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Concise Review

Stem Cell-Induced Cell Motility: A Removable Obstacle on the Way to Safe Therapies?

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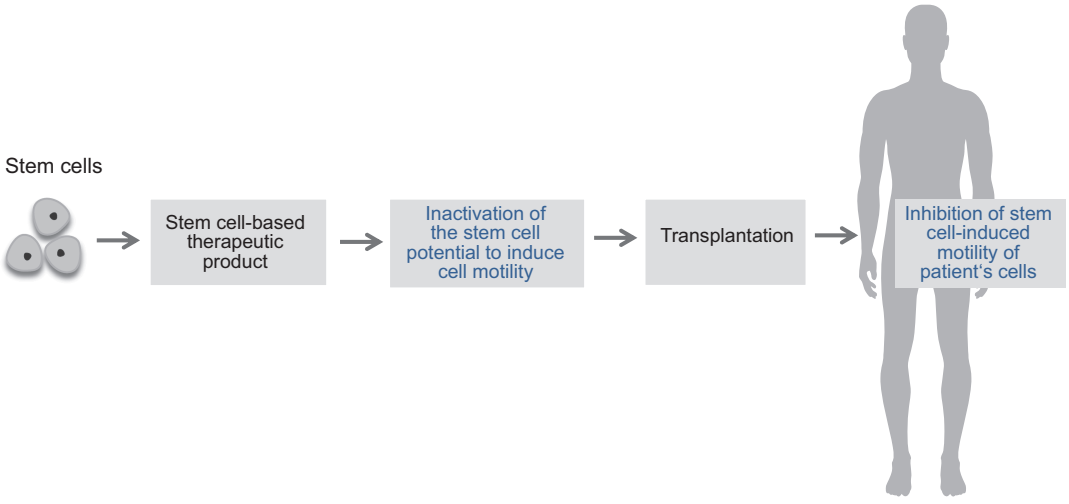
Abstract

It is the hope of clinicians and patients alike that stem cell-based therapeutic products will increasingly become applicable remedies for many diseases and injuries. Whereas some multipotent stem cells are already routinely used in regenerative medicine, the efficacious and safe clinical translation of pluripotent stem cells is still hampered by their inherent immunogenicity and tumorigenicity. In addition, stem cells harbor the paracrine potential to affect the behavior of cells in their microenvironment. On the one hand, this property can mediate advantageous supportive effects on the overall therapeutic concept. However, in the last years, it became evident that both, multipotent and pluripotent stem cells, are capable of inducing adjacent cells to become motile. Not only in the context of tumor development but generally, deregulated mobilization and uncontrolled navigation of patient's cells can have deleterious consequences for the therapeutic outcome. A more comprehensive understanding of this ubiquitous stem cell feature could allow its proper clinical handling and could thereby constitute an important building block for the further development of safe therapies.

Key words: invasion; motility; multipotent stem cells; pluripotent stem cells; safety; stem cell-based therapy.

Graphical Abstract

The stem cell-mediated, uncontrolled mobilization of adjacent cells represents a concern regarding the therapeutic application of multipotent and pluripotent stem cells. Both, the processing of the therapeutic product to deactivate this property and the interference with the recipient's cells to minimize their responsiveness could be envisaged to counterbalance unwanted side effects, enhancing the efficacy and safety of stem cell-based therapies.



Significance Statement

Multipotent stem cells as well as pluripotent hESCs and hiPSCs share the potential to mobilize adjacent cells in their microenvironment. This recently explored ubiquitous stem cell property is an obvious concern with regard to the efficacy and safety of stem cell-based therapies. This article discusses the respective literature and highlights the importance to establish guidance for its management. The putative benefit of prior-to-usage characterization of stem cell-based products and of developable biochemical interventions in the course of transplantation is discussed.

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Introduction

Stem cells are defined by their capacity for self-renewal and their specific differentiation potential. Multipotent stem cells, including human hematopoietic stem cells (hHSCs) and mesenchymal stem cells (hMSCs), can give rise to a limited spectrum of lineage-specific cell types, whereas pluripotent stem cells, such as human embryonic stem cells (hESCs) and human-induced pluripotent stem cells (hiPSCs), can differentiate into cell types of all three embryonic germ layers.[1] Regarding their biological features, fetal stem cells derived from the fetus proper or from fetal extra-embryonic tissues are discussed to represent intermediates between adult and pluripotent stem cells.[2] For example, human amniotic fluid stem cells (hAFSCs) are not tumorigenic but harbor the potential to give rise to derivatives of all embryonic germ layers.[3,4]

