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Mesenchymal Stem Cells as Vehicles for Targeted Therapies

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1. Introduction

1.1 Challenges of conventional drug delivery

Traditional drug delivery systems include oral drug administration, injection, infusion, and topical administration, where the drug is applied to body surfaces such as the skin or mucous membranes. Many of the conventional drug delivery systems distribute the pharmaceutical compound proportionally to the regional blood flow through the systemic blood circulation. Consequently, the drug is delivered indiscriminately throughout the whole body to diseased and healthy tissues. As a result, patients suffer from side effects due to the non-specific delivery of the drug. Systemic delivery of a drug with body-wide distribution also results in a limited availability of the therapeutic agent at the site of interest, lowering the ability of the drug to produce a beneficial effect. To compensate for the low availability of the drug at the affected site, the drug has to be administered in large quantities, resulting in increased drug toxicity as well as high therapy costs. Another drawback of systemic drug delivery is the short circulation half-life of many drugs, which leads to the administration of high drug concentrations or high dosing frequencies (Branco & Schneider, 2009).

Pain and discomfort caused by frequent drug applications are another challenge of conventional drug delivery, especially for children and the elderly. First steps have been made in the development of micro needle injection and needle-free injection to reduce the pain and inconvenience of injections (Brunner, 2004; Stoeber & Liepmann, 2002). As beneficial as mechanical improvements in drug delivery will be for the patient's comfort and compliance, they will not reduce the number of administrations or the amount of required therapeutic. They also will not affect drug toxicity or effectiveness. To maximize the therapeutic effect of a drug, the appropriate concentration of the drug has to be available at the right location and time, while sparing healthy tissues. Therefore, new tools are needed that enable the delivery of drugs directly to the diseased area, and/or release the therapeutic agent in a controlled way.

In this chapter, vehicles for the targeted delivery of drugs will be discussed, with special focus on the potential use of human mesenchymal stem cells (hMSCs) for targeted therapies. Besides the development of targeted drug delivery tools, efforts in the medical field attempt to increase the efficiency of conventional applications. An example is topical drug delivery, which has profited from the introduction of new topical applications including transdermal patches (Brunner, 2004), use of microneedles (Henry et al., 1998), electroporation techniques

(Escobar-Chavez et al., 2009), and the development of pulmonary delivery methods (Brunner, 2004). Additional methods to improve conventional drugs include sustained and controlled release technologies and enhanced absorption technology to provide more efficient drug absorption and increased bioavailability, as well as reduce pain from administration and improve ease of use (Brunner, 2004). These topics will not be further addressed in this chapter.

1.2 Advantages of targeted drug delivery

The ultimate goal of drug delivery is the efficient and timely transport of a drug to a diseased tissue, within the therapeutic and outside the toxic range, while sparing any healthy tissue. To progress towards this end, controlled drug delivery systems are being developed that can 1) control the rate of drug release, 2) control the location of drug release (spatial/targeted delivery), 3) or achieve both, temporal and spatial control of drug delivery (Hilt, 2010). Controlled delivery systems require the ability to localize and target drug action, extend drug action at a predetermined rate, and provide a physiologically/therapeutically based drug release system, which controls the rate of drug release based on the physiological/therapeutic needs of the body (Ding et al., 2006).

Targeted drug delivery seeks to concentrate the medication in the tissues of interest while reducing the relative concentration of the medication in the remaining tissues. As a result, a high local drug concentration and low systemic exposure is achieved which helps to improve the drug's effectiveness while lowering its damaging effects on healthy tissue. Especially in cancer therapy requiring highly toxic drugs, there is a great need for vehicles that transport drugs in a safe manner, very specifically to the diseased sites.

Targeted drug delivery can rely on passive or active mechanisms. Passive targeting is mediated by the enhanced permeability and retention (EPR) effect, which is based on the longevity of the pharmaceutical carrier in the blood and its accumulation in pathological sites with compromised vasculature (Haley & Frenkel, 2008; Ruoslahti et al., 2010). Delivery tools relying on passive targeting mechanisms have limited target specificity, as passive targeting depends on the EPR effect, and thus on the degree of vascularization and angiogenesis of the targeted site. In cancer therapy, passive targeting makes extravasation of nanocarriers dependent on tumor type and tumor location. Active targeting, in contrast, is based on the attachment of specific ligands to the surface of pharmaceutical carriers to recognize and bind pathological target cells. The targeting ligands can be monoclonal antibodies, antibody fragments or non-antibody ligands, some of which will be discussed in the review. Despite the discrimination of active and passive mechanisms, it is important to keep in mind that active targeting cannot be separated from the passive because it occurs only after passive accumulation in the targeted site (Bae, 2009; Danhier et al., 2010).

In summary, targeted drug delivery systems have several advantages over common systemic drug delivery methods (see Figure 1). The ability to convey the therapeutically active molecule only to the site of action, without affecting other organs and tissues, increases the therapeutic index, and allows for a lower required drug dose or dose frequency. This in turn increases the safety profile of the drug, and reduces side effects and risks, as less healthy tissue is targeted (Ruggiero et al., 2010). Equally important is the impact on the patient's comfort, which will improve as a result of lower drug dose and side effects. Finally, the economic benefits of decreased drug use should be appreciated. The next section provides an overview of recent progress in the development of active and passive

systems for the targeted delivery of drugs, with a brief description of intracellular targeting strategies.

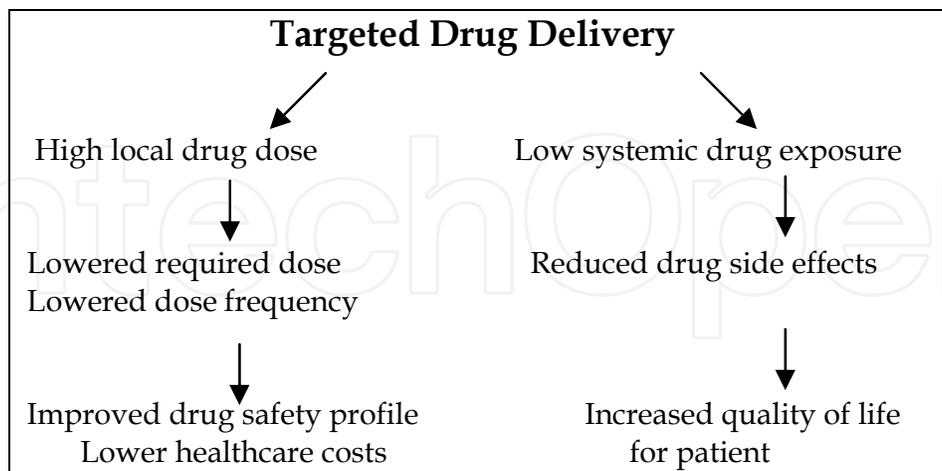


Fig. 1. Advantages of targeted delivery tools over conventional drug delivery methods

2. Recent progress in the development of targeted drug delivery tools

2.1 Nanoparticles

Among other targeted drug delivery systems, nanoparticles have recently drawn strong interest in the medical community because of their utility as carriers. Nanoparticles come in a variety of sizes and shapes, like spheres, tubes, shells, and branched structures, and include among others, liposomes, quantum dots, nanospheres, nanocapsules, nanotubes, dendrimers, micelles, and fullerenes. One important aspect of nanoparticles is their limited size of up to 100nm (although the upper limit can vary in the literature), which enables them to pass through fenestrations of compromised leaky endothelium. As leaky epithelium is characteristic for tumors and their environment, nanoparticles can accumulate at tumor sites mediated by the EPR effect, and therefore can be used as a carrier for cancer therapeutics to the tumor (Danhier et al., 2010; Haley & Frenkel, 2008; Lowery et al., 2011). Besides being used in a passive drug delivery process, nanoparticles can be coupled to ligands which interact with their receptors at the target cell site and used in an active targeting process (Haley & Frenkel, 2008). Therapeutics can be encapsulated, entrapped, or attached to the nanoparticle surface and delivered to the tumor by the nanoparticles.

