JPET Fast Forward. Published on June 7, 2019 as DOI: 10.1124/jpet.119.259796
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JPET # 259796

Title Page

Engineered Mesenchymal Stem Cells (MSCs) for Targeting Solid Tumors: Therapeutic Potential beyond Regenerative Therapy

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Running Title Page

a) Running Title: Engineered MSCs for tumor targeted delivery

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c) Number of text pages: 35
Number of tables: 3
Number of figures: 3
Number of references: 130
Number of words in the abstract: 111
Number of words in the introduction: 340
Number of words in the manuscript: 4871
Number of words in the concluding remarks: 99

d) Keywords: Mesenchymal Stem Cells; Tumor Homing; Targeted Drug Delivery; Chemotherapy; Nanoparticles.

e) List of non-standard abbreviations:

Mesenchymal stem cell (MSC), interleukin (IL), tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), C-X-C chemokine receptor (CXCR), cluster of differentiation (CD), intercellular adhesion molecule (ICAM), vascular cell adhesion molecule (VCAM), stromal cell-derived factor (SDF), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), transforming growth factor (TGF), major histocompatibility complex (MHC), interferon (IFN), P-glycoprotein (Pgp), nanoparticle (NP), polylactide (PLA), lipid nanocapsule (LNC), poly(L-lactide-co-glycolide) (PLGA), transactivator of transcription (TAT), mesoporous silica nanoparticle (MSN), hyaluronic acid (HA), near-infrared (NIR), Fluorescein isothiocyanate (FITC), positron emission tomography (PET), magnetic resonance (MR), lipids, doxorubicin, gold nanorods and iron oxide nanocluster (LDGI), photothermal (PT), photoacoustic (PA), triple negative breast cancer (TNBC), N-azidoacetylmannosamine-tetraacylated (Ac_4ManNAz), dibenzyl cyclooctyne (DBCO), hematopoietic cell E-selectin/L-selectin ligand (HCELL), C-X-C motif chemokine (CXCL), carbon nanotube (CNT), doxorubicin (DOX), platelet-derived growth factor receptor (PDGFR), tissue inhibitors of metalloproteinase (TIMP), Investigational New Drug (IND), Biologics License Application (BLA), Food and Drug Administration (FDA), National Institutes of Health (NIH), standardized good manufacturing practices (cGMP), fetal bovine serum (FBS), International Society for Stem Cell Research (ISSCR), oncolytic measles virus encoding thyroidal sodium iodide symporter (MV-NIS), herpes-simplex-virus thymidine kinase (HSV-TK), amyotrophic lateral sclerosis (ALS), Cyanine5.5(Cy5.5).

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Abstract: Mesenchymal stem cells (MSCs) have previously demonstrated considerable promise in regenerative medicine based on their ability to proliferate and differentiate into cells of different lineages. More recently, there has been a significant interest in using MSCs as cellular vehicles for targeted cancer therapy by exploiting their tumor homing properties. Initial studies focused on using genetically modified MSCs for targeted delivery of various pro-apoptotic, anti-angiogenic and therapeutic proteins to a wide variety of tumors. However, their use as drug delivery vehicles has been limited by poor drug load capacity. This review discusses various strategies for the non-genetic modification of MSCs that allows their use in tumor-targeted delivery of small molecule chemotherapeutic agents.

Significance Statement: There has been a considerable interest in exploiting the tumor homing potential of MSCs to develop them as a vehicle for the targeted delivery of cytotoxic agents to tumor tissue. The inherent tumor-tropic and drug-resistant properties make MSCs ideal carriers for toxic payload. While significant progress has been made in the area of the genetic modification of MSCs, studies focused on identification of molecular mechanisms that contribute to the tumor tropism along with optimization of the engineering conditions can further improve their effectiveness as drug delivery vehicles.

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Introduction

Mesenchymal stem cells (MSCs) are adult, multipotent progenitor cells known for their self-renewal ability, and have shown considerable potential in regenerative medicine. More recently, their use in anticancer therapies has gained considerable interest owing to their tumor homing capabilities. Targeted delivery of chemotherapeutics for the treatment of cancer is a goal that has not yet been fully realized due to various physiological and pathological obstacles. Development of cell based active targeting approaches using macrophages (Choi et al., 2007; Cheng et al.,

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2010; Choi et al., 2012), red blood cells (Chambers and Mitragotri, 2007), neural stem cells (Cheng et al., 2013; Schnarr et al., 2013), MSCs (Roger et al., 2010) and T cells (Huang et al., 2015) has created a new avenue for targeting cancer therapies to malignant tissues. Of these cell-based strategies, MSCs (Nakamizo et al., 2005; Muller et al., 2006; Tang et al., 2010; Ahmed and Lesniak; Sadhukha et al., 2014) and macrophages (Batrakova et al., 2007; Brynskikh et al., 2010; Zhao et al., 2011) are particularly interesting owing to their potential to efficiently infiltrate specific tumor types (Touboul et al., 2013). MSCs have been genetically engineered using viral and non-viral vectors to secrete cytokines that improve host immune response against cancer cells as well as other proteins that can directly mediate tumor cell death (Hodgkinson et al., 2010). For example, MSCs have been genetically modified to express interleukin (IL)-2 (Nakamura et al., 2004), IL-12 (Ryu et al., 2011), and IL-18 (Xu et al., 2009), CXCR4 (Yang et al., 2013), prodrug-activating enzymes against tumors (Amara et al., 2014), tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) (Mueller et al., 2011) and interferon-β (Ling et al., 2010). In addition, MSCs have been engineered to carry traditional anticancer cytotoxic drugs (Sadhukha et al., 2014). Many of these newer efforts have focused on using nano delivery systems to incorporate small molecules in MSCs. This review will discuss the progress that has been made in the development of MSCs as tumor targeted therapeutics, with emphasis on non-genetic modification of MSCs to deliver small molecule chemotherapeutics.

1. MSCs: origin, sources and traditional uses

MSCs were first discovered by A.J. Friedenstein in the early 1970s (V. Afanasyev et al., 2009), and since then have been successfully isolated from numerous tissues including bone marrow (Stewart et al., 1999; Chamberlain et al., 2007), adipose (Wagner et al., 2005; Zhang et al., 2011; Pendleton et al., 2013), peripheral blood (Ab Kadir et al., 2012), skin (Bartsch et al., 2005;

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Riekstina et al., 2008), brain (Kang et al., 2010), dental tissues (Gronthos et al., 2002) and endometrium (Cheng et al., 2017). They are characterized by the presence of several surface markers such as CD105, CD73, CD90 and the absence of certain markers such as CD45, CD34, CD14 or CD11b, CD19, and HLA-DR (Baghaei et al., 2017).