hHSCs represent the most commonly used stem cell entity in the clinic worldwide. Whereas hHSCs for the treatment of hematological cancers entered the clinical arena already decades ago, hMSCs-based therapeutic approaches are still waiting for their first approval by the FDA. However, such approvals are foreseeable taking into account that many hundreds of hMSC-based clinical trials are currently already listed on ClinicalTrials.gov. And in the last years, the first clinical trials using pluripotent stem cells to treat, for example, macular degeneration, myocardial infarction, spinal cord injuries, or type 1 diabetes mellitus, were inaugurated.[1] In this context, it is important to note that their inherent immunogenicity and tumorigenic potential are still considered major hurdles for the translation of hESCs and hiPSCs to the bedside. This is of particular relevance when residual pluripotent stem cells are present in the final therapeutic product transplanted into the patient.[5,6] Nevertheless, stem cells already triggered a paradigm shift in regenerative medicine and will certainly pave the way to the establishment of new therapeutic concepts for many of humanities most life-threatening diseases. This assumption gains additional support by the recent developments regarding next-generation stem cell approaches, which tremendously expand their therapeutic utility. Apart from the fact that stem cells or stem cell derivatives can function as therapeutic products themselves, they will also more and more serve as gene therapy mediators (of the effects of transduced exogenous genes or of gene editing approaches) and as drug-delivery vehicles (eg, of prodrug-converting enzymes, oncolytic viruses or promoters of apoptosis).[1]

The physiological equilibrium of the human body essentially depends on the capacity of destined cells to migrate and to cross basement membranes, to spread within tissues, and to enter and exit the vasculature to infiltrate distant tissues and organs. Cell migration and invasion (the latter is also referred to as 3D migration) are of crucial relevance during development and for tissue homeostasis, wound healing, and organ repair. On the other hand, deregulated mobilization of cells from their physiological site of operation as well as their uncontrolled motility is not only a hallmark of cancer development but also plays an important role in the genesis of many other human diseases.[7-9] Accordingly, the recently elucidated feature of stem cells to trigger mobilization of cells in their microenvironment raises serious concerns in the context of all therapeutic applications of stem cell-based products. In this article, we want to highlight the importance of reinforcing research endeavors to obtain a better understanding of the underlying molecular mechanisms of stem cell-induced mobilization processes. A so obtained more comprehensive picture of this phenomenon will finally allow to establish guidance for its management in the context of stem cell-based therapies.

Stem Cells Promote Mobilization of Adjacent Cells

Multipotent Stem Cell-Promoted Motility

Recently, mounting research attempts demonstrated that stem cells harbor the paracrine potential to induce migration and invasion of adjacent cells in their local environment herein referred to as target cells. In this review, we summarize and discuss the respective literature to finally suggest strategies to handle this phenomenon in the course of stem cell-based therapies. In Table 1 as well as in the text below, we organized this presentation according to the different multipotent (this section) and pluripotent (next section) stem cell entities harboring this potential. Upon reasonable grounds, stem cells derived from cancer tissues or cancer stem cells are in no way considered for the development of therapeutic products and we have therefore excluded them from the here presented synopsis. In addition, in Table 1, we present the different non-tumorigenic and tumorigenic target cells and the experimental approaches that have been used to prove the mobilizing stem cell potential in the cited studies.

Human and rat bone marrow-derived mesenchymal stem cells (BM-MSCs) were reported to promote the invasion of colorectal cancer cells, colorectal cancer stem cells, and glioblastoma cells.[10-12] Furthermore, hBM-MSCs induce migration of ovarian cancer cells[13] and also promote invasion of hepatocellular carcinoma cells in vitro as well as their in vivo metastatic potential,[14-16] for which the induction of lymphovascular invasion is an obligatory prerequisite.[7-9] Wound healing migration assays, transwell invasion assays, and in vivo co-injection and xenograft experiments in rodents revealed that BM-MSCs increase in vitro motility and in vivo metastasis of osteosarcoma cells.[15,17-21] In these studies, the wound healing assay has been used as a well scalable and reproducible experimental approach to study cell motility. However, it is important to note that in vivo the closure of wounds is the result of complex movements of many different cell types. Wound healing is known to depend on the motility of, for example, fibroblasts and immune cells in the granulation tissue.[9] Accordingly, we believe it to be very interesting to further investigate a putative role of cell-induced mobilization of target cells in wound healing processes.

Without doubt, a pioneering study on MSC-induced cell motility was published by Karnoub et al in the year 2007. Xenograft studies revealed that hBM-MSCs greatly induced the metastatic potency of otherwise weakly metastatic human breast carcinoma cells when these two cell types were injected as a cell mixture to form a tumor. This stem cell-mediated paracrine induction of cancer cell motility and metastasis was found to be reversible and depend on chemokine signaling.[22] Until now it has been shown that hBM-MSCs induce breast cancer, follicular lymphoma, colon cancer, oral tongue squamous cell carcinoma, nasopharyngeal carcinoma, and lung cancer cell motility.[22-37] Importantly, mouse and human BM-MSCs have also been demonstrated to induce the motility of non-transformed endothelial progenitor cells in vitro and in vivo.[38-40] In this context, we assume it to be very interesting

Table 1. The potential of stem cells to induce motility of adjacent cells.