The small size of nanoparticles also has a drawback. It results in the fast clearance of nanoparticles by the mononuclear phagocyte system (MPS), also called reticulo-endothelial system (RES), which is predominantly distributed in liver, lung, spleen, and bone marrow. Unless there is desired drug delivery to those organs, nanoparticles have to be surface modified with molecules like polyethylene glycol (PEG) to escape the MPS (Haley & Frenkel, 2008). PEG creates a steric barrier and prevents the interaction of nanoparticles with opsonins and phagocytic cells (Ishihara et al., 2009). Yang et al. (Yang et al., 2007) showed that the PEGylated liposomal formulation of paclitaxel significantly reduced the uptake by the MPS, while accumulation of liposomes at the tumor site, as well as biological half-life, were increased. Several drugs using nanoparticle carriers are already in preclinical and clinical use (Adisheshaiah et al., 2009; Bawa, 2008; Haley & Frenkel, 2008; Kim et al., 2010; Lowery et al., 2011; Ochekepe et al., 2009; Tuscano et al., 2010; Zhang et al., 2008), like

PEGylated doxorubicin (Doxil), and PEGylated daunorubicin (DaunoXome). Table 1 lists examples of approved nanoparticle-based therapeutics in clinical use.

| Brand Name | Description | Nanostructure | Approved Indications |
|----------------|--|---------------------------------|--|
| Abelcet | Amphotericin B lipid complex injection | Liposomes | <ul style="list-style-type: none"> Invasive fungal infections in patients refractory or intolerant to amphotericin B |
| Abraxane | Albumin-bound nanoparticle formulation of Paclitaxel (Taxol) | Protein nanoparticles | <ul style="list-style-type: none"> Breast cancer after failure of combination chemotherapy for metastatic disease or relapse within 6 months of adjuvant chemotherapy |
| Adagen | Pegademase bovine | Polymeric nanoparticles | <ul style="list-style-type: none"> Adenosine deaminase deficiency in patients with severe combined immunodeficiency disease who failed or are not suitable candidates for bone marrow transplantation |
| AmBisome | Liposomal amphotericin B | Liposomes | <ul style="list-style-type: none"> Systemic or disseminated infections due to <i>Candida</i>, <i>Aspergillus</i>, or <i>Cryptococcus</i> in patients who are refractory to or intolerant of conventional amphotericin B therapy, or have renal impairment Visceral leishmaniasis |
| Amphotec | Amphotericin B lipid complex | Lipid colloidal dispersion | <ul style="list-style-type: none"> Invasive aspergillosis in patients refractory, or intolerant to amphotericin B |
| Copaxone | Glatiramer acetate injection | Polymeric nanoparticles | <ul style="list-style-type: none"> Relapsing-remitting multiple sclerosis, including patients who have experienced a first clinical episode and have MRI features consistent with multiple sclerosis |
| DaunoXome | Daunorubicin citrate liposome injection | Liposomes | <ul style="list-style-type: none"> Advanced AIDS-related Kaposi's sarcoma |
| Depocyt | Cytarabine liposome injection | Liposomes | <ul style="list-style-type: none"> Intrathecal treatment of lymphomatous meningitis |
| Diprivan | Propofol liposomes | Liposomes | <ul style="list-style-type: none"> Induction and maintenance of anesthesia |
| Doxil / Caelyx | Doxorubicin HCl liposome injection | Liposomes | <ul style="list-style-type: none"> Progressed or refractory ovarian cancer AIDS-related Kaposi's sarcoma in patients with intolerance to, or failure of prior systemic chemotherapy Myeloma in combination with bortezomib in patients who have not previously received bortezomib and have received at least one prior therapy |
| Elestrin | Estradiol gel incorporating calcium phosphate nanoparticles | Calcium phosphate nanoparticles | <ul style="list-style-type: none"> Moderate-to-severe vasomotor symptoms (hot flashes) associated with menopause |

| Brand Name | Description | Nanostructure | Approved Indications |
|-------------------------------------|--|--------------------------|--|
| Epaxal* (Switzerland, Canada) | Hepatitis A vaccine adjuvanted with immunopotentiating reconstituted influenza virosomes | Liposomes | • Active immunization against hepatitis A |
| Estrasorb | Estradiol micellar nanoparticles | Liposomes | • Vasomotor symptoms in menopausal women |
| Feridex | Ferumoxides injectable solution | Iron oxide Nanoparticles | • Contrast agent for magnetic resonance imaging of liver lesions |
| Macugen | Pegylated anti-VEGF aptamer | Polymeric nanoparticles | • Neovascular age-related macular degeneration |
| Myocet* (Europe, Canada) | Nonpegylated liposomal doxorubicin | Liposomes | • Metastatic breast cancer in combination with cyclophosphamide |
| Oncaspar | Pegaspargase | Polymeric nanoparticles | • First-line treatment of patients with acute lymphoblastic leukemia as a component of a multiagent chemotherapy regimen |
| Neulasta | Pegfilgrastim (PEG-rmetHuG-CSF) | Polymeric nanoparticles | • Decrease incidence of infection manifested by febrile neutropenia in patients with non-myeloid malignancies receiving myelosuppressive anti-cancer drugs associated with a clinically significant incidence of febrile neutropenia |
| Pegasys | Peginterferon alfa-2a | Polymeric nanoparticles | • Chronic hepatitis C in patients coinfecting with hepatitis C and HIV |
| PEGIntron | Peginterferon alfa-2b | Polymeric nanoparticles | • Chronic hepatitis C infection with compensated liver disease |
| Resovist | Carboxydextran superparamagnetic iron oxide formulation | Iron oxide nanoparticles | • Contrast agent for magnetic resonance imaging of liver lesions |
| Somavert | Pegvisomant | Polymeric nanoparticles | • Acromegaly in patients with inadequate response to surgery and/or radiation therapy and/or other medical therapies, or for whom these therapies are not appropriate |
| Triglide | Nanocrystalline fenofibrate | Nanocrystals | • Primary hypercholesterolemia, mixed dyslipidemia, and hypertriglyceridemia, for use in conjunction with diet |
| Verigene | Gold nanoparticles | Gold nanoparticles | • <i>In vitro</i> diagnostics: genetic test for warfarin sensitivity |

* Not U.S. Food and Drug Administration (FDA) approved

Table 1. Examples of approved nanoparticle-based therapeutics in clinical use

2.2 Ligand-targeted therapeutics

Ligand-targeted therapeutics are based on the selective delivery of drugs to target cells by associating drugs with molecules that bind to antigens or receptors uniquely expressed or over-expressed on the target cell relative to normal cells (Allen, 2002). Such targeting ligands can be monoclonal antibodies, antibody fragments, and non-antibody ligands (Danhier et al., 2010). The drug of interest is directly conjugated to these targeting ligands, or loaded onto high capacity drug carriers, which are directly conjugated to targeting proteins or derivatized for interactions with specific adapters that are conjugated to the targeting protein (Backer et al., 2002). Ligand-targeted delivery systems rely on active targeting mechanisms, which help to improve target specificity, as the target ligands can act as “homing devices”, improving the selective delivery of drug to specific tissue and cells (Danhier et al., 2010).

Non-antibody targeting ligands include small molecules (folic acid, galactose), peptides (Arginine-Glycine-Aspartic acid (RGD), Vascular Endothelial Growth Factor (VEGF) peptide), aptamers (pegaptanib), and proteins, like transferrin and luteinizing hormone releasing hormone (Allen, 2002; Yu et al., 2010). Despite the advantage that non-antibody ligands are often readily available and inexpensive to manufacture, many of them bind relatively non-selectively to target and non-target tissue (Allen, 2002). For this reason, antibody ligands with higher cell selectivity, e.g. Anti-Human Epidermal Growth Factor Receptor 2 (HER2/neu/ERBB2), Anti-Vascular Endothelial Growth Factor Receptor (VEGFR), Anti-CD20, and Anti-CD33, have gained research attention (Allen, 2002; Park et al., 1997, 2002). However, the fact that antibody-targeted therapies rely on the expression of specific antigens is at the same time a drawback, as antigen expression is likely to change between patients, type of disease and time (Loebinger & Janes, 2010).