MSCs are multipotent and can primarily differentiate into adipocytes (Dennis et al., 1999; van Vliet et al., 2007), osteoblasts (Friedenstein et al., 1966; Bruder et al., 1997; Bruder et al., 1998), and chondrocytes (Solchaga et al., 2011). However, the differentiation potential of MSCs is not just limited to mesodermal lineages (Kadiyala et al., 1997), and can differentiate into epithelial cells, hepatocytes (endodermal), and neural (ectodermal) cell lineages when provided appropriate signals. This ability to differentiate into various cell types has resulted in their extensive use in regenerative medicine and tissue engineering (Park et al., 2015; Fitzsimmons et al., 2018). In fact, the utilization of MSCs has been considered one of the most exciting advances in stem cell transplantation (Garcia-Castro et al., 2008). There are currently over 700 clinical trials investigating the use of MSCs as clinical therapeutics (Wang et al., 2018a). MSCs have been investigated for direct repair of various tissues such as bone (Lin et al., 2017), heart (Thakker and Yang, 2014), kidney (Morigi et al., 2016) and skin (Laverdet et al., 2014). MSCs have shown positive results in studies involving hematopoietic transplantation (Zhao and Liu, 2016), arthritis therapy (Ansboro et al., 2017), intervertebral disk repair (Noriega et al., 2017) , and many others. In addition to growth and differentiation, other characteristics of MSCs such as migration (De Becker and Riet, 2016), stable long-term transduction (Liu et al., 2018), and lack of immunogenicity (Morandi et al., 2008) suggest that these cells can be utilized for therapeutic uses beyond regenerative medicine.

2. MSCs for tumor targeted delivery

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Obstacles to successful treatment of malignant tumors include the development of tumor drug resistance and the insufficient tumor selectivity of anticancer drugs leading to acute and long-term host morbidities (Housman et al., 2014). Encapsulation in nano delivery systems can enable passive targeting of the drug to the tumor tissue and addresses these obstacles to some extent. However, passive targeting is inefficient and does not result in uniform distribution of the drug within the tumor tissues. Active targeting approaches utilize ligands capable of binding surface antigens or receptors that are overexpressed on tumor cells (Bazak et al., 2015). However, active targeting is, in reality, not an active process, since the delivery system must first accumulate passively in the tumor, followed by binding to tumor cells (Rosenblum et al., 2018). Thus, active or ligand-based targeting suffers from some of the same limitations as passive targeting. MSCs have been shown to actively traffic to both primary tumors and metastases (Layek et al., 2016), in response to inflammatory signals secreted by neutrophils and macrophages infiltrating the tumor (Sun et al., 2014). MSCs have been genetically modified to express peptides and proteins with anti-tumor properties. For example, significant anti-cancer effect against tumors of brain, ovary, pancreas, liver, kidney, breast or prostate and pulmonary metastases were observed with MSCs genetically engineered to express suicidal genes (thymidine kinase, cytosine deaminase, carboxylesterase) (Kucerova et al., 2008; Song et al., 2011; Yin et al., 2011). Thus, it is possible to achieve true active tumor targeting with MSCs. In fact, several anticancer therapies utilizing MSCs are in various stages of clinical development (Table 1). In addition, recent studies show that MSCs infiltrate tumor tissue uniformly and thus improve the intra-tumoral distribution of the therapeutic agents they carry (Bexell et al., 2012).

Tumor homing potential of MSCs is attributed to the presence of surface-associated chemokine receptors such as CXCR4 and their interaction with chemokines secreted on the surface of tumor

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cells (Gao et al., 2007). Although the mechanism of tumor tropism of MSCs is not fully understood, trans-endothelial migration of MSCs towards tumors is considered to be similar to that of leukocytes, which involves steps such as rolling, adhesion and extravasation (Figure 1). In addition, MSCs express various adhesion molecules such as intercellular adhesion molecules (ICAMs) (eg. ICAM-1 and ICAM-2) and vascular cell adhesion molecule-1 (VCAM-1), which also contribute to their intrinsic migratory properties (Hernanda et al., 2014). Tumors are enriched with various chemokines such as stromal cell-derived factor-1α (SDF1-α), vascular endothelial growth factor A (VEGF-A) (Ball et al., 2007), basic fibroblast growth factor (bFGF) (Schmidt et al., 2006), and transforming growth factor (TGFβ) (Ponte et al., 2007), which act as chemo-attractants for MSCs.

2.1 Characteristics enabling the use of MSCs as targeted carriers

As discussed earlier, several characteristics of MSCs such as ability to self-renew, ease of isolation, and tumor tropism make them ideally suited for tumor targeting. In addition, MSCs have demonstrated low immunogenicity *in vivo* (Lee et al., 2014). They have been found suitable for allogenic use due to lack of MHC class II molecules and relatively low levels of MHC class I molecules (Squillaro et al., 2016). MSCs are easily expanded *in vitro* due to their natural adherence to tissue culture flasks. Further, MSCs have limited replicative lifespan (Kim and Park, 2017), ensuring low risk of malignant transformations following transplantation *in vivo*. Also, the use of MSCs is devoid of ethical considerations unlike in the case of embryonic stem cells (Mahla, 2016).

2.2 Engineered MSCs for tumor-targeted drug delivery

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Engineered cell-based systems have emerged as excellent platforms for addressing various challenges faced by synthetic delivery systems including site-specific delivery. Cells can be engineered to present natural biomolecules or synthetic ligands to overcome various physiological barriers and control their interaction with the tumor microenvironment.

2.2.1 Genetic modification of MSCs

Over the past few decades, genetically engineered MSCs have been successfully used to express therapeutic peptides and proteins for the treatment of both localized and metastatic tumors. For instance, MSCs expressing IL-12 showed potent anticancer activity against melanoma, breast cancer, and hepatoma (Chen et al., 2008). Similarly, MSCs expressing interferon gamma (IFN-γ) were shown to inhibit tumor growth in mouse neuroblastoma (Relation et al., 2018) and lung carcinoma (Yang et al., 2014) models. Genetic modification of MSCs is usually performed using viral and non-viral vectors. Transient gene expression and low-transfection efficiency are the limitations observed in genetic modification of MSCs using the non-viral vectors (Park et al., 2015). Moreover viral transduction methods for genetic engineering of MSCs pose high risk of chromosomal instability (Takeuchi et al., 2007), insertional mutagenesis (Bokhoven et al., 2009) and proto-oncogene activation (Modlich et al., 2009) in spite of inherent high transfection efficiency.