Stem cells	Target cells	Proof	References
Multipotent			
hBM-MSCs	Human colorectal cancer cells	CIA	10
rBM-MSCs	Human colorectal cancer stem cells	TMA, TIA	11
rBM-MSCs	Rat glioblastoma cells	WHMA, TIA	12
hBM-MSCs	Human ovarian cancer cells	TMA	13
hBM-MSCs	Human hepatocellular carcinoma cells	WHMA, TIA, in vivo	14
hBM-MSCs	Human hepatocellular carcinoma cells	WHMA, TIA	15
hBM-MSCs	Human hepatocellular carcinoma cells	WHMA, TMA, TIA, in vivo	16
hBM-MSCs	Human osteosarcoma cells	TIA, in vivo	17
hBM-MSCs	Human osteosarcoma cells	TIA, in vivo	18
hBM-MSCs	Human osteosarcoma cells	TIA	19
hBM-MSCs	Human osteosarcoma cells	WHMA	20
SD-hBM-MSCs	Human osteosarcoma cells	WHMA	20
rBM-MSCs	Rat osteosarcoma cells	In vivo	21
hBM-MSCs	Human osteosarcoma cells	WHMA, TIA	15
hBM-MSCs	Human breast cancer cells	In vivo	22
hBM-MSCs	Human breast cancer cells	TMA	23
hBM-MSCs	Human breast cancer cells	TMA	24
hBM-MSCs	Human breast cancer cells	In vivo	25
mBM-MSCs	Human breast cancer cells	In vivo	25
mBM-MSCs	Mouse breast cancer cells	TMA	26
hBM-MSCs	Human breast cancer cells	TMA	27
hBM-MSCs	Human breast cancer cells	In vivo	28
hBM-MSCs	Human breast cancer cells	CIA	29
hBM-MSCs	Human breast cancer cells	TIA	30
hBM-MSCs	Human follicular lymphoma cells	TMA	31
hBM-MSCs	Human colon cancer cells	In vivo	32
hBM-MSCs	Human oral tongue squamous cell carcinoma	MOIM	33
hBM-MSCs	Human nasopharyngeal carcinoma cells	TMA	34
hBM-MSCs	Human primary lung cancer cells	In vivo	35
hBM-MSCs	Human lung adenocarcinoma cells	TMA, TIA	36
hBM-MSC	Human non-small cell lung cancer cells	TIA	37
mBM-MSCs	Mouse endothelial progenitor cells	In vivo	38
hBM-MSCs	Human endothelial progenitor cells	TIA	39
hBM-MSCs	Human and mouse endothelial progenitor cells	TMA, in vivo	40
hADSCs	Human endothelial cells	WHMA, TIA	41
hADSCs	Human breast cancer cells	TIA	42
hADSCs	Human breast cancer cells	TMA, TIA	43
hADSCs	Human cervical cancer cells	TIA	44
hBM-SSCs	Human prostate cancer cells	In vivo	45
mBM-HPCs	Mouse lung carcinoma and melanoma cells	In vivo	46
hUC-HPCs	Human melanoma cells	In vivo	47
hUC-HPCs	Human colorectal cancer cells	TIA, in vivo	48
hUC-HPCs	Human breast cancer cells	TIA	49
hAFSCs	Human primary lung fibroblast	TIA	50
hAFSCs	Human primary skin fibroblast	TIA	50
hAFSCs	Human primary cardiac fibroblasts	TIA	50
hAFSCs	Human primary chondrocytes	TIA	50
hAFSCs	Human mammary epithelial cells	TIA	50
hAFSCs	Human primary hepatocytes	TIA	50
hAF-MSCs	Human endothelial cells	WHMA, TMA	51
hUC-MSCs	Human breast cancer cells	WHMA, TMA	52
hUC-MSCs	Human breast cancer cells	WHMA, TMA, TIA	53
hUC-MSCs	Human hepatocellular carcinoma cells	TIA, in vivo	54
hUC-MSCs	Human lung cancer cells	WHMA, TIA	55
hUC-MSCs	Human trophoblasts	TMA, TIA	56
hPMSCs	Human trophoblasts	TMA	57
hP-MSCs	Human primary trophoblasts	TIA	58
hP-MSCs	Human endothelial progenitor cells	TMA	39

Table 1. Continued

Stem cells	Target cells	Proof	References
Pluripotent			
hESCs	Human primary lung fibroblast	TIA	50
hESCs	Human primary cardiac fibroblasts	TIA	50
hESCs	Human primary chondrocytes	TIA	50
hESCs	Human mammary epithelial cells	TIA	50
hESCs	Human primary hepatocytes	TIA	50
hESCs	Mouse cells adjacent to teratoma	In vivo	50
hiPSCs	Human primary lung fibroblast	TIA	50
hiPSCs	Human primary cardiac fibroblasts	TIA	50
hiPSCs	Human primary chondrocytes	TIA	50
hiPSCs	Human mammary epithelial cells	TIA	50
hiPSCs	Human primary hepatocytes	TIA	50

