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Mini-review

Targeted antitumor therapy mediated by prodrug-activating mesenchymal stromal cells

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ABSTRACT

Mesenchymal stromal cells (MSCs) were introduced as tumor-targeted vehicles suitable for delivery of the gene-directed enzyme/prodrug therapy more than 10 years ago. Over these years key properties of tumor cells and MSCs, which are crucial for the treatment efficiency, were examined; and there are some critical issues to be considered for the maximum antitumor effect. Moreover, engineered MSCs expressing enzymes capable of activating non-toxic prodrugs achieved long-term curative effect even in metastatic and hard-to-treat tumor types in pre-clinical scenario(s). These gene-modified MSCs are termed prodrug-activating MSCs throughout the text and represent promising approach for further clinical application. This review summarizes major determinants to be considered for the application of the prodrug-activating MSCs in antitumor therapy in order to maximize therapeutic efficiency.

Introduction

Non-malignant cells within the tumor microenvironment including mesenchymal stromal cells (MSCs) substantially affect major hallmarks of tumor cells [1]. Bidirectional signaling between tumor cells and MSCs alters phenotype, proliferative and metabolic activities of both cell types [2,3]. MSCs regulate cancer stem cell functions within a stem cell niche [4,5]. Tight biological interaction of MSCs with tumor cells led to a hypothesis that gene-modified MSCs with a capability to enzymatically convert non-toxic prodrug to toxic metabolite(s) may target drug-resistant cancer cells, including cancer stem cells, tumor- and metastasis-initiating cells [6,7]. Recently, the first clinical trial employing the prodrug-activating MSCs was initiated [8]. There are several key factors, which affect a long-term therapeutic potential of the MSCs-mediated enzyme/prodrug therapy: intrinsic drug resistance to delay suicide effect in the gene-modified MSCs [9], intrinsic tumor cell properties [10,11], ratio of the therapeutic to target cells, timing of the prodrug administration and initial tumor burden [12].

Key properties of mesenchymal stromal cells

The MSCs are found *in situ* within all mammalian supportive stromal tissue compartments maintaining tissue homeostasis and stabilizing local vasculature [13]. Bone marrow, adipose tissue and umbilical cord are the most commonly used sources of MSCs [14]. MSCs represent multipotent cells originally expanded from a plastic-adherent subpopulation [15]. MSCs proliferate as a heterogeneous population of fibroblast-like cells at varying stages of commitment to differentiation characterized by ecto-5′-nucleotidase CD73, Thy-1 cell surface antigen CD90 and endoglin CD105 markers. External factors such as donor age, cell source and culture conditions affect proliferation of MSCs [16], which were reported to retain their multipotency for 30–40 cell divisions in culture [17,18]. MSCs possess beneficial properties for clinical use: immune-privileged status, homing abilities, availability, expandability, genotypic and phenotypic stability, and safety record in clinical trials [9,19]. Their ability to migrate and reach damaged tissues in response to paracrine signaling molecules from the injured tissue increased the interest in their use in regenerative medicine [20]. In 2016 there were over 400 clinical trials that scrutinized therapeutic effects of the MSCs [21].

Novel MSC markers for their prospective isolation, such as nerve growth factor receptor (NGFR, CD271) and gangliosides GD1 and

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GD2, were suggested in order to distinguish MSCs from fibroblasts [22–24]. Fibroblasts are defined as adherent cells capable of synthetizing and remodeling the extracellular matrix (ECM) involved in wound healing, fibrosis, inflammation and tumor microenvironment [25,26]. There are contradictory reports on their capability of trilineage differentiation [27–29], or immunophenotypic profile due to the resemblance to the MSCs [30]. In contrast to fibroblasts, the adipose tissue-derived MSCs (AT-MSCs) secrete high levels of vascular endothelial growth factor A (VEGFA), hepatocyte growth factor (HGF) and angiopoietin-1 (Ang-1) [29]. Halfon et al. [31] showed opposing expression of activated leukocyte cell adhesion molecule (ALCAM, CD166) and transmembrane protein tetraspanin (CD9) in MSCs and fibroblasts. Increased expression of vascular cell adhesion molecule 1 (VCAM1, CD106), integrin subunit alpha 11 (ITGA11) and insulin-like growth factor (IGF-2), but decreased levels of matrix metallopeptidases 1 (MMP1) and 2 (MMP2) was detected in the MSCs in comparison to fibroblasts. Importantly, we have shown a functional difference between MSCs and fibroblasts in the antitumor therapy. The adipose tissue-derived MSCs and fibroblasts were transduced to express a transgene, but the gene-modified fibroblasts were incapable of delivering the transgene into tumors; and thus proven inefficient as vehicles for tumor-targeted gene therapy [32].

Mesenchymal stromal cells in tumors

MSCs derived from local and distant tissues contribute to tumor stroma. Host-derived cells such as pericytes (positive for the proteoglycan NG2 and α-smooth muscle actin αSMA) and endothelial cells (positive for the platelet and endothelial cell adhesion molecule 1 CD31) were recruited from local adipose tissue to stromal compartment. The majority of fibroblastic cells positive for fibroblast-activating protein FAP and fibroblast-specific protein FSP were recruited from bone marrow progenitor populations of the host [33].