2.2.2. Non-genetic modification of MSCs

The successes achieved with genetically modified MSCs suggest that MSCs can also serve as potential carriers for tumor-specific delivery of small molecule therapeutics including cytotoxic agents. Pessina et al. initially demonstrated that exposure of MSCs to paclitaxel led to incorporation of the drug in MSCs. The paclitaxel-primed cells exhibited dose-dependent

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antiangiogenic and antitumor activity *in vitro* (Pessina et al., 2011). However, the use of MSCs as cytotoxic drug carriers has been limited by the overexpression of drug efflux transporters such as P-glycoprotein (Pgp), resulting in poor drug loading capacity (Dai et al., 2013; Sadhukha et al., 2014). Additionally, small molecules are subjected to rapid diffusional clearance out of the cells.

To overcome these drawbacks, a number of groups including ours have started investigating nanoparticle (NP) encapsulated forms of drugs, which can increase the drug carrying capacity of MSCs. NPs not only conceal drug molecules from the efflux transporters but also limit their diffusional clearance (Sadhukha et al., 2014; Zhang et al., 2015; Layek et al., 2016; Yao et al., 2017; Layek et al., 2018; Wang et al., 2018b; Moku et al., 2019). Table 2 summarizes the various studies that have reported engineering of MSCs with NPs for applications such as targeted tumor therapy, imaging, and cell tracking. Drug-loaded NPs can be incorporated in MSCs either via intracellular uptake or cell surface conjugation. As MSCs home to the tumor site, NP encapsulated drug can be released locally at the tumor site over a prolonged duration and inhibit tumor growth. In this section, we describe the recent advances in nanoengineering of MSCs for tumor targeted drug delivery.

2.2.2.1. Nanoengineering via cellular uptake

MSCs have been engineered with various types of NPs (organic and inorganic matrices) to enhance their drug loading and therapeutic efficacy. Early studies utilized MSCs loaded with magnetic and fluorescently labeled NPs for use in diagnostic applications. Roger et al. demonstrated that coumarin-6 dye loaded polylactide NPs (PLA-NPs) and lipid nanocapsules (LNCs) (Roger et al., 2010) were efficiently taken up by MSCs in a concentration and time-dependent manner without affecting the viability and differentiation of MSCs. However, these

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studies did not investigate loading of chemotherapeutic agents in MSCs. The ability to load small molecule drugs is critical for clinical translation of MSCs (Krueger et al., 2018).

Achieving high payload capacity (amount of drug that can be loaded per cell) is crucial to achieving therapeutic drug concentrations in the tumor tissue. On the other hand, loading a cytotoxic drug can adversely affect MSC viability and tumor tropism. Thus, loading of drug containing NPs in MSCs needs to be carefully optimized to achieve effective tumor targeting. Factors that have been shown to be important in the intracellular uptake of NPs in other mammalian cells have a similar influence on the uptake of NPs in MSCs. It is widely established that NPs are taken up by endocytosis. It was first proposed by Panyam et al. that NPs formulated from certain polymers such as poly(L-lactide-co-glycolide) escape the endosomal compartment, resulting in higher intracellular retention of the encapsulated drug (Panyam et al., 2002). Another important attribute of NPs is protection of the encapsulated drug from efflux transporters present on the MSCs, which in turn results in higher uptake and greater payload capacity (Figure 2). Several groups have demonstrated the presence of the efflux transporter P-glycoprotein (Pgp) on the surface of MSCs (Dai et al., 2013; Sadhukha et al., 2014). It has been demonstrated that uptake of NPs inside MSCs is concentration, size and time dependent (Dai et al., 2013). The uptake is also influenced by the surface charge of NPs. The studies carried out by Dai et al. demonstrated that the migration of MSCs was compromised by loading of NPs, which could be attributed to the positive surface charge of chitosan NPs. In addition, the modification process required the use of MSCs at low passages, which may indicate the need for further optimization of this technology.

Sadhukha et al. reported an effective tumor-targeting strategy that relied on engineering MSCs with paclitaxel loaded PLGA NPs (nanoengineered MSCs) (Sadhukha et al., 2014). In this study,

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MSCs demonstrated both concentration and time dependent uptake of NPs, with very little effect on cell viability. In addition, the nanoengineering process did not affect the migration or differentiation potential of MSCs *in vitro* and *in vivo*. Nanoengineered MSCs also exhibited a dose-dependent cytotoxicity against A549 human lung adenocarcinoma cells and MA148 human epithelial ovarian carcinoma cells *in vitro*. Most importantly, intravenous injection of nanoengineered MSCs resulted in selective tumor accumulation and retention in an orthotopic lung tumor model, while free NPs were predominantly distributed in the liver and spleen. In a follow up study, Layek et al. evaluated the efficacy of nanoengineered MSCs in the A549 orthotopic lung tumor model (Layek et al., 2018). Nanoengineered MSCs actively homed to the tumor sites and were capable of creating cellular drug depots that released the drug payload over an extended time period. Despite significantly lower total doses of paclitaxel used, nanoengineered MSCs led to more effective tumor growth inhibition and superior survival than solution or NP-encapsulated forms of paclitaxel. Pharmacokinetic and biodistribution studies suggested improved antitumor efficacy of nanoengineered MSCs was primarily due to the higher drug exposure of paclitaxel in the tumor tissue. Furthermore, the common side effects of paclitaxel such as leukopenia were mitigated when the drug was delivered using nanoengineered MSCs, since the dose required to achieve improved anticancer efficacy was much lower compared to that with paclitaxel solution and paclitaxel loaded PLGA NPs. Taken together, these studies established the clinical potential of MSCs as delivery carriers for small molecule drugs.

In a similar study, Wang et al constructed nanoengineered MSCs with paclitaxel loaded PLGA NPs (Wang et al., 2018b). Nanoengineered MSCs showed enhanced and sustained release of paclitaxel compared to free paclitaxel primed MSCs. Also, the nanoengineering process was found to have minimal effect on migration, cell cycle, and differentiation potential of MSCs.

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Additionally, nanoengineered MSCs demonstrated superior efficacy in an orthotopic rat glioma model following contralateral injection compared to that with free paclitaxel primed MSCs or paclitaxel loaded PLGA NPs.