Abbreviations: hBM-MSCs, human bone marrow-derived mesenchymal stem cells; rBM-MSCs, rat bone marrow-derived mesenchymal stem cells; SD-hBM-MSCs, serum deprived human bone marrow-derived mesenchymal stem cells; mBM-MSCs, mouse bone marrow-derived mesenchymal stem cells; hADSCs, human adipose-derived stem cells; hBM-SSCs, human bone marrow-derived skeletal stem cells; mBM-HPCs, mouse bone marrow-derived hematopoietic progenitor cells; hUC-HPCs, human umbilical cord blood-derived hematopoietic progenitor cells; hAFSCs, human amniotic fluid stem cells; hAF-MSCs, human amniotic fluid-derived mesenchymal stem cells; hUC-MSCs, human umbilical cord blood-derived mesenchymal stem cells; hPMSCs, human placental multipotent mesenchymal stromal cells; hP-MSCs, human placenta-derived mesenchymal stem cells; hESCs, human embryonic stem cells; hiPSCs, human induced pluripotent stem cells; CIA, collagen invasion assay; TMA, transwell migration assay; TIA, transwell invasion assay; WHMA, wound healing migration assay; MOIM, myoma organotypic invasion model.

that the same stem cell type can induce the motility of both transformed and non-transformed target cells. As we discuss in the section "Routes to Translation", a more comprehensive picture of the underlying molecular mechanisms of stem cell-induced mobilization processes can build the basis for the establishment of biochemical strategies to block this stem cell potential in the course of clinical applications. With regard to that, it is of the highest relevance to investigate whether one stem cell entity makes use of different motility-inducing mechanisms in different target cells.

Human adipose-derived stem cells (hADSCs) also represent a stem cell entity exhibiting the capacity to induce invasion of both, tumorigenic and non-tumorigenic target cells. hADSCs have been reported to efficiently promote the invasion of human endothelial cells[41] and of breast cancer and cervical cancer cells.[42-44] And finally, human bone marrow-derived skeletal stem cells (hBM-SSCs) were demonstrated to control the motility of human prostate cancer cells in an in vivo mouse metastasis model.[45]

In our opinion, the fact that apart from MSCs the totally different entity of hematopoietic progenitor cells (HPCs) also harbor a motility-inducing potential, strongly suggests this feature to be common to multipotent stem cells. In this context, a groundbreaking report was published by Kaplan et al in the year 2005. The authors demonstrated that vascular endothelial growth factor receptor 1 (VEGFR1)-positive bone marrow-derived hematopoietic progenitor cells (BM-HPCs) can home to pre-metastatic tumor sites to form cellular clusters. These BM-HPCs clusters have been found to form a pre-metastatic niche and to mobilize and attract tumor cells. Strikingly, removement of these VEGRF1-positive progenitor cells from the bone marrow blocked the formation of these pre-metastatic niches and downregulated tumor metastasis in mice.[46] Furthermore, VEGFR1-positive, human umbilical cord blood-derived hematopoietic progenitor cells (hUC-HPCs) can significantly induce the invasive growth of co-injected human melanoma cells in mice.[47] And CD133-positive hUC-HPCs enhance the in vitro invasive potential and the in vivo metastatic behavior of human colorectal cancer cells tested in xenograft assays in mice[48] as well as human breast cancer cell invasion.[49]

For a long time, the amniotic fluid was primarily seen as a protective shock absorber and liquid container contributing to the regulation of the temperature of the fetus and allowing fetal movements and growth. That totally changed when it became clear that amniotic fluid contains both, terminally differentiated cells and stem cells. For many years, the investigation of amniotic fluid-derived hAFSCs is a major research focus of our group. These broadly multipotent fetal stem cells are genomically stable, can efficiently be grown in vitro, harbor the potential to form embryoid bodies, and to differentiate into cells of all three embryonic germ layers, but are not tumorigenic.[4] Performing transwell invasion assays we demonstrated that the multipotent Oct-4/c-Kit (CD117)-positive hAFSC lines AFSC-A1, AFSC-H1, and AFSC-Q1 share the capacity to promote 3D migration of poorly invasive primary human lung fibroblasts. Beyond that, hAFSCs harbor the potential to induce invasion of primary skin fibroblasts, cardiac fibroblasts, chondrocytes, mammary epithelial cells, and hepatocytes.[50] Another study showed that exosomes from human amniotic fluid-derived mesenchymal stem cells (hAF-MSCs) promote the migratory potential of human endothelial cells.[51] And also human umbilical cord blood-derived mesenchymal stem cells (hUC-MSCs) trigger enhancement of the motility of breast cancer, hepatocellular carcinoma, lung cancer, and trophoblast cells.[52-56] Furthermore, human placental multipotent mesenchymal stromal cells (hPMSCs), which are a subpopulation of villous stromal cells expressing mesenchymal stem cell markers, can promote trophoblast migration[57] and human placenta-derived mesenchymal stem cells (hP-MSCs), originating from the chorionic plate, efficiently induce the invasive motility of primary human trophoblasts.[58] And finally, hP-MSCs also promote the migration of human endothelial progenitor cells.[39]

Taken together, these data highlight that more or less all multipotent stem cell types currently under investigation for clinical applications share the property to induce mobilization of target cells. Based on this summary of the literature we conclude that a more comprehensive molecular understanding of this feature could have a tremendous positive impact on the establishment of new and safe multipotent stem cell-based therapeutic concepts.