To enable the delivery of highly potent cytotoxic agents to antigen-expressing cells, antibody-drug conjugates (ADCs) were designed, which take advantage of the site specificity of antibodies. The key components of an ADC are 1) the cytotoxic agent, 2) a monoclonal antibody targeting a tumor-enriched or tumor specific antigen, and 3) a linker that covalently binds these components together (Alley et al., 2010; Chari, 2008; Krop et al., 2010). Unfortunately, the clinical success of ADCs so far has been very limited. Only gemtuzumab ozogamicin (Pfizer), an anti-CD33 monoclonal antibody linked to calicheamicin, had been approved by the FDA in the year 2000 for the treatment of patients with acute myeloid leukemia, but the product was voluntarily withdrawn from the US market in 2010 after results from a clinical trial raised concerns about the product's safety and clinical benefit (Beck et al., 2010, 2011). Several ADC therapies are in clinical testing, including trastuzumab-DM1 (T-DM1) for breast cancer, brentuximab vedotin (SGN-35) for Hodgkin lymphoma (HL) and anaplastic large cell lymphoma (ALCL), and inotuzumab ozogamicin (CMC-544) for non-Hodgkin lymphoma (NHL) (Alley et al., 2010). T-DM1 is an antibody-drug conjugate which uses the HER2-binding antibody trastuzumab to deliver the potent antimicrotubule agent DM1 to HER2-expressing cells (Krop et al., 2010). A first clinical study with HER2-directed ADC in patients with HER2-positive metastatic breast cancer showed a clinical benefit rate (objective response plus stable disease at 6 months) of 73% among 15 patients treated at the maximum tolerated-dose. The confirmed response rate among patients with measurable disease in this group was 44%. Based on those results, Burries et al. (Burris et al., 2011) evaluated T-DM1 treatment in patients with HER2-positive metastatic breast cancer who experienced progression on HER2-directed therapy in a single-

arm phase II study (study ID: TDM4258g). Among 112 treated patients, an objective response rate (ORR) of 26% was observed by independent assessment, which is comparable to the ORR of other HER2 therapies (Burriss et al., 2011). The fact that response rates were higher among patients with confirmed HER2-positive tumors and among patients whose tumor HER2 expression levels were above the median, emphasizes the need for patient prescreening for HER2 target expression levels to obtain optimal results.

Another ADC in advanced clinical development is SGN-35 (Seattle Genetics and Millennium Pharmaceuticals), comprised of an anti-CD30 monoclonal antibody linked to monomethyl auristatin E. CD30, is a defining marker of HL, but also expressed on other cancers, including ALCL. HL and ALCL patients were treated intravenously with SGN-35 every 3 weeks for up to 16 cycles. With an objective response rate of 75% in HL and 86% in ALCL, SGN-35 represents the most active ADC reported. In February 2011, a biologics license application was submitted to the FDA (Beck et al., 2011; Deutsch et al., 2011). Many other ADCs are in early clinical trials and future results will reveal their benefit for clinical use.

2.3 Cell penetrating peptides

The transport of a therapeutic from the site of administration to the site of interest is not the only phase in drug delivery that can be controlled. After arrival of the drug at the site of a diseased tissue, it might also be necessary to control the transport of the drug across the plasma membrane of the targeted cells. Vehicles that facilitate and control intracellular transport are being developed and include physical delivery strategies, like electroporation, and biochemical delivery strategies, like cell-penetrating peptides (CPPs). CPPs are also named protein transduction domains and comprise short and usually basic amino acids-rich peptides originating from proteins able to cross biological barriers (Chou et al., 2011; Hassan & Elshafeey, 2010; Vives et al., 2008). They are able to act as vectors for the delivery of chemically conjugated biomolecules like peptides or oligonucleotides, and allow for viral-free transduction which eliminates the risk of virus vector induced complications. Besides the protein transduction domains (penetratin and TAT (48-60)), there are chimeric CPPs (MPG, transportan), synthetic CPPs (oligoarginine), and peptidic vectors designed from structure-activity studies on already known CPPs (Pip2b, stearylated-Tp10). The mechanisms of internalization of CPPs are still controversial, and might be diverse, depending on CPP, cell type and cell cargo (Hassan & Elshafeey, 2010; Sawant & Torchilin, 2011).

The most frequently used CPP is the TAT peptide (TATp), derived from the transcription activator protein encoded by human immunodeficiency virus type 1 (Torchilin, 2008). TATp can be covalently linked to many drug classes, including large protein molecules, and was used to transduce attached cargoes into cells of all organ types (Sawant & Torchilin, 2011). Responsible for the transduction ability of the TAT protein is the positive charge in the transduction domain which extends from residue 47 to 57. As for other CPPs, different mechanisms have also been proposed for the endocytic uptake of TATp, including classical clathrin-mediated endocytosis and clathrin-independent lipid raft-mediated caveolae endocytosis (Torchilin, 2008).

Still, there are some challenges that must be overcome, like sequestration and entrapment of internalized material within endocytic vesicles, before CPPs can become a valuable clinical tool. Tools to enhance endosomal escape of CPP-attached cargoes are developed and include the use of pH sensitive proteins, fusogenic lipids, membrane disruptive peptides, polymers, and lysomotropic agents. Another drawback is the lack of selectivity of certain CPPs, which

raises concerns about drug-induced toxicity in normal tissues (Chou et al., 2011; Sawant & Torchilin, 2011). The problem might be solved by combining target specific drug carriers with CPPs, to assure both delivery of the drug to the target cell and delivery of the drug from the outside of the cell into the cytoplasm of the target cell.

2.4 Cells as delivery vehicles

The ability of hematopoietic and non-hematopoietic cells to migrate to sites of injury, inflammation, and infection makes them attractive for investigation as a potential drug delivery vehicle. Cells can be loaded with drugs or modified to produce them, and then be used to carry the drug to the site of interest. The cell modifications can be done in many ways, including genetic cell engineering, and culture-induced modifications.

Genetically-modified cells used in clinical trials are mainly autologous hematopoietic cells which are isolated from the patient, modified and reintroduced into the patient. Among hematopoietic cells, lymphocytes are the most commonly genetically modified cell population used in clinical trials, with lymphocytes expressing T-cell receptor (TCR) or Interleukin-12 (IL-12) for the treatment of advanced melanoma, and lymphocytes expressing Anti-P53 TCR, Anti-carcinoembryonic antigen (CEA), Anti-melanoma antigen family (MAGE)-A3/12 TCR, Anti-HER2, and Anti-NY ESO-1 (a cancer/testis antigen) TCR for the treatment of other metastatic cancers (see clinicaltrials.gov). The disadvantage of hematopoietic cells as delivery vehicle, and in transfusion medicine in general, is their immunogenicity. To avoid immune reactions of the patient's immune system against the introduced cells, and attacks of the transplanted material against the recipient's body, autologous cells or ABO- and human leukocyte antigen (HLA)-matched cells must be used. An alternative to the use of hematopoietic cells are non-hematopoietic mesenchymal stem cells (MSCs), which are known for their low immunogenicity. The ability to use MSCs in an allogeneic setting eliminates the time consuming step of collecting stem cells from the patient and allows for the use of frozen, off-the-shelf cell products. The delay between diagnosis and availability of cells would be eliminated. In the following sections, MSCs in general and their use as potential drug delivery vehicles are discussed.