Most of the nanoengineering strategies described previously rely on simple endocytosis to load drug encapsulated NPs into MSCs. The rapid exocytosis of internalized NPs may result in insufficient drug loading and retention. To enhance drug loading in MSCs, Moku et al. evaluated PLGA NPs surface functionalized with transactivator of transcription (TAT) peptide (Moku et al., 2019). It was found that TAT functionalization not only enhanced the intracellular drug accumulation of NPs but also increased their retention. Additionally, treatment with nanoengineered MSCs led to significantly ($p < 0.05$) higher tumor growth inhibition and improved survival in an orthotopic mouse model of lung cancer compared to those with free or NPs encapsulated paclitaxel.

In addition to the tumor-targeted delivery, nanoengineering MSCs have also been used as diagnostic aids for imaging tumors. For example, MSCs loaded with mesoporous silica nanoparticles (MSNs) coated with hyaluronic acid-based polymer (HA-MSNs) and containing imaging agents (FITC, Gd^{3+} and ^{64}Cu and NIR dye ZW800) were used for optical, positron emission tomography (PET), and magnetic resonance (MR) imaging (Huang et al., 2013). Another interesting example is a study conducted by Xu et al., in which MSCs loaded with plasmonic-magnetic hybrid NPs (LDGI: lipids, doxorubicin, gold nanorods and iron oxide nanocluster) were fabricated for chemotherapy, photothermal (PT) therapy, and photoacoustic (PA) imaging of triple negative breast cancer (TNBC) tumors (Xu et al., 2018). LDGI hybrid NPs were efficiently taken up by the MSCs and the iron oxide nanoclusters of the LDGI upregulated CXCR4 expression, enhancing the migration of MSCs towards cancer cells. Light

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irradiated-disassembly of the LDGI NPs facilitated the PT therapy and drug release. *In vitro* studies with MDA-MB 231 breast cancer cell lines demonstrated significant anti-cancer effect upon NIR laser irradiation. Intratumoral and intravenous administration of the MSC-LDGI in tumor models also showed improved migration and penetration to the tumor site, compared with LDGI alone. Therefore, this nanohybrid-loaded MSC delivery system could be developed as a synergistic approach combining chemotherapy, PT therapy and PA imaging for effective cancer treatment.

2.2.2.2 Nanoengineering via surface anchoring

Despite several efforts to improve the therapeutic efficacy of nanoengineered MSCs, inadequate drug loading capacity still restricts their use in small molecule drugs delivery. To overcome the limited drug loading capacity of MSCs, cell surface conjugation or dual drug-loading approaches comprising both endocytosis and membrane-conjugation have been investigated. NPs can be attached to the surface of MSCs by covalent conjugation or by physical association mediated by electrostatic and hydrophobic interactions. For instance, Yao et al. reported a novel nanoengineering strategy involving endocytosis of doxorubicin conjugates accompanied by cell surface anchoring of the drug conjugates via avidin-biotin complex formation (Yao et al., 2017). This strategy resulted in significantly higher drug loading than each of the individual modes. Such modified MSCs predominantly migrated to the lung where the foci of metastatic tumor were present. Importantly, these drug-carrying MSCs significantly inhibited tumor growth and prolonged the survival of tumor-bearing mice compared to free doxorubicin and doxorubicin conjugates.

In another study, Li et al. demonstrated the anchoring of doxorubicin loaded silica nanorattles to MSCs using a cytomembrane antibody-antigen interaction (Li et al., 2011). Silica nanorattles

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were conjugated to antibodies against antigens such as CD90 and CD73, which are expressed on bone marrow derived MSCs (Crisan et al., 2008). Nanorattles were retained within the MSCs for at least 48 hours, which is a sufficient duration for tumor accumulation of the engineered MSCs. Furthermore the engineered MSCs showed selective migration towards U251 glioma tumor cells and efficient doxorubicin delivery with greater and prolonged tumor distribution. It was concluded that this strategy has the potential to improve tumor targeting and to reduce systemic toxicity.

Glycoengineering of MSCs to express synthetic functional groups such as azides is another exciting strategy that can be used to anchor drug loaded NPs on MSC membrane. In metabolic glycoengineering, N-azidoacetylmannosamine-tetraacylated (Ac_4ManNAz) or other azide-containing sugar is metabolized by the cytidine-5′-monophospho-N-acetylneuraminic acid biosynthesis pathway of living cells into N-azidoacetyl neuraminic acid, leading to the cell surface expression of azido-sialic acid containing N_3-linked glycoproteins (Du et al., 2009). Layek et al. demonstrated that culturing MSCs in Ac_4ManNAz supplemented media effectively generated azide-bearing sialic acid on the cell surface without affecting their viability, migration, and differentiation potential (Layek et al., 2016). Azide groups on the surface have been used to conjugate DBCO-functionalized, paclitaxel loaded NPs to MSC, which allowed for significantly increased NP loading in MSCs (Layek et al., 2019). Incorporation of DBCO-functionalized NPs on MSCs did not affect the viability and migration of MSCs. Such nanoengineered MSCs demonstrated greater tumor inhibition and improved survival than the free or NP encapsulated paclitaxel in an orthotopic ovarian tumor mouse model.

Glycoengineered MSCs can also be used as tumor-localized anchors for a novel two-step tumor-targeting strategy. In a previous study, systemic administration of azide-expressing MSCs

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created a highly-dense artificial receptor (azide groups) pool at tumor tissues, which then enhanced the tumor-targeting ability of dibenzyl cyclooctyne (DBCO)-functionalized NPs via copper-free click chemistry (Figure 3). This approach overcomes the lack of tumor specificity observed with conventional ligand-based techniques as these azide targets are not naturally expressed in the living system. Administration of azide labeled MSCs followed by paclitaxel-loaded DBCO-functionalized NPs significantly inhibited tumor growth and enhanced survival compared to paclitaxel loaded NPs in an orthotopic metastatic ovarian tumor model. Although the azide groups expressed on cell surfaces gradually diminish due to the intracellular hydrolysis of glycans by neuraminidase after internalization (Du et al., 2009; Yoon et al., 2017), their presence on the cell surface has been detected at least for 14 days (Lee et al., 2016). Thus, the azide groups can be used for repeated targeting of NPs to the tumor tissue.

Surface glycoengineering has been previously used to modify MSC homing properties to other tissues as well. Sackstein et al. employed glycoengineering approaches for converting native CD44 glycoform available on MSCs surface to express hematopoietic cell E-selectin/L-selectin ligand (HCELL) (Sackstein et al., 2008). Glycoengineering did not affect the viability or potency of MSCs and enhanced binding affinity to E-selectin, thereby increasing their migration ability to bone marrow. Similar strategies of cell-surface glycoengineering were used safely and at reduced MSC payload to direct MSCs to ischemia-reperfusion sites in the porcine heart for improved clinical efficacy and successful regenerative therapy (Lo et al., 2016).

