 ligand. J Clin Invest 1999;104:155–162.
[90] Mueller LP, Luetzkendorf J, Widder M, Nerger K, Caysa H, Mueller T. TRAIL-transduced multipotent mesenchymal stromal cells (TRAIL-MSC) overcome TRAIL resistance in selected CRC cell lines *in vitro* and *in vivo*. Cancer Gene Ther 2011;18:229–239.
[91] Mohr A, Albarenque SM, Deedigan L, et al. Targeting of XIAP combined with systemic mesenchymal stem cell-mediated delivery of sTRAIL ligand inhibits metastatic growth of pancreatic carcinoma cells. Stem Cells 2010;28:2109–2120.
[92] Deng Q, Zhang Z, Feng X, et al. TRAIL-secreting mesenchymal stem cells promote apoptosis in heat-shock-treated liver cancer cells and inhibit tumor growth in nude mice. Gene Ther 2014;21:317–327.
[93] Lathrop MJ, Sage EK, Macura SL, et al. Antitumor effects of TRAIL-expressing mesenchymal stromal cells in a mouse xenograft model of human mesothelioma. Cancer Gene Ther 2015;22:44–54.
[94] Zhu Y, Cheng M, Yang Z, et al. Mesenchymal stem cell-based NK4 gene therapy in nude mice bearing gastric cancer xenografts. Drug Des Dev Ther 2014;8:2449–2462.
[95] Martinez-Quintanilla J, Bhere D, Heidari P, He D, Mahmood U, Shah K. Therapeutic efficacy and fate of bimodal engineered stem cells in malignant brain tumors. Stem Cells 2013;31:1706–1714.
[96] Mesnil M, Yamasaki H. Bystander effect in herpes simplex virus-thymidine kinase/ganciclovir cancer gene therapy: role of gap-junctional intercellular communication. Cancer Res 2000;60:3989–3999.
[97] Karjoo Z, Chen X, Hatefi A. Progress and problems with the use of suicide genes for targeted cancer therapy. Adv Drug Deliv Rev 2016;99:113–128.
[98] Freeman SM, Abboud CN, Whartenby KA, et al. The "bystander effect": tumor regression when a fraction of the tumor mass is genetically modified. Cancer Res 1993;53:5274–5283.
[99] Miletic H, Fischer Y, Litwak S, et al. Bystander killing of malignant glioma by bone marrow-derived tumor-infiltrating progenitor cells expressing a suicide gene. Mol Ther 2007;15:1373–1381.
[100] Kim SW, Kim SJ, Park SH, et al. Complete regression of metastatic renal cell carcinoma by multiple injections of engineered mesenchymal stem cells expressing dodecameric TRAIL and HSV-TK. Clin Cancer Res 2013;19:415–427.
[101] Kucerova L, Altanerova V, Matuskova M, Tyciakova S, Altaner C. Adipose tissue-derived human mesenchymal stem cells mediated prodrug cancer gene therapy. Cancer Res 2007;67:6304–6313.
[102] NguyenThai QA, Sharma N, Luong do H, et al. Targeted inhibition of osteosarcoma tumor growth by bone marrow-derived mesenchymal stem cells expressing cytosine deaminase/5-fluorocytosine in tumor-bearing mice. J Gene Med 2015;17:87–99.
[103] Kosaka H, Ichikawa T, Kurozumi K, et al. Therapeutic effect of suicide gene-transferred mesenchymal stem cells in a rat model of glioma. Cancer Gene Ther 2012;19;572–578.
[104] Abrate A, Buono R, Canu T, et al. Mesenchymal stem cells expressing therapeutic genes induce autochthonous prostate tumour regression. Eur J Cancer 2014;50:2478–2488.
[105] Von Luttichau I, Notohamiprodjo M, Wechselberger A, et al. 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.
[106] Bao Q, Zhao Y, Niess H, et al. Mesenchymal stem cell-based tumor-targeted gene therapy in gastrointestinal cancer. Stem Cells Dev 2012;21:2355–2363.
[107] Conrad C, Niess H, Huss R, et al. Multipotent mesenchymal stem cells acquire a lymphendothelial phenotype and enhance lymphatic regeneration *in vivo*. Circulation 2009;119:281–289.
[108] Wang HH, Cui YL, Zaorsky NG, et al. Mesenchymal stem cells generate pericytes to promote tumor recurrence via vasculogenesis after stereotactic body radiation therapy. Cancer Lett 2016;375:349–359.
[109] Bagley RG, Weber W, Rouleau C, et al. Human mesenchymal stem cells from bone marrow express tumor endothelial and stromal markers. Int J Oncol 2009;34:619–627.
[110] Conrad C, Husemann Y, Niess H, et al. Linking transgene expression of engineered mesenchymal stem cells and angiopoietin-1-induced differentiation to target cancer angiogenesis. Ann Surg 2011;253:566–571.

[111] Yan C, Yang M, Li Z, et al. Suppression of orthotopically implanted hepatocarcinoma in mice by umbilical cord-derived mesenchymal stem cells with sTRAIL gene expression driven by AFP promoter. Biomaterials 2014;35:3035–3043.
[112] Spitzweg C, Morris JC. The sodium iodide symporter: its pathophysiological and therapeutic implications. Clin Endocrinol (Oxf) 2002;57:559–574.
[113] Hingorani M, Spitzweg C, Vassaux G, et al. The biology of the sodium iodide symporter and its potential for targeted gene delivery. Curr Cancer Drug Targets 2010; 10:242–267.
[114] Dwyer RM, Ryan J, Havelin RJ, et al. Mesenchymal Stem Cell-mediated delivery of the sodium iodide symporter supports radionuclide imaging and treatment of breast cancer. Stem Cells 2011;29:1149–1157.
[115] Knoop K, Kolokythas M, Klutz K, et al. Image-guided, tumor stroma-targeted 131I therapy of hepatocellular cancer after systemic mesenchymal stem cell-mediated NIS gene delivery. Mol Ther 2011;19:1704–1713.
[116] Knoop K, Schwenk N, Dolp P, et al. Stromal targeting of sodium iodide symporter using mesenchymal stem cells allows enhanced imaging and therapy of hepatocellular carcinoma. Hum Gene Ther 2013;24:306–316.
[117] Knoop K, Schwenk N, Schmohl K, et al. Mesenchymal stem cell-mediated, tumor stroma-targeted radioiodine therapy of metastatic colon cancer using the sodium iodide symporter as theranostic gene. J Nucl Med 2015;56:600–606.
[118] Niess H, von Einem JC, Thomas MN, et al. Treatment of advanced gastrointestinal tumors with genetically modified autologous mesenchymal stromal cells (TREAT-ME1): study protocol of a phase I/II clinical trial. BMC Cancer 2015;15:237.

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