3. Mesenchymal Stem Cell biology

The therapeutic potential of MSCs is linked to a broad spectrum of MSC biological activities such as anti-inflammatory, immunomodulative and tissue reparative activities via paracrine mechanisms. Besides those activities, MSCs have the unique ability to home to sites of inflammation/injury and tumors, which makes them useful for the delivery of therapeutics to these sites. Cells are an ideal vehicle for targeted drug delivery, since they can be loaded with therapeutic agents and have the ability to migrate to sites of disease. This section describes current understanding of MSCs, their characteristics and biological activities, and current experience with MSCs in clinical trials that supports their use for targeted drug delivery.

3.1 Background on MSCs

The ability to generate an embryo from a single fertilized oocyte or to regenerate tissues upon injury or natural physiological turnover is a direct result of stem cells. As the embryo first develops, an undifferentiated mass of totipotent embryonic stem cells (ESCs) will form

a multicellular organism. As development proceeds, totipotent ESCs disappear as more restricted somatic stem cells (SSCs) give rise to the tissues and organs. Although cell diversification is mostly completed at or shortly after birth, organs must have a mechanism to replenish cells as they die as a result of natural homeostasis or injury. Therefore, after birth life-long reservoirs of SSCs are present in the body. Major characteristics of stem cells that distinguish them from all other cells include (1) self-renewal, or the ability to generate at least one daughter cell with characteristics similar to the initiating cell; (2) multi-lineage differentiation potential of a single cell; and (3) *in vivo* functional reconstruction of a given tissue. Adult SSCs fulfill these criteria, however the degree of self-renewal and differentiation potential are restricted in comparison to ESCs. There are several types of SSCs in the body including MSCs.

MSCs were described as precursors of fibroblasts, which were isolated from bone marrow by Friedenstein in 1970 (Friedenstein et al., 1970). Upon culture at low density either as whole bone marrow or after cell separation over a density gradient, the cultured cells form characteristic colonies derived from a single precursor, referred to as colony forming unit fibroblasts or CFU-F. After ectopic transplantation under the kidney capsule, these cells gave rise to a broad spectrum of differentiated connective tissues including bone, cartilage, adipose and myelosupportive stroma (Owen, 1988; Prockop, 1997). Based on these observations it was proposed that these mesenchymal origin tissues are derived from a common precursor cell residing in bone marrow, termed the mesenchymal stem cell. These observations also led to the development and wide use of the colony forming unit-fibroblasts (CFU-F) assay used to estimate MSC frequency among bone marrow nucleated cells. Using this technique, MSCs have been identified as a rare population of cells in bone marrow, representing ~0.001-0.01% of the nucleated cells (Pittenger & Martin, 2004). Estimation of MSC frequency in bone marrow using CFU-F shows that MSC number declines with age (Caplan, 2007), which correlates with poor mesenchymal tissue healing. This poor capacity for healing is evident, for example, in broken bones in elderly individuals.

Adult, tissue specific stem cells are found in specialized niches in their corresponding tissues of origin. For example, hematopoietic stem cells (HSCs) can be found in bone marrow and epidermal stem cells are located in mammalian hair follicles. In contrast, cell types originated from MSCs are present through the entire body, and it has been shown that MSCs can be isolated from virtually all organs. There are three hypotheses regarding the location of MSCs in the body. In the first, MSCs are located in only one specific organ or tissue, from which they can migrate to other sites via the blood circulation to replenish the cell population. However, the number of MSCs circulating in blood is extremely low or undetectable. The difficulty establishing MSC culture from peripheral blood votes against this possibility. The second possibility is that MSCs are present in different tissues: MSCs have been successfully isolated from various tissues in addition to bone marrow. These tissues include adipose, periosteum, tendon, muscle, synovial membrane, skin and many others. When cultured *in vitro*, MSCs derived from different tissues show very similar characteristics and functionality, suggesting that different tissue-intrinsic stem cells might behave as MSCs when characterized *in vitro*. And the third possibility is that the MSC niche *in vivo* is the perivascular zone of blood vessels, and pericytes have all characteristics of MSCs. In this scenario, MSCs can actively participate in tissue repair after the release from blood vessels upon the damage in any tissue. Several experimental data support this hypothesis (da Silva Meirelles et al., 2008).

3.2 MSC immune privilege

One of the most significant advantages of MSCs is that they can be used allogeneically. *In vivo* studies have demonstrated that tissues of mesodermal origin including bone, cartilage, and connective tissues, derived from MSCs, can be successfully transplanted without matching with a low incidence of acute rejection (Bacsich & Wyburn, 1947; Girdler, 1997). Thus, the mesodermal origin of MSCs suggests they are not recognized as foreign by the recipient immune system. Data accumulated *in vitro* and *in vivo* support the concept of MSCs as universally tolerated stem cells, rationalizing the transplantation of allogeneic MSCs without donor-recipient HLA matching. The universality is based on the low immunogenicity profile of MSCs. *In vitro* characterization has shown that MSCs constitutively express low levels of HLA class I molecules and do not express HLA class II molecules on the cell surface and co-stimulatory molecules like CD40, 80 and 86, which are essential for initiation of the immune response (Klyushnenkova et al., 2005; Tse et al., 2003). The absence of co-stimulatory molecules may lead to tolerance induction instead of rejection of allogeneic MSCs. The absence of host immune response against allogeneic MSCs has been demonstrated *in vitro* (Bartholomew et al., 2002; Di Nicola et al., 2002; Tse et al., 2003) and *in vivo* in animals (Atoui et al., 2008; Chen et al., 2009) and humans (Kebriaei et al., 2009; Le Blanc et al., 2004; Prasad et al., 2011; Sundin et al., 2007).

3.3 MSC biological activities

3.3.1 Regulation of hematopoiesis (stromal support)

The critical role of bone marrow stroma for homing and long-term maintenance of hematopoiesis in mammalian bone was demonstrated early on by Friedenstein (Friedenstein et al., 1974). MSCs secrete a variety of cytokines, chemokines, and growth factors supporting hematopoietic cell expansion and maturation (Caplan, 2007; Deans & Moseley, 2000). Stromal Derived Factor-1 (SDF-1), which is produced by bone marrow stromal cells and mediates HSC homing and engraftment to the bone marrow, is one example of such chemokines. MSCs also play a critical role in megakaryocyte development: MSCs express thrombopoietin (TPO), IL-6, IL-11, Leukemia inhibitory factor (LIF) and Stem Cell Factor (SCF), which are critical regulators of megakaryopoiesis (Cheng et al., 2000). In addition to the regulation of megakaryopoiesis, MSCs also support cells of myeloid and lymphoid lineages. The ability of MSCs to support hematopoiesis was used in patients with hematopoietic cell graft failures. Co-administration of MSCs together with hematopoietic stem cells enhanced engraftment and accelerated neutrophil, platelet, and lymphoid cell recovery (Ball et al., 2007; Fouillard et al., 2003, 2007; Koc et al., 2000; Lazarus et al., 2005; Le Blanc et al., 2007). However, administration of MSCs together with hematopoietic cells resulted in high chimerism for hematopoietic cells in both blood and bone marrow, and microchimerism for MSCs – the majority of MSCs remained of host origin (Bacher et al., 2010; Bartsch et al., 2009). The low number of engrafted donor MSCs in bone marrow suggests that the support of hematopoietic cell engraftment and recovery is unlikely to be due to a stromal support function of donor MSCs. This MSC effect can be mediated rather by the MSC's ability to modulate immune response, and thus, to prevent the hematopoietic graft rejection.