An important consideration in using MSCs for drug delivery is the potential pro-tumorigenic effects of MSCs when they come in contact with tumor cells (Cuiffo and Karnoub, 2012; Rodini et al., 2018; Zong et al., 2018). To address this issue, nanoengineered systems that can kill the MSCs following their tumor accumulation are being evaluated. Kim et al. demonstrated the

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concept of co-destruction of lung tumor cells by MSCs loaded with nanodrug conjugates (Kim et al., 2018). The authors reported that bone marrow-derived MSCs showed specific targeting towards lung tumors compared to others such as breast and brain tumors, and this was attributed to the affinity of MSCs towards CXCL12 and IL-8 secreted by lung cancer cells. MSCs were nanoengineered by conjugation with carbon nanotube (CNT)-doxorubicin (DOX) either through surface engineering by CD73, CD90 or through intracellular uptake. Surface engineered MSCs showed 9-fold higher drug uptake and better tumor homing ability relative to that observed for MSCs in which nanotubes were incorporated through intracellular uptake. Surface engineered MSCs demonstrated higher and prolonged cytotoxicity in H1975 lung cancer cells *in vitro* and significant reduction in tumor growth in A549 lung tumors *in vivo*. It was shown that potential destruction of surface engineered MSCs by secreted chemokines from the dead tumor cells resulted in a bystander effect and improved tumor growth inhibition at a 100-fold lower dose when compared to that with conventional chemotherapeutic approaches.

3. Challenges and future directions (Regulatory considerations for the clinical translation of MSC based cancer therapies)

A key concern with the use of MSCs for drug delivery is their potential to promote tumor growth. Most studies evaluating the use of MSCs for the delivery of anticancer agents have demonstrated tumor suppressive effects. However, other studies suggest that MSCs (in the absence of anticancer agents) can promote tumor growth when co-injected with cancer cells or under hypoxic conditions. Extracellular vesicles released from MSCs have been shown to contain tumor supportive proteins such as PDGFR-β, TIMP-1 etc. (Djouad et al., 2003; Djouad et al., 2006; Zhu et al., 2006; Karnoub et al., 2007; Muehlberg et al., 2009; Vallabhaneni et al., 2015; Lee and Hong, 2017). MSCs were shown to support the growth of tumor vasculature by either

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secreting pro-angiogenic factors such as VEGF (Kinnaird et al., 2004; Potapova et al., 2007) or differentiation into pericytes, which further promote the growth of vasculature (Al-Khaldi et al., 2003). Hwang et al found that MSCs promote the recruitment of tumor fibroblasts, which are known to promote tumor growth (Hwang et al., 2008). In addition, MSCs are considered to possess immunosuppressive properties and promote tumor metastasis (Krampera et al., 2003; Karnoub et al., 2007). Interestingly, MSCs were shown to inhibit tumor capillary growth by intercalating between endothelial cells, establishing gap junctional communications and increased generation of reactive oxygen species (Otsu et al., 2009). The authors concluded that non-functionalized MSCs can be cytotoxic to tumor cells at high numbers. These contradictory observations may indicate the context dependent effects of MSCs. Therefore, it is crucial that the underlying mechanisms behind these observations are further delineated to enable effective clinical translation of MSC based therapies for targeting cancer.

Another potential limitation is that MSCs harvested from different tissues such as bone marrow or adipose share some similar features including morphology and cell surface markers but may exhibit differences in other biological properties such as differentiation capabilities (Xu et al., 2017). Altered *in vivo* phenotype could result in variations in the effectiveness of administered therapies. Thus, it is essential to establish standardized procedures and perform stringent quality control to ensure consistency of MSCs being used (Le Blanc and Davies, 2018).

The number of Investigational New Drug (IND) / Biologic License Application (BLA) submissions to FDA and registered clinical trials of MSC-based products has increased exponentially in the past decade (Olsen et al., 2018). According to NIH database, there are 703 MSC-based clinical trials in different disease areas as of May 1st, 2019 with known status (ClinicalTrials.gov). Several MSC based products have been approved globally as shown in

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Table 3. Despite considerable interest in utilizing MSCs for varied therapeutic purposes, several regulatory hurdles limit the effective clinical translation of MSC-based cell therapies. One of the major hurdles for clinical application of MSCs is the lack of clear understanding of their *in vivo* fate and dose response relationship in humans. There is a clear need to develop effective analytical methods to track MSCs in the body to establish their pharmacokinetic and pharmacodynamics profiles. The fact that MSCs can be isolated and expanded from a plethora of tissues in the body and that MSCs from different sources have slightly varied biological effects potentially increase the regulatory burden. Diversity in the source of MSCs also occurs in allogeneic donors, and there is a trend to move towards autologous sources of MSCs. However, this could increase the cost of MSC-based therapy. MSCs derived from bone marrow of patients with leukemia or multiple myeloma may be contaminated with clonogenic tumor cells and could lead to relapse in these patients (Champlin, 2003).

Additionally, standardized good manufacturing practices (cGMP) need to be established for the production of MSCs. Various cell culture factors such as fetal bovine serum (FBS) concentration, oxygen content and additional growth factor supplements vary widely, leading to a significant variability in the properties of manufactured MSCs (Mendicino et al., 2014). As previously discussed, there is a lack of clear understanding of the molecular mechanisms and pathways associated with the tumor-suppressive or tumor-promoting role of MSCs. The FDA has issued various guidelines for cell and gene therapies to ensure that the products conform to the desired standards and are safe and effective for use (Center for Biologics Evaluation and Research, 1998; Center for Biologics Evaluation and Research, 2011). Also, the International Society for Stem Cell Research (ISSCR) has published “The ISSCR Guidelines for the Clinical Translation of

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Stem Cells" for promoting medication innovation, protecting patients, preserving rigor, and ensuring integrity in stem-cell based therapies (Hyun et al., 2008).

4. Concluding remarks

MSCs have emerged as excellent candidates for cell-based therapy in the fields of tissue engineering, regenerative medicine, and targeted delivery of therapeutics owing to their multipotency, self-renewal and homing abilities. In order to further advance MSC-based therapies, novel approaches are being explored for engineering MSCs with genes, proteins and chemotherapeutics. Nano-engineering MSCs with drug-loaded NPs by either surface conjugation or through internalization can increase the drug-payload that can be delivered to the tumor tissue. Identifying the molecular mechanisms that contribute to the tumor tropism along with tuning the engineering conditions can further improve their effectiveness as drug delivery vehicles.