Pluripotent Stem Cells Induce Invasive Motility

Groundbreaking reports, such as those describing BM-HPCs- and BM-MSCs-induced tumor cell motility were already

published in the years 2005 and 2007, respectively.[22,46] These and other pioneering investigations paved the way for a productive and ever-expanding research field aiming at a better understanding of the paracrine potentials of multipotent stem cells. In contrast, the first demonstration that also pluripotent stem cells harbor the potential to mobilize target cells was published by our group only recently.[50] In a set of functional experiments we could prove that the widely used hESC lines ESC-WA01 (H1), ESC-WA09 (H9), and ESC-WA19 as well as the OCT4/SOX2/NANOG/LIN28-transduced hiPSCs lines iPSC-IMR90-1, iPSC-IMR90-3, iPSC-Foreskin-2, and iPSC-Foreskin-4, the OCT4/SOX2/NANOG/LIN28/c-Myc/KLF4/SV40LT-transduced hiPSCs lines iPSC-DF6-9-9T.B and iPSC-DF19-9-7T and the OCT4/SOX2/KLF4/c-MYC (Yamanaka factors)-transduced hiPSC lines iPSC-DYR0100 (Foreskin) and iPSC-HYR0103 (Liver) harbor the potential to induce invasion of primary non-transformed, non-immortalized human fibroblasts. Transwell invasion assays further revealed that hESCs and hiPSCs can also promote the invasion of a variety of other target cells, including primary human cardiac fibroblasts, chondrocytes, mammary epithelial cells, and hepatocytes. Furthermore, mouse studies revealed that hESC induce invasion of adjacent cells in vivo. Manipulating this process in the context of teratoma formation experiments demonstrated that stem cell-triggered tumor development depends on this mechanism.[50] These findings underpin the doctrine that this potential is an inherent property common to all pluripotent stem cells irrespective of their origin or mode of generation (Table 1).

Both, multipotent and pluripotent stem cells are considered as powerful tools for the development of innovative cell therapies against many diseases and injuries.[1-6] However, the above-reviewed findings on stem cell-induced motility could raise concerns with regard to the efficacy and safety of such stem cell-based therapeutic concepts. As discussed in the next section, to our opinion, three different objectives are conceivable to encounter such concerns.

Routes to Translation

In the context of clinical applications, the used therapeutic product can consist of the stem cell type itself, of a stem cell-derived cell type generated via targeted differentiation, or of a usually undesirable mixture of both (Fig. 1a). Irrespective of whether naturally occurring multipotent stem cells or differentiated and/or genetically modified multipotent or pluripotent stem cell-derived products are intended to be transplanted, a prior-to-usage multiparametric in vitro testing represents a compulsory requirement in the course of the approval procedure. The currently used strategies basically rely on the investigation of cell-autonomous characteristics and functions in traditional mono-cell culture assays, which leave the cellular components of the therapeutic microenvironment out of consideration (Fig. 1b). Especially, residual tumorigenic pluripotent stem cells in the therapeutic product, which escaped the differentiation process, constitute a serious safety concern. Regardless of the putative risk of malignant tumor development, the development of benign neoplasms could already be highly destructive to surrounding normal or regenerating tissues in the patient. Accordingly, it is a declared goal that remaining non-differentiated pluripotent stem cells should be eliminated from the stem cell-derived product before transplantation in humans. Different strategies, such as cell sorting, pluripotent stem cell-killing agents, or transfection with stem cell-specific suicide genes, are currently under investigation. However, it is already evident that none of these approaches will be perfect and only some hundred highly tumorigenic stem cells are sufficient to generate tumors.[1,4-6]

We here want to draw attention to the putative clinical impact of the only recently explored motility-inducing potential, which can be attributed to more or less all human stem cell types currently under consideration for clinical use (Table 1). Across all assumable variants of applications, including the usage of naturally occurring stem cells or the administration of multipotent or pluripotent stem cell-derived differentiated cell products, irrespective of whether they are intended to directly fulfill regenerative tasks or they should function as vehicles for gene therapy or drug-delivery, this feature should be taken into account on the way to establish therapies of improved functionality and safety. For example, BM-MSCs induce the motility of many cells including a variety of different tumor cells (Table 1). Accordingly, great caution should be taken with a view to the currently emerging intentions to use them as anti-inflammatory and immuno-regulatory therapeutics (these effects are also mediated in a non-contact fashion by the secretion of soluble factors and extracellular vesicles) or as tumor-selective targeting carriers for the delivery of therapeutic agents. To encounter the associated well-justified concerns, three different objectives are conceivable.