3.3.2 Tissue protection and repair

Accumulated data in animal models indicate that MSCs have the potential to protect and repair tissues in the body by several different mechanisms. First, mesenchymal tissue repair

can occur via MSC differentiation into cells of mesenchymal tissues. Since MSCs can be differentiated into distinctive mesenchymal phenotypes, they have been used for mesenchymal tissue regeneration by implanting MSCs *in vivo* into different tissue sites in tissue specific scaffolds. For example, MSCs can be delivered to bone or cartilage repair sites in calcium phosphate porous ceramics or hyaluronan and polymeric scaffolds for bone and cartilage repair, respectively (Bruder et al., 1998; Kon et al., 2000; Murphy et al., 2003; Solchaga et al., 2005). This approach resulted in well integrated, newly differentiated tissues (Kadiyala et al., 1997; Kon et al., 2000; Murphy et al., 2003), and showed that MSC-based tissue engineering is feasible for clinical use. MSCs have also been shown potential to transdifferentiate into mature cells of non-mesenchymal origin. Reported data indicate that MSCs can be transdifferentiated into hepatocytes (Ong et al., 2006; Sato et al., 2005), islet beta cells (Moriscot et al., 2005; Sun et al., 2007), endothelial cells (Oswald et al., 2004) and neural (Phinney & Isakova, 2005) or kidney tissues (Yokoo et al., 2005). These data show plasticity of MSCs and point to the possible use of MSCs for regenerative medicine of non-mesenchymal tissues. In addition to the differentiation mechanism, the paracrine mechanism plays an important role in MSC-mediated tissue protection and repair. A set of MSC-derived factors with proangiogenic/proarteriogenic activities is shown by Kinnaird et al. (Kinnaird et al., 2004). Under hypoxic conditions MSCs promote proliferation and migration of endothelial and smooth muscle cells via secretion of VEGF and basic Fibroblast Growth Factor (bFGF), augmenting collateral remodeling that is critical for recovery from tissue ischemia. The effects of MSC-secreted biological active molecules can be direct, indirect, or both: direct by triggering intracellular signaling, or indirect by triggering another cell in the vicinity to secrete other biologically active factors. This indirect effect has been termed a “trophic” effect (Caplan & Dennis, 2006). In a variety of animal models, including myocardial infarction and stroke, MSC-mediated trophic effects are the primary mechanism involved in tissue repair (Caplan & Dennis, 2006).

3.3.3 MSC-mediated immunomodulation

An important function of MSCs is their role as potent immunomodulators. It was first observed by Osiris scientists, as well as others, that MSCs can inhibit T-cell proliferation both *in vitro* and *in vivo* (Bartholomew et al., 2002; Di Nicola et al., 2002). Subsequently, further studies have demonstrated that MSCs are able to regulate the immune system through cells of both the innate (macrophages, dendritic and natural killer cells) and adaptive (T- and B-cells) immune systems (Newman et al., 2009). A simplified schematic representation of MSC effects on different subsets of immune cells reflecting our current knowledge is captured in Figure 2. The ability of MSCs to inhibit immune response and down regulate secretion of inflammatory cytokines suggests that MSCs have the potential to treat inflammatory immune-mediated diseases such as graft versus host disease (GvHD), organ rejection, and autoimmune diseases. However, MSCs are not constitutively immunosuppressive. In a non-inflammatory environment, MSCs express low levels of COX-2 (cyclooxygenase 2), Prostaglandin E2 (PGE2), Transforming growth factor β (TGF- β), Indoleamine 2,3-dioxygenase (IDO), and other factors that can inhibit immune response, however, pro-inflammatory cytokines such as Interferon γ (IFN- γ) and Tumor Necrosis Factor- α (TNF- α) dramatically up-regulate the secretion of anti-inflammatory factors by MSCs (Aggarwal & Pittenger, 2005; English et al., 2007; Krampera et al., 2006; Meisel et al.,

2004; Ryan et al., 2007). These *in vitro* data support a hypothesis of dynamic MSC response to inflammatory stimuli released from activated immune cells. *In vivo* animal data further demonstrate that MSCs require an ongoing immune response to exert their immunosuppressive functions (Renner et al., 2009). The dynamic response to cells and factors present in the microenvironment is an important feature and benefit of MSCs. Such regulated immunosuppressive activity of MSCs will help to avoid treatment-related complications that are common for traditional immunosuppressive drugs, particularly high rate of infections and multiple organ toxicities.

The MSCs' ability to regulate hematopoiesis, protect and repair tissues, and regulate immune reactions equips the cells with a great therapeutic potential. The therapeutic effects are well documented in animal models and are investigated in ongoing clinical trials (Parekkadan & Milwid, 2010). Whether those biological activities still play a role after MSCs modification for the transport of therapeutic drugs has to be determined. Especially for the treatment of inflammatory diseases it could be beneficial to combine the immunomodulatory effect of the MSCs with the therapeutic effect of MSC-delivered drug. Also, tissue repair activities would be beneficial in combination with therapeutic drugs, when injured tissue is targeted.

3.4 MSC biodistribution and migration ability

3.4.1 MSC biodistribution

Understanding the biodistribution of MSCs in the body in its healthy, or baseline state is important for the development of MSCs as targeted drugs. For this reason, MSC biodistribution after infusion was studied in healthy animals (Allers et al., 2004). Allers et al. infused human bone marrow-derived technetium-99m (^{99m}Tc)-labeled MSCs intravenously into unconditioned mice. Fifteen minutes after infusion, radioactivity was detected in lungs and heart, suggesting blood vessel circulation of the infused labeled cells. Three hours later, MSCs were scattered in the body, but still accumulated in the lungs and also in the liver. The MSCs became temporarily entrapped in the lungs, probably as a consequence of significant differences in the diameter of MSC and inner lung capillary lumen. Whole-body scanning 24 hours after infusion revealed no or scarce radioactivity in the body, except for lungs, liver, and spleen. A similar pattern of short-term distribution of MSCs was observed in rats after syngeneic transplantation of indium-111-oxine-labeled rat MSCs (Gao et al., 2001). After intraarterial (IA) and intravenous (IV) infusion, radioactivity associated with MSCs was first detected in the lungs and secondarily in the liver and other organs. Forty-eight hours later, the radioactivity was observed primarily in the liver with considerable amounts detected in the lungs and kidneys (Gao et al., 2001). The long-term fate of systemically infused autologous and allogeneic MSCs was studied in non-human primates (Devine et al., 2003). Following lethal total body irradiation, which causes major damage to the bone marrow, baboons received green fluorescent protein (GFP)-labeled baboon MSCs by IV infusion. Tissue collection after 9 to 21 months after infusions showed that allogeneic and autologous MSCs appeared to distribute in a similar manner. The highest concentrations of engrafted cells per microgram of deoxyribonucleic acid (DNA) were found in gastrointestinal tissues including colon, duodenum, jejunum, and ileum. Kidney, lung, liver, thymus, and skin also harbored high amounts of DNA equivalents. Estimated levels of engraftment ranged from 0.1% to 2.7%. The data show that MSCs not only migrate to certain tissues, but can also engraft in low numbers at those sites.