Authorship contributions

Wrote or contributed to the writing of review: Cheng, Nethi, Rathi, Layek and Prabha

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Funding support received from Minnesota Ovarian Cancer Alliance, Betty and Terry Noble Gift for Ovarian Cancer Research and National Institute of Health [Grant No. RO1EB022558] to S.P.

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Legends for figures

Figure 1. The physical parameters and cell surface molecules of MSCs cooperate to induce active homing of MSCs to tumors. Endothelial cells are green; MSCs are blue; and tumor cells are pink. Although the mechanisms of MSC homing are poorly understood, they likely involve partially overlapping steps of deceleration in the blood flow (which may be partially physical – here depicted as bulging endothelial cells), rolling, adhesion, transmigration through the endothelium, and migration into surrounding tissues. The possible molecular determinants are indicated. The elucidation of the MSC homing mechanism to tumors will facilitate the development of drugs for inhibition of this process (endogenous MSCs), or for understanding how ex vivo cultured MSCs can be engineered to be used as effective and specific drug delivery vehicles. Reproduced from Droujinine IA, Eckert MA and Zhao W (2013) To grab the stroma by the horns: from biology to cancer therapy with mesenchymal stem cells. *Oncotarget* **4**:651-664.

Figure 2. Hypothetical mechanism of action of nanoengineered MSCs. Once nanoengineered MSCs home to tumors, while free drug is effluxed out of the MSCs by efflux transporters, nanoparticles can enter the cells by endocytosis, escape into the cytoplasm and release the drug inside the cells. The free drug inside the cells can be effluxed out into the surrounding tumor cells to elicit anti-tumor response.

Figure 3. In vivo tumor-tropism of glycoengineered MSCs (orthotopic ovarian tumor). (a) MA148-Luc ovarian tumor bearing animals were injected with MSC-Cy5.5 intraperitoneally and imaged at different time points. Tumor free animals injected intraperitoneally with MSC-Cy5.5 were used as control. Representative bioluminescence image (at 1 day) for (i) tumor free and (viii) tumor bearing animal and representative fluorescence images at different time interval for (ii-vii) control and (ix-xvi) tumor bearing animals is shown. (b) Tissue distribution of glycoengineered MSCs at the end of the study. The animals were euthanized at 4 weeks and the organs were collected and imaged. Bioluminescence and fluorescence images respectively of (i, x) kidneys, (ii, xi) brain, (iii, xii) liver, (iv, xiii) abdominal wall, (v, xiv) spleen, (vi, xv) lungs, (vii, xvi) heart, (viii, xvii) ovarian tumor, and (ix, xviii) abdominal tumor are shown. (c) Quantitative fluorescence intensity from the different organs at the end of the study (10 days for control and 28 days for tumor bearing animals). Data represents mean ± SD; n = 3 for treated and 2 for control; *$p < 0.05$ compared to control ovary. Reproduced with permission from Layek B, Sadhukha T and Prabha S (2016) Glycoengineered mesenchymal stem cells as an enabling platform for two-step targeting of solid tumors. *Biomaterials* **88**:97-109.

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Table 1. Ongoing/ completed Clinical Studies using MSCs as Cancer therapeutics (ClinicalTrials.gov; ClinicalTrialsRegister)

Delivery System	Route of Administration	Sponsor	Indications	Development Phase	Status	NCT Number
MSCs secreting interferon beta (IFNβ)	Intraperitoneal	M.D. Anderson Cancer Center, Dallas TX	Ovarian Cancer	Phase 1	Active, not recruiting	NCT02530047
Oncolytic measles virus encoding thyroidal sodium iodide symporter (MV-NIS) infected Adipose Tissue Derived MSCs	Intraperitoneal	Mayo Clinic, Rochester MN	Recurrent Ovarian Cancer	Phase ½	Recruiting	NCT02068794
Bone marrow-derived autologous MSCs infected with ICOVIR5, an oncolytic adenovirus (CELYVIR)	Intravenous	Hospital Infantil Universitario Niño Jesús, Madrid, Spain	Metastatic and Refractory Solid Tumors	Phase ½	Completed	NCT01844661
MSCs genetically modified to express TNF-related apoptosis-inducing ligand (TRAIL)	Intravenous	University College, London	Lung Adenocarcinoma	Phase ½	Recruiting	NCT03298763
Autologous human MSCs genetically modified to express Herpes-simplex-virus thymidine kinase (HSV-TK), which catalyzes the phosphorylation of the prodrug ganciclovir to the toxic compound ganciclovir triphosphate	Intravenous	Apceth GmbH & Co. KG, Germany	Advanced Gastrointestinal Cancer	Phase ½	Completed	2012-003741-15 (EudraCT number)

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Table 2. Nanoengineered MSCs used for tumor targeted drug delivery.

Delivery system	Engineering technique	Tumor model	Route of administration	Effect	Reference
Paclitaxel loaded PLGA NPs	Endocytosis	A549 Lung Adenocarcinoma and Lewis Lung Carcinoma	Intravenous injection	• Improved biodistribution in lung tumors • Improved anti-tumor efficacy • Reduced dose dependent toxicity	(Layek et al., 2018)
DOX-polymer conjugates	Receptor dependent endocytosis	Intracerebral Glioma	Intracranial administration	• Enhanced penetration and sustained release of doxorubicin in tumor • Improved survival	(Zhang et al., 2015)
Biotinylated DOX-polymer conjugates	Endocytosis and membrane anchoring via avidin-biotin complex	Metastatic Pulmonary Breast Cancer	Intravenous injection	• Improved payload capacity • Improved survival and tumor inhibition	(Yao et al., 2017)
TAT-functionalized paclitaxel loaded PLGA NPs	Cell penetrating peptide mediated endocytosis	A549 Lung Adenocarcinoma	Intravenous injection	• Improved payload capacity • Improved survival and tumor inhibition	(Moku et al., 2019)
Plasmonic-magnetic hybrid NPs (lipids, doxorubicin, gold nanorods and iron oxide nanoclusters)	Endocytosis	Triple Negative Breast Cancer (TNBC)	Intratumoral and intravenous injection	• Enhanced migration and penetration • Improved antitumor efficacy	(Xu et al., 2018)
Antibody conjugated carbon nanotube (CNT)-doxorubicin conjugate	Cell surface anchoring via antibody–antigen recognitions	A549 lung adenocarcinoma	Intravenous injection	• Improved survival and tumor inhibition	(Kim et al., 2018)
Antibody conjugated doxorubicin-loaded silica nanorattles	Cell surface anchoring via antibody–antigen recognition	Glioma	Intratumoral administration	• Improved penetration and tumor kill	(Li et al., 2011)
Doxorubicin loaded PLGA NPs	Simple endocytosis	Metastatic Lung tumor	Intravenous injection	• Enhanced targeting and permeation into tumor nest • Significant reduction of lung metastases	(Zhao et al., 2017)

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Table 3. Marketed MSC based Products.