First, the cross-talk of stem cell-based therapeutic products with target cells of the designated transplantation site could routinely be monitored complementary to the traditionally used mono-cell culture biosafety approaches. For this purpose, we want to suggest the use of the well-established and widely used transwell assay, which can be applied to study migration (without pre-coating of the insert with extracellular matrix) and invasion (with pre-coating) (the latter is depicted in Fig. 1c). This is a perfectly scalable and easy-to-handle assay to monitor cell behavior upon indirect co-culture. Since we want to suggest using it routinely prior to the clinical application, the fact that the transwell assay is a very cost-effective tool is of the highest relevance. It can be applied in high-throughput formats using multiwell plates to compare the effects of a great number of therapeutic stem cell products on basically all types of putative target cells, pieces of tissues, or even organoids, under defined experimental settings. It is a highly reproducible approach, which enables the precise quantification of stem cell-mediated effects via the analysis of the percentage of motile target cells, which crossed the membrane. The high sensitivity of this assay allows the accurate detection of as little as one motile cell out of 2.5×10^4 cells. The apparent simplicity of adapting the evaluation period enables convenient investigation of both short- and long-term effects. And finally, the separate chambers create optimal conditions to investigate motility-associated cellular signaling or, for example, the putatively induced secretion of matrix metalloproteinases (MMPs) playing a pivotal role in 3D migration-associated proteolytic matrix degradation.[9,50,59] Very likely attributable to the fact that in the bottom chamber all different stem cell types can be grown in their individual culture medium under maintenance conditions, the vast majority of so far published studies demonstrating stem cell-induced motility used this kind of assay (Table 1).

Second, a more comprehensive picture of the molecular mechanisms involved in stem cell-induced mobilization processes could function as a door-opener for putative