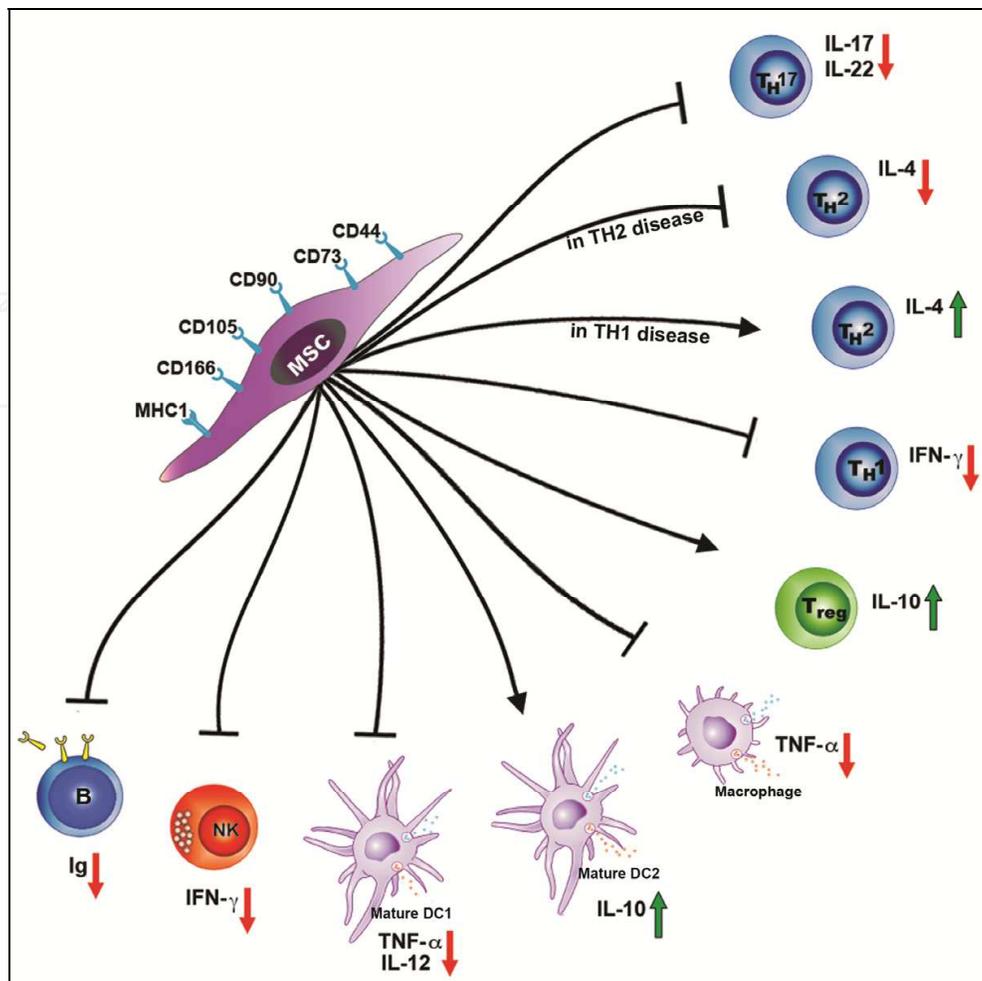


Fig. 2. MSC effects on different types of immune cells. MSCs have both immunostimulative and suppressive activity, which is driven by type of immune cells and presence of cellular and molecular signals in local tissue microenvironment. Abbreviations: B = B-cells; CD = cluster of differentiation, molecules expressed on cell surface; DC1 and DC2 = dendritic cells types 1 and 2; IFN- γ = interferon gamma; Ig = immunoglobulin; IL-4, 10, 12, 17 and 22 = interleukin 4, 10, 12, 17, and 22; MHC1 = major histocompatibility complex class I; MSC = mesenchymal stem cell; NK = natural killer cells; Th1, 2, and 17 = T helper cells 1, 2, and 17; TNF- α = tumor necrosis factor alpha; Treg = regulatory T-cells. Red arrows - decrease/inhibition. Green arrows - increase/stimulation. Black stealth arrows - stimulation of immune cells; black lines with blunt ends- suppression of immune cells

3.4.2 Migration of MSCs towards injured tissue

MSCs reside in various tissues including bone marrow, adipose tissues, amniotic membrane, and the umbilical cord (Motaln et al., 2010). In case of tissue damage, MSCs can be mobilized by signals such as cytokines and chemokines released from the damaged tissue and migrate to the sites of injury to participate in wound repair and tissue regeneration (Ramirez et al., 2006). Animal studies demonstrated that MSCs migrate to injured sites in the body, including the heart (Assis et al., 2010; Detante et al., 2009; Kraitchman et al., 2005; Wu et al., 2003), kidney (Herrera et al., 2007; Morigi et al., 2004), skin (Li et al., 2006), and bone (Horwitz et al., 1999; Mackenzie & Flake, 2001; Mosca et al., 2000). In rats bearing Lewis cardiac allografts, Wu et al. (Wu et al., 2003) found that IV injected β -galactosidase (lacZ)

labeled MSCs can migrate into lesions of chronic rejection in the cardiac grafts and home to the bone marrow. In a myocardial infarction model in rats, Assis et al. (Assis et al., 2010) showed that systemically delivered ^{99m}Tc -labeled hexamethylpropyleneamine oxime (^{99m}Tc -HMPAO) and 4',6-diamidino-2-phenylindole (DAPI)-labeled MSCs migrate to the infarcted area. One hour after MSC injection, the radioactivity in infarcted hearts was 23-fold higher than in control hearts, and a week later DAPI-labeled MSCs were still detected in the infarcted areas of the heart (Assis et al., 2010). In a similar study, ^{99m}Tc -hMSCs were injected into the saphenous vein of rats one week after cerebral ischemia (Detante et al., 2009). After initial entrapment of the cells in the lungs, they were able to migrate towards the ischemic brain lesion. Finally, the MSCs were sequestered in the spleen and eliminated predominantly by the kidneys. MSC migration was also studied in mice with induced renal injury. After IV injection of MSCs into syngeneic female mice one day after induction of kidney injuries, MSCs were detected in the context of the well-differentiated tubular epithelial lining. MSCs strongly protected renal function as reflected by significantly lower blood urea nitrogen values (Morigi et al., 2004). In a canine model, MSCs were also shown to migrate to the bone marrow after myeloablation of dogs via total body irradiation (Mosca et al., 2000). After MSC transfusion, 58% of the bone marrow samples analyzed were transgene positive. Engrafted MSCs were viable at least 6 months after infusion. The animal studies clearly show the MSCs' ability to migrate to sites of injury to participate in tissue repair processes and demonstrate their potential as vehicles for gene delivery.

The migration ability of MSCs was also studied in human patients with osteogenesis imperfecta (Horwitz et al., 2002). Osteogenesis imperfecta is a genetic disorder of mesenchymal cells in which generalized osteopenia leads to bony deformities, excessive fragility with fracturing, and short stature mostly due to a mutation in one of the two genes encoding type I collagen. Intravenous infusions of allogeneic, gene-marked, marrow-derived MSCs in patients with osteogenesis imperfecta resulted in the migration of the cells to the bone, skin, and marrow stroma, and their engraftment in one or more of these sites (Horwitz et al., 2002).

3.4.3 MSC migration to cancer tissue

MSCs do not only show tropism to sites of injury, but also to sites of tumorigenesis. In both cases, inflammatory mediators are involved in recruitment of MSCs. Factors involved include cytokines and growth factors like Epidermal Growth factor (EGF), Hepatocyte Growth Factor (HGF), Insulin like Growth Factor 1 (IGF-1), IL-1- β , IL-8, Platelet Derived Growth Factor (PDGF), SDF-1, TGF- β , TNF- α , and VEGF (Birnbbaum et al., 2007; Forte et al., 2006; Ji et al., 2004; Klopp et al., 2007; Motaln et al., 2010; Nakamizo et al., 2005; Ponte et al., 2007; Ries et al., 2007; Xu et al., 2010). Many of the same inflammatory mediators secreted by wounds are also found in tumor microenvironment and thought to be involved in attracting MSCs to these sites (Spaeth et al., 2008). Dvorak actually described the tumor as an unhealed wound that produces a continuous source of inflammatory mediators (Dvorak, 1986). Inflammation is a component present during all steps of tumor development and in all types of tumors (Sansone & Bromberg, 2011; Spaeth et al., 2008; von Herten et al., 2011; Wallace et al., 2010).

The migration of MSCs to tumors is well documented (Loebinger et al., 2009a, 2009b; Nakamizo et al., 2005; Studeny et al., 2002, 2004; Xin et al., 2007). Loebinger et al. studied MSC migration in metastatic xenograft cancer models (Loebinger et al., 2009a, 2009b). Metastatic lung tumors were produced by the delivery of MDA-MB-231 cells into the lateral tail vein of mice, and visualized as focal regions of increased signal with magnetic resonance

imaging (MRI). Thirty-five days after setup of the animal model, human MSCs double-labeled with DiI and iron nanoparticles were injected into the lateral tail vein of the animals. MRI one hour after injection showed a decrease in signal intensity caused by the iron oxide in MSCs in areas of metastatic deposits detected in pre-MSC delivery images (Loebinger et al., 2009b). The *in vivo* experiments confirmed results from *in vitro* transwell migration studies, which had demonstrated tumor homing of iron nanoparticle-labeled and unlabeled MSCs. The migration potential of MSCs towards tumors could also be shown for murine osteosarcoma (Xu et al., 2009), murine fibrosarcoma (Xiang et al., 2009), and murine glioma models (Nakamizo et al., 2005).