Products	Types of MSCs	Indications	Company	Country of Approval	Year of Approval
Queencell®	Autologous adipose derived	Connective tissue disorder	Anterogen Ltd.	South Korea	2010
Cartistem®	Allogeneic umbilical cord blood	Traumatic and degenerative osteoarthritis	Medipost Ltd.		2011
Cellgram®-AMI (Hearticellgram®-AMI)	Autologous bone marrow derived	Acute myocardial infraction	FCB-Pharmicell Ltd.		2011
Cupistem®	Autologous adipose derived	Crohn's fistula	Anterogen Ltd.		2012
Neuronata-r®	Autologous bone marrow	Amyotrophic Lateral Sclerosis (ALS)	Corestem Inc.		2014
TEMCELL® HS	Allogeneic	Acute radiation injury	JCR Pharmaceuticals Ltd., Licensee of Mesoblast Ltd.	Japan	2016
Stempeucel®	Allogeneic stromal	Critical Limb Ischemia	Stempeutics Research Pvt. Ltd.	India	2016
Alofisel® (Darvadstrocel)	Allogeneic adipose tissue	Complex perianal fistulas in adult patients with non-active/mildly active luminal Crohn's disease	Takeda Pharmaceutical Company Ltd.	Europe	2018

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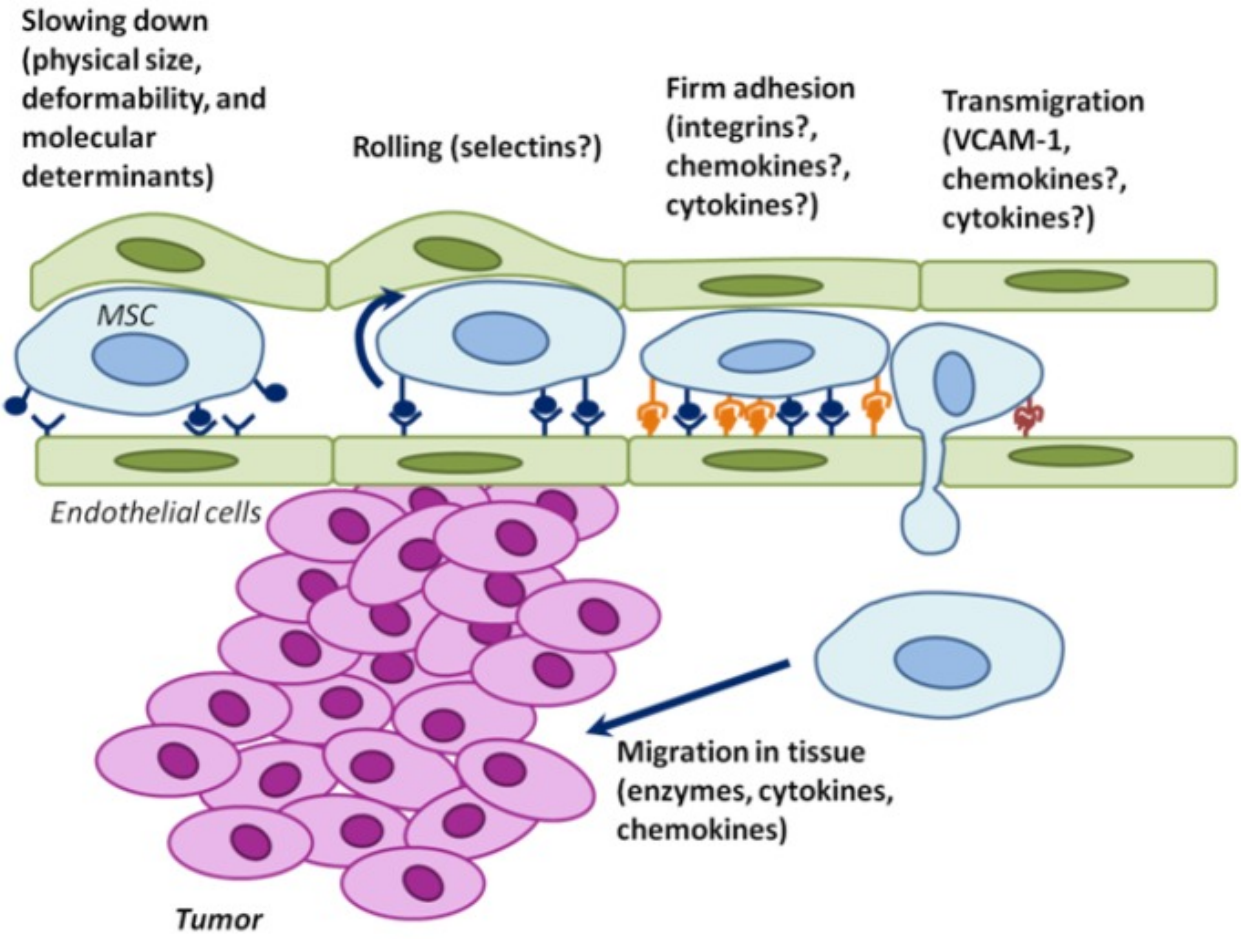