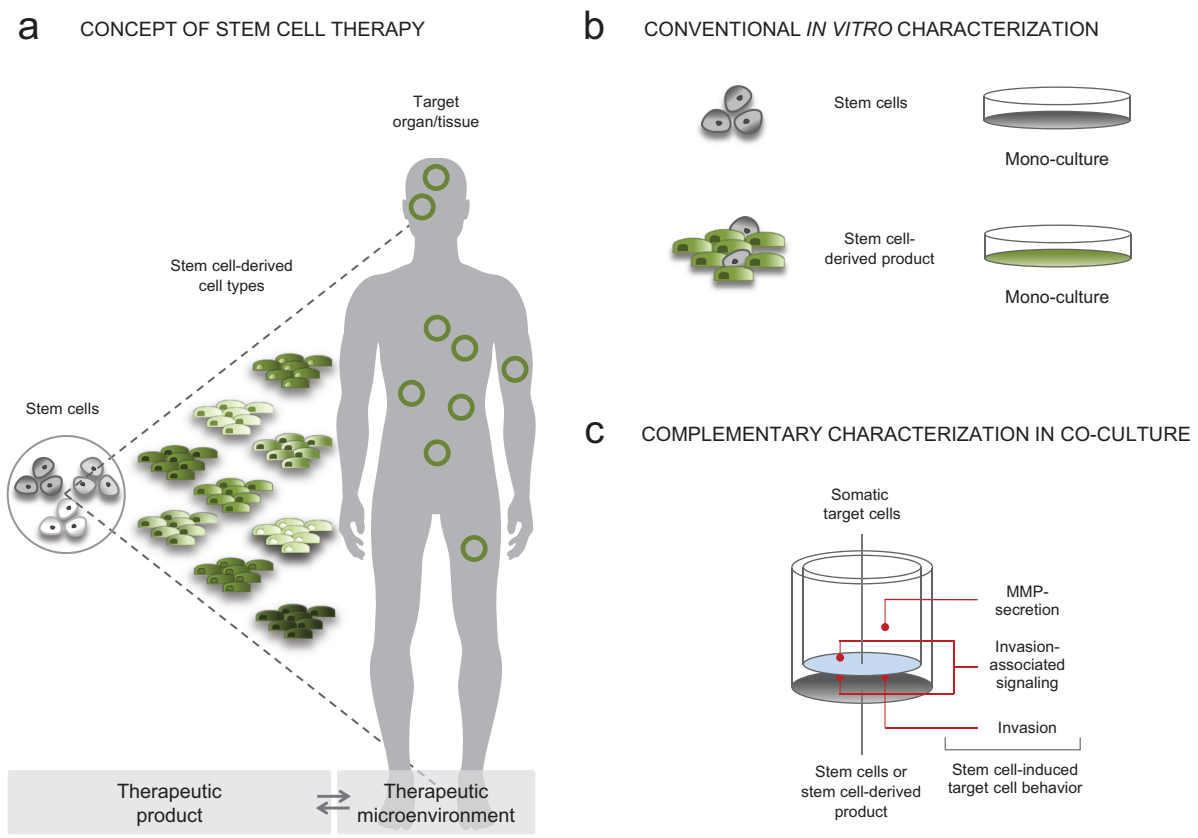

Figure 1. The key determinants of stem cell-based therapy and their evaluation during in vitro biosafety analysis. (a) The main factors in transplantation therapy include the starting stem cells and the stem cell-derived differentiated cell types which can constitute a therapeutic product. The third component involves the target organ or tissue representing the local environment with which the transplanted cells interact. (b) Current strategies to evaluate the efficacy and safety of stem cells or stem cell-derived products in vitro typically rely on cell characterization in traditional mono-cell culture, primarily assessing cell-autonomous states, functions, and behaviors. (c) In vitro co-culture to investigate the impact of stem cells or stem cell-derived products, potentially including residual stem cells, on the behavior of somatic cells (eg, MMP secretion, invasion-associated signaling, invasion) in their local environment represents a complementary approach to characterize and predict stem cell function in vivo. Abbreviation: MMP, matrix metalloproteinases.

mechanistic interventions. Elucidation of the underlying paracrine signaling cascades could allow to screen for potent inhibitors of the motility-inducing effects on target cells. Fortunately, for numerous of the published mobilizing cross-talks between specific stem cells and somatic target cells basic knowledge on the involved paracrine signaling mechanisms already exists. And actually, for several of these reported biochemical processes inhibitors have already earlier been established, which in the context of other purposes underwent drug approvals and are on the way to be licensed for routine clinical use in humans. For example, the observation that multipotent hAFSCs and pluripotent hESCs and hiPSCs induce invasion through insulin-like growth factor 1/2 (IGF1/2)-mediated paracrine induction of mechanistic target of rapamycin complex 1 (mTORC1) to activate MMPs[50] could be envisaged for biochemical interventions using prominent mTOR inhibitors already routinely used in a variety of clinical applications.[60] Paracrine neuregulin 1/human epidermal growth factor receptor 3 (HER3) signaling has been shown to be involved in hBM-MSCs-triggered 3D migration.[10] Furthermore, BM-MSC-induced motility can be blocked by inhibitors of the platelet-activating factor receptor (PAFR), such as ginkgolide B, which also exhibits therapeutic action and is therefore already used in preliminary clinical trials for several diseases,[13] by SB265619, a small molecular inhibitor of C-X-C chemokine receptor type 2 (CXCR2),[26] by zoledronic acid,[27] and by inhibitors of C-X-C chemokine receptor type 4 (CXCR4) and of aquaporin (AQP1), developed as anti-tumor agents.[15,61] hADSCs induce invasive behavior by paracrine targeting of the hepatocyte growth factor (HGF)/mesenchymal-epithelial transition factor (c-Met) pathway.[44] hUC-MSCs and hUC-MSC-derived extracellular vesicles drive breast cancer motility via activation of the extracellular signal-regulated kinases (ERK) pathway, which can be blocked by the clinically used ERK inhibitor UO126.[52,53] The pro-migratory effects of hPMSCs mediated by HGF-induced adenosine 3′,5′-cyclic monophosphate (cAMP) production and Ras-related protein 1 (Rap1) activation was shown to be affected by the well-known protein kinase A (PKA) inhibitor H89, and by treatment with Rap1 siRNA or a c-Met blocking antibody.[57] Needless to say that the efficacy of all possibly usable inhibitors must undergo a series of defined investigations, conceivably including transwell assays and animal trials.

Third, in addition to blocking the motility-inducing capacity in the stem cell product prior to its clinical usage, it could also be assumed to interfere with the stem cell-induced deregulated mobilization and navigation of target cells in vivo. In fact, the in vivo impact of such interfering approaches has already been demonstrated in specific experimental settings. For example, hBM-MSCs induce the motility

of endothelial progenitor cells via activating CXCR2 and its downstream signaling pathway. In mouse experiments, local delivery of the well-known and pre-clinically tested CXCR2 antagonist SB225002 triggered a substantial decrease in the motility of the murine target cells in vivo.[40] hUC-HPCs significantly induce melanoma cell motility in a liver injection mouse model mimicking the in vivo invasive growth of cells. This process could significantly be inhibited in vivo by treatment with agents, which are already undergoing preclinical trial evaluations for many conditions, such as LY-294002, a specific inhibitor of the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) pathway, or PD98059, a specific inhibitor of the mitogen-activated protein kinase kinase (MEK)/ERK pathway.[47] In addition, it was demonstrated that hESC-driven teratoma development is accompanied by the activation of mTORC1 and MMPs in the tumor microenvironment triggering the invasive attraction of adjacent murine cells in the teratoma. This entire cascade was stringently downregulated in vivo via depletion of endogenous IGFs in hESCs before injection.[50] These findings could encourage studies to investigate the effects of systemic or local in vivo administrations of, for example, clinically approved mTOR inhibitors. Importantly, this downregulation upon IGF depletion also had a pronounced negative impact on the growth of the teratoma in vivo. Since no effects on the tumor formation incidence, the differentiation status of the teratoma, or cell survival were observed, these in vivo experiments allow to draw the conclusion that hESC-related tumor growth, what is probably the most serious concern associated with the clinical usage of pluripotent stem cells, depends on the paracrine stem cell property to induce uncontrolled mobilization and 3D navigation of adjacent host cells.[50] Consequently, blocking this stem cell potential could not only prevent the patient's cells to cause deleterious side effects via uncontrolled invasion processes but could also persistently restrict unrestrained malignant growth of stem cell-induced neoplasms. Accordingly, it is not implausible that complementary to the attempts to eliminate undifferentiated tumorigenic pluripotent stem cells from the therapeutic cell product, strategies to block stem cell-induced invasion processes could be of significant anti-tumorigenic benefit for a sustained therapeutic concept.