The number of MSCs that reaches a site of injury or tumorigenesis after systemic administration may not always be sufficient to have a therapeutic effect. In those cases, ways have to be found to enhance MSC migration. As radiation increases the expression of inflammatory mediators, it was argued that it might also enhance the recruitment of MSCs. Klopp et al. addressed the question by irradiation of murine breast carcinomas and showed that migration of MSCs to the tumor environment can indeed be enhanced by irradiation. Twenty-four hours after unilateral irradiation of 4T1 breast carcinomas, MSCs expressing firefly luciferase were injected intravenously into the animals. Forty-eight hours post irradiation, levels of MSC engraftment were 34% higher in tumors receiving 2 Gy ($p = 0.004$) than in the contralateral unirradiated limb. Immunohistochemistry also revealed higher levels of MSCs in the parenchyma of radiated tumors. Irradiated 4T1 cells resulted in increased expression of the cytokines, TGF- β 1, VEGF, and PDGF-BB, known to be involved in MSC migration (Klopp et al., 2007). Similar results were obtained in murine colon cancer xenograft models (Zielske et al., 2009). Thus, low dose irradiation might be a potential clinical tool to increase the tropism for and engraftment of MSCs in the tumor microenvironment. Another option to increase the *in vivo* migratory and adhesion capacity of MSCs is the activation of MSCs with proinflammatory cytokines like TNF- α prior to treatment (Dwyer et al., 2007; Spaeth et al., 2008).

3.5 Safety of human MSCs in clinical trials

The clinical use of MSCs started in the 1990's (Horwitz et al., 1999; Koc et al., 2000; Lazarus et al., 1995). There were no adverse events linked to MSCs, and some clinical benefits were observed after MSC infusions. Today more than 100 clinical trials are registered at clinicaltrials.gov. These clinical trials are using bone marrow, adipose or placenta-derived MSCs and are covering a wide spectrum of diseases (see Table 2). More than half of the registered trials utilize autologous MSCs. The routes of delivery include both systemic and local administration.

Osiris Therapeutics has experience with numerous clinical trials utilizing expanded human allogeneic MSCs (Prochymal® (remestemcel-L)), which allow for immediate treatment of patients with no delay due to cell processing that occurs with the use of autologous MSCs. Human MSCs have been used by Osiris for the treatment of immunologic, gastrointestinal, cardiac, and orthopedic indications. Prochymal® is being evaluated in Phase III clinical trials for several indications, such as acute GvHD and Crohn's disease. Chondrogen™, an injectable formulation of MSCs, is under investigation for the treatment of arthritis in the knee. Altogether, more than 1,300 patients have been treated in Osiris clinical trials. With much of the data from double-blinded, placebo-controlled studies, the results provide strong support for the positive safety profile of MSCs.

| MSC Type | MSC Source | Therapeutic Areas |
|--|---|--|
| <ul style="list-style-type: none"> • Autologous • Allogeneic | <ul style="list-style-type: none"> • Bone Marrow • Adipose • Placental-derived | <ul style="list-style-type: none"> • Inflammatory and immune-mediated • Cardiovascular • Orthopedic • Gastrointestinal/Liver/Pancreatic • Neurological • Pulmonary • Nephrological • Dermatological • Hematological • Metabolic • Genetic |

Table 2. MSCs in clinical trials

Valuable safety information has been obtained from these studies because of the diverse patient population, including adult and pediatric patients, and the wide range of dosing regimens studied in Osiris clinical trials. No infusional toxicities were observed in both adult and pediatric populations. Overall review of safety data, including adverse events, has detected no trends or signals in the events experienced by patients attributed to administration of MSCs (Hare et al., 2009; Kebriaei et al., 2009; Lazarus et al., 2005; Prasad et al., 2011). Consistent with Osiris' data, the safe use of MSCs has also been reported by other investigators (e.g. Chen et al., 2004; Ciccocioppo et al., 2011; Duijvestein et al., 2010; Le Blanc et al., 2008; Lucchini et al., 2010; Williams et al., 2011). The safety of MSCs, together with the ability to use unmatched allogeneic MSCs and the potential of MSCs to home to the sites of inflammation or injury, makes these cells a promising candidate for drug delivery vehicles.

4. MSCs - A drug delivery tool

The unique ability of MSCs to migrate to sites of inflammation, modulate immune and inhibit inflammatory responses, and prevent and repair tissue damage, make MSCs an attractive cell therapy for the treatment of diseases with inflammatory components (Newman et al., 2009). It is those features, as well as MSCs' low immunogenicity profile allowing for allogeneic, off-the-shelf use, that makes MSCs promising as a cell therapy. Table 3 summarizes the important features of MSCs for use in drug delivery.

| Characteristics supporting the use of MSCs as a vehicle for drug delivery |
|---|
| <ul style="list-style-type: none"> • Selective MSCs homing to sites of inflammation and cancer • Low immunogenicity profile of MSCs which allows for allogeneic use of MSCs • Allogeneic use of MSCs enables development of "off-the-shelf" drugs • Established biodistribution and toxicology profile • Positive safety profile of MSCs in clinical trials to date • Easy availability of MSCs from adult bone marrow donors and other sources • Potential for GMP-compliant, large-scale manufacturing processes • Cryopreservation for long-term storage of MSC products |

Table 3. Features of MSCs for use as a drug delivery system

4.1 MSCs as vehicle for therapeutic drugs

4.1.1 MSCs as vehicle for cancer therapeutics

Recent data providing evidence that MSCs migrate to sites of tumorigenesis in some instances suggest another therapeutic area for MSCs: the use of MSCs as a vehicle for the targeted delivery of cytotoxic agents to tumor tissue (Hall et al., 2007; Hu et al., 2010). Cells, like MSCs, which are able to target cancer cells, and are, at the same time, non-immunogenic and non-toxic to the host are the ideal vehicle for tumor-selective drug delivery. Several preclinical studies support the rationale for genetically modified MSC to deliver therapeutics to tumor sites. Successful animal models include sarcoma, melanoma, carcinoma, and several cancer metastasis models. Table 4 gives an overview of preclinical studies focusing on the delivery of genetically modified MSCs for cancer therapy.

| Genes Delivered By MSCs | Treated Tumor | Reference |
|---|--|--|
| Cytosine deaminase in combination with 5-FC | Colon cancer Melanoma | (Kucerova et al., 2007) (Kucerova et al., 2008) |
| CD : UPRT | Human prostate tumor | (Cavarretta et al., 2010) |
| CRAd | Ovarian carcinoma Intracranial glioma Tumor metastasis | (Komarova et al., 2006) (Sonabend et al., 2008) (Stoff-Khalili et al., 2007) |
| CX3CL1 (Fractalkine) | Tumor metastasis | (Xin et al., 2007) |
| EGFRvIII | Glioma | (Balyasnikova et al., 2010) |
| Interferon- α | Tumor metastasis | (Ren et al., 2008a) |
| Interferon- β | Glioma Tumor metastasis Tumor metastasis | (Nakamizo et al., 2005) (Ren et al., 2008b) (Studený et al., 2004) |
| Interleukin-2 | Glioma Melanoma | (Nakamura et al., 2004) (Stagg et al., 2004) |
| Interleukin-12 | Tumor metastasis Ewing sarcoma tumors Melanoma | (Chen et al., 2008) (Duan et al., 2009) (Elzaouk et al., 2006) |
| iNOS | Fibrosarcoma | (Xiang et al., 2009) |
| NK4 (adenovirus) | Tumor metastasis | (Kanehira et al., 2007) |
| (Delta)24-RGD (adenovirus) | Glioma | (Yong et al., 2009) |
| TRAIL | Tumor metastasis Glioma Carcinoma | (Loebinger et al., 2009a) (Sasportas et al., 2009) (Mohr et al., 2008) |

Abbreviations: CD: UPRT = cytosine deaminase: uracil phosphoribosyltransferase; CRADs = conditionally replicating adenoviruses; EGFRvIII = mutant epidermal growth factor receptor; 5FC = 5-fluorocytosine; iNOS = inducible nitric oxide synthase; TRAIL = tumor necrosis factor related apoptosis-inducing ligand

Table 4. Preclinical studies of genetically engineered MSCs for the delivery of anti-cancer agents to tumors.