MSCs are mobilized from bone marrow and other tissues to the peripheral blood under normoxia, hypoxia, inflammation and injury. Their homing results from an interplay between active recruitment via chemokines and inflammatory processes and passive entrapment in the vasculature [34,35]. Tropism of MSCs to solid tumors is mediated by interaction of chemokine (C-X-C motif) ligand 12/stromal cell-derived factor 1 (CXCL12/SDF-1α) with its cognate receptor CXCR4; and also activation of transforming growth factor β (TGFβ) receptor. Furthermore, platelet-derived growth factor (PDGF), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), and interleukin-6 (IL-6) secreted by tumor cells also contribute to MSCs tropism to tumors. Tumor engraftment is promoted by selectins, selectin ligands, VCAM1 and endothelial stimulation with tumor necrosis factor α (TNF-α). MSCs form a part of the stem cell niche: they expand the putative breast cancer stem cell population via cytokine loop involving IL-6 and CXCL7 after transplantation into bone marrow [36]. MSCs are able to regulate proliferation and self-renewal of the aldehyde dehydrogenase-positive (ALDH+) cancer stem cells [37]. Human MSCs promote establishment of metastatic niche via secretion of C-C motif chemokine ligand 5 (CCL-5), SDF-1α and other chemokines in a paracrine and endocrine manner as comprehensively summarized in Refs. [5,38].

MSCs together with other components of the tumor microenvironment protect tumor cells from the effect of chemo-therapeutic agents [39]. Although the MSCs are inherently chemoresistant to commonly used chemotherapeutics [40,41], endogenous MSCs become activated during treatment and secreted factors decrease chemotherapeutic response [42,43]. Drug resistance conferred by the MSCs involves SDF-1α/CXCR4 signaling activation, secreted platinum-induced fatty acids and increased pool of cancer-initiating cells [44]. Paclitaxel resistance in MSCs was attributed to cell cycle regulation. MSCs cultivated under hypoxia have increased expression of ATP-binding cassette (ABC) efflux pump ABCG2 rendering protection under these cultivation conditions [45]. The expression of ALDH as an intracellular detoxification enzyme protecting long-living cells against oxidative stress may also contribute to inherent MSCs chemoresistance. Cells with high ALDH activity ($ALDH^{hi}$) represent a rare fraction of human bone morrow (<0.8% of mononuclear cells) or umbilical cord blood (<0.5% of mononuclear cells) cells. $ALDH^{hi}$ cells are highly enriched in expandable progenitor cell population with hematopoietic, endothelial and mesenchymal colony–forming functions [46].

Exogenously delivered MSCs capable of crossing the blood-brain barrier preferentially home to and survive in pre-established tumors making them suitable delivery vehicles for proteins of interest even into the brain malignancies [18,32]. Exogenous MSCs persisted in tumor or inflamed regions for more than 7 days, but not in healthy tissues [47]. Tumorotropic properties make them favorable for use in targeted cancer therapy as a cellular delivery vehicles [48,49]. Engineered prodrug-activating gene-modified MSCs have shown cytotoxic and therapeutic antitumorigenic potential in several hard-to-treat tumor types including colorectal carcinoma [32], aggressive melanoma [12,18,50], triple-negative breast cancer [10,51], human medullary carcinoma [52,53], glioblastoma [10,11,54], prostate carcinoma [55] and metastatic ovarian cancer [56].

Gene modifications of mesenchymal stromal cells

Retroviral or lentiviral vectors are used to achieve long-term stable expression of transgene(s) in MSCs [57,58]. Transduction efficiency of the MSCs was close to 90 ± 5% in our experiments [18]. Vector incorporation into DNA did not affect the key properties of the MSCs, although the enzymatic function of the constitutively expressed transgene increased proliferation and chemosensitivity in engineered MSCs [9]. Recently, novel methods of gene editing were introduced to circumvent the risk of insertional mutagenesis or oncogene activation upon viral transduction by highly specific programmable nucleases, which generate site-specific cleavage [59]. Zinc finger nuclease (ZFN)-mediated gene addition was achieved in 30–40% of the MSCs and resulted in stable expression of the erythropoietin gene from the chemokine [C-C motif] receptor 5 (CCR5) gene locus in MSCs [60]. Even though only 7%-positive MSCs were nucleofected with the targeting vectors, cell sorting based on GFP required only 24 h to harvest the modified MSCs in contrast to drug screening, which required 2–4 weeks [61]. Genome editing can be performed by clustered regularly interspaced short palindromic repeats (CRISPR) and the CRISPR-associated (Cas) protein 9, which is an RNA-guided endonuclease [62]. The Cas9 can be guided to any genomic location, inducing double strand breaks allowing for non-homologous end joining or homologous recombination of genomes. Vector for the homologous recombination was prepared to overexpress hepatocyte growth factor and insulin-like growth factor 1 in engineered MSCs through CRISPR/Cas9 system to examine a therapeutic effects on liver fibrosis. It remains to be determined, how the overexpression of specific genes affects the MSCs function or clonal selection [63]. Vanoli et al., who used a strategy combining CRISPR-Cas9 technology and homology-directed repair to produce oncogenic chromosomal translocation, have shown that gene-edited MSCs retained surface markers characteristic of mesenchymal progenitors: CD44, CD73, and CD105 [64]. A nuclease-deficient Cas9 (dCas9) guided by target-specific short guide (sg)RNAs to the upstream promoter region of endogenous genes can upregulate gene expression. Multiple sgRNAs could synergistically activate genes, which may be used to enhance