Figure 1

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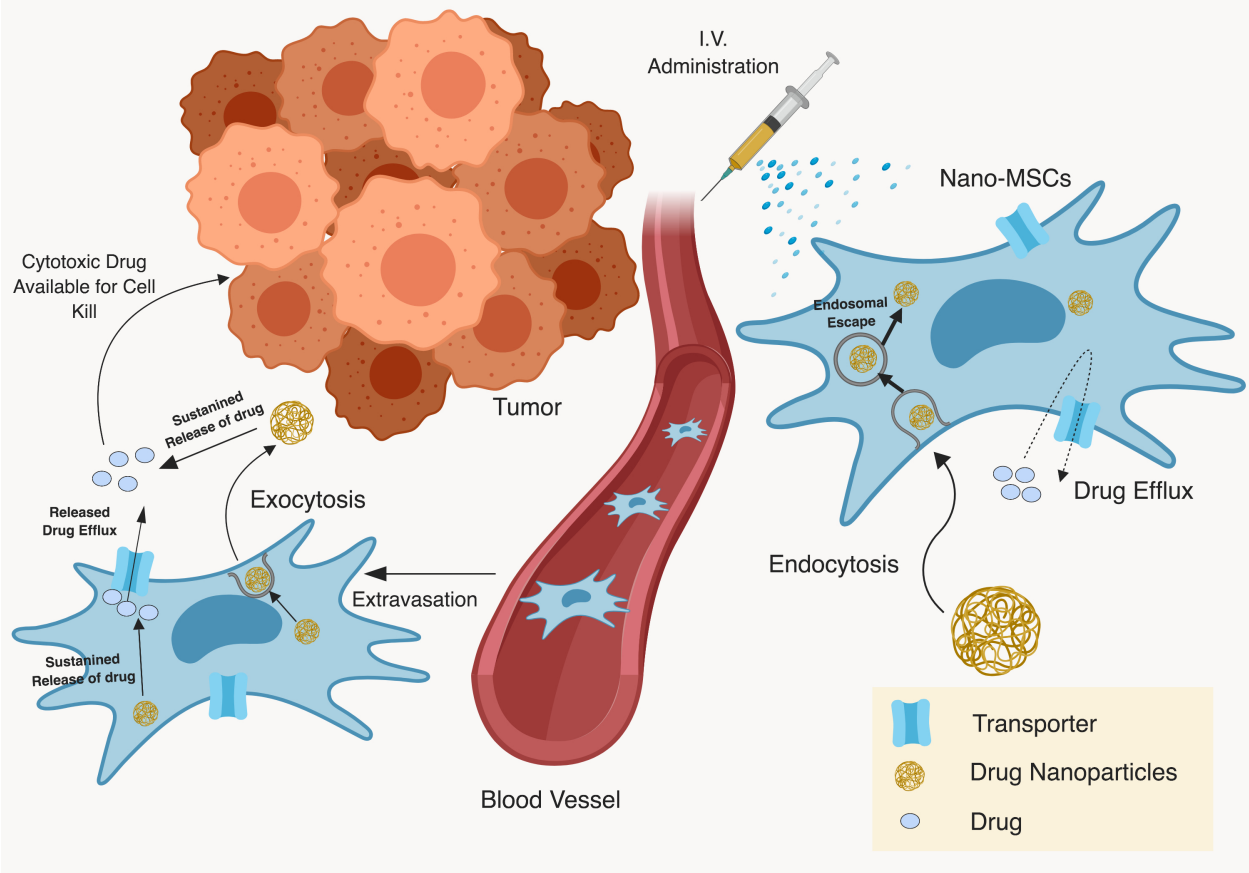

Figure 2

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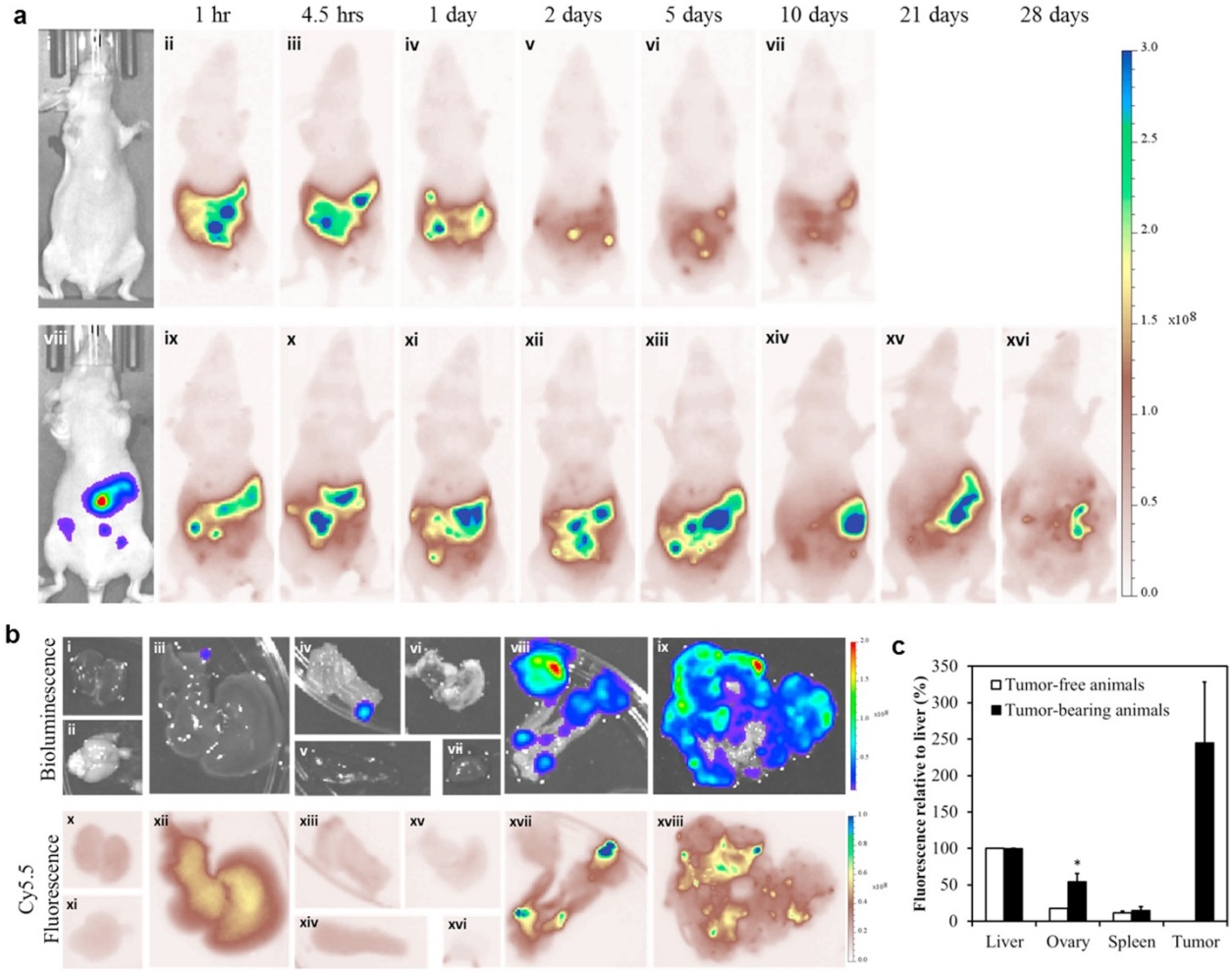

Figure 3

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