In summary, with the here presented discussion we don't want more but also no less than to emphasize that further investigations on this ubiquitous feature of stem cells could significantly contribute to pave the routes to their clinical translation. Although there is obviously still a long way to go, it appears to be assumable that patients could once benefit from the inhibition of this motility-inducing capacity of stem cells and stem cell-derived products prior to their usage, from co-administration of medications to block the invasive reaction of the patient's cells, or from the combination of both (Fig. 2).

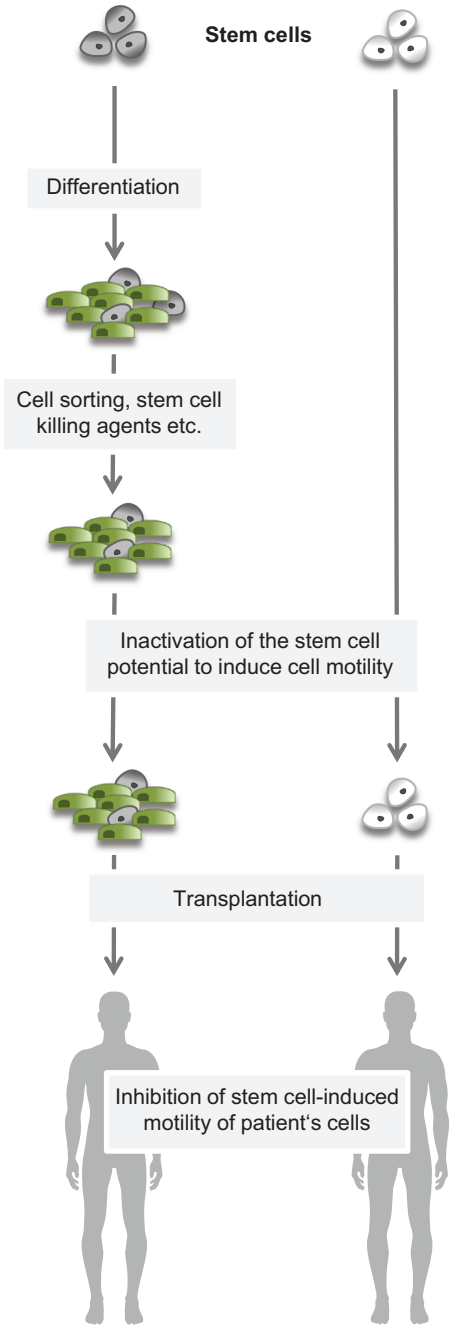

Figure 2. Design and development of stem cell-based therapies. Stem cells are either directly used for therapy or can be differentiated into a desired cell type. For the latter, remaining undifferentiated stem cells are attempted to be eliminated from the preclinical cell product at highest possible efficiency via, for example, cell sorting or stem cell-killing agents. As a complementary approach, the potential to induce motility of somatic target cells could be inactivated to allow the transplantation of a safer therapeutic product into patients for cell therapy. Finally, cell product transplantation could be accompanied by the application of agents which block the mobilization of somatic cells in the patient.

Conclusion

Stem cells are well under way to fundamentally affect the paradigm of regenerative medicine. Although this development has already gathered a tremendous momentum, it is evident that there are still several obstacles to be removed. For the benefit of patients, a variety of ameliorations improving functionality, specificity, and safety of stem cell-based therapeutic concepts are still indispensable. In this respect, the proper management of the here discussed stem cell-induced mobilization of cells at the putative transplantation site is evidently just one of many relevant building blocks. Still, this feature is shared by so many human stem cell entities and appropriate prior-to-usage characterizations and interventions could be assumed to be straightforward, readily applicable, cost-effective to implement, and of

proportionate therapeutic benefit. Accordingly, the achievement of the goal to reduce deleterious therapeutic side effects would very likely benefit from the elaboration of a more comprehensive picture of the phenomenon of stem cell-induced motility.

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Conflict of Interest

The authors declared no potential conflicts of interest.

Author Contributions

M.R. and M.H. wrote and edited the manuscript.

Data Availability

No new data were generated or analyzed in support of this research.

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