Among others, recent efforts focused on the delivery of anti-proliferative and pro-apoptotic therapeutics, like Interferon- β (IFN- β) (Chawla-Sarkar et al., 2001; Johns et al., 1992). The therapeutic efficacy of IFN- β had been limited by its toxicity associated with systemic administration (Menon et al., 2009). To be able to minimize toxicity and increase the local concentration of IFN- β , MSCs were selected as delivery vehicle. Murine MSCs were engineered to release IFN- β and injected via tail vein into immunocompetent mice with prostate cancer lung metastasis (Ren et al., 2008b). Following IFN- β -expressing MSC therapy, the mice showed a reduction of tumor volume in the lung, increased tumor cell apoptosis, decreased tumor cell proliferation and blood vessel counts, and an increase in the natural killer cell activity. The systemic level of IFN- β was not significantly elevated by the targeted cell therapy (Ren et al., 2008b).

MSCs expressing IFN- β were also used for targeted delivery of interferon to metastatic breast carcinoma and melanoma models (Studený et al., 2004). To establish pulmonary metastases, mice were injected MDA-MB-231 tumor cells in the lateral tail vein. Eight days after tumor cell injection, mice started treatment with recombinant IFN- β , IFN- β -MSCs, or MSC-Gal by intravenous injection (Studený et al., 2004). Whole lung weight was used as a surrogate endpoint of MDA-MB-231 tumor burden in the lung. Tumor mice treated intravenously with MSC-IFN- β cells had significantly smaller lungs than untreated control mice injected with tumor cells only (mean lung weight 0.408 g versus 0.977 g; $p = 0.021$). By contrast, there was no statistically significant difference in the mean lung weight of mice treated with recombinant IFN- β or MSC-Gal cells and control mice injected with tumor cells only. Intravenous administration of human IFN- β -MSCs also prolonged the survival of animals with established metastases of MDA-MB-231 breast carcinoma (median survival 60 days versus 37 days in control mice, $p < 0.001$) and A375SM melanoma tumor in the lung (median survival 73.5 days versus 30 days in control mice, $p < 0.001$) (Studený et al., 2004).

Another therapeutic with encouraging preclinical results in MSC-targeted cancer therapy is the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), a transmembrane protein, which induces apoptosis in various tumor cell types (Hao et al., 2001; Kagawa et al., 2001). Loebinger and coauthors (Loebinger et al., 2009a) showed that directly delivered TRAIL-expressing MSCs were able to significantly reduce tumor growth ($p < 0.001$) in subcutaneous xenograft experiments. In a pulmonary metastasis model, systemically delivered TRAIL-expressing MSCs localized to lung metastasis, and the controlled local delivery of TRAIL completely cleared lung metastases in 38% of mice compared to none of the controls ($p < 0.05$) (Loebinger et al., 2009a). Anti-tumorigenic effects of MSC-delivered recombinant TRAIL were also reported from human glioma models (Sasportas et al., 2009). The ability of MSCs to target primary tumors and their metastases suggests an important therapeutic role for MSCs as drug delivery vehicles in the future.

4.1.2 MSCs as vehicle for prodrug gene therapy

A very promising approach to reduce cancer drug toxicity is prodrug gene therapy, based on the delivery of genes encoding enzymes that convert nontoxic prodrugs into toxic antimetabolites (Menon et al., 2009). One of the chemotherapeutic agents of interest for prodrug gene therapy is the prodrug 5-Fluorocytosine. This prodrug is converted to the potent chemotherapeutic substrate 5-fluorouracil by the bacterial and/or yeast cytosine deaminase enzyme. 5-Fluorouracil has been used successfully for colorectal and pancreatic cancer therapy for about 40 years. Despite the fact that it is a potent chemotherapeutic agent, its high toxicity results in severe side effects in treated patients. The development of a

prodrug, which is only converted into the toxic chemotherapeutic agent at the site of tumorigenesis, is an important step to achieve lower toxicity of 5-fluorouracil. Kucerova et al. combined MSC-driven targeted delivery of the prodrug converting enzyme cytosine deaminase: uracil phosphoribosyltransferase with the systemic administration of the prodrug 5-fluorocytosine (Kucerova et al., 2007, 2008). The administration of MSCs expressing the prodrug converting enzyme (CDy-AT-MSCs) in combination with systemic delivery of 5-Fluorocytosine, inhibited subcutaneous human colon cancer growth in immunocompromised mice. By day 18, significant inhibition of tumor growth of up to 69% was observed in all animals injected with CDy-AT-MSC. The animals did not show any signs of toxic side effects of the therapeutic regimen.

Also in a murine melanoma model, systemic administration of CDy-AT-MSC resulted in cell homing into subcutaneous melanoma and mediated tumor growth inhibition (Kucerova et al., 2008). A similar study on pancreatic cancer was recently conducted by Cararetta et al. (Cavarretta et al., 2010). After induction of PC3 tumors in mice, the animals received systemic administration of CDy-AT-MSCs and were daily treated with 5-Fluorocytosine. On day 24, average tumor volume had decreased in all treated animals compared to control animals. In a second group with repeated CDy-AT-MSC injections, complete regression of the tumor was observed at day 36 in three out of six mice. The studies confirm that MSC have the potential to travel to site of tumorigenesis and to effectively deliver prodrug converting enzymes. Targeted delivery of prodrug-converting enzymes in combination with the systemic delivery of the according prodrug might substantially reduce side effects of otherwise highly toxic therapeutics.

4.1.3 MSCs as vehicle for biological pacemaker genes

In addition to the development of MSCs as delivery vehicles for cancer therapeutics, much research in MSC delivery tools focuses on the delivery of biological pacemakers for the treatment of heart diseases. Electronic pacemakers, the standard of care for heart block and other electrophysiological abnormalities, still have shortcomings, like limitations on exercise tolerance and cardiac rate-response to emotion; limited battery life, interference with neural stimulators, metal detectors, and MRI equipment; effects on electrophysiological or contractile function; and sizing challenges for growing pediatric patients (Rosen et al., 2004, 2008). Biological pacemakers that would create a stable physiological rhythm, not require electronic equipment and adapt to changes in activity and emotion, would be an attractive alternative. MSCs might serve as a platform for the delivery of pacemaker genes. First proof of concept was collected in a canine study by Potapova et al. (Potapova et al., 2004). Human MSCs were engineered to express the biological pacemaker gene mHCN2 and administered into the left ventricular wall of adult dogs. The animals were subjected to a pericardiectomy and within ten days, vagal stimulation was performed to induce atrioventricular block and to analyze whether escape pacemaker function occurred. Five of six animals receiving hMSCs expressing the biological pacemaker and the enhanced green fluorescent protein (EGFP) reporter gene developed rhythms originating from the left ventricle and pace-mapped to the injection site. Only two out of four control animals, which had received MSCs expressing EGFP alone, developed right ventricular escape rhythms. Dogs who had received the biological pacemaker gene developed idioventricular rhythms with rates approximating on average 61 beats per minute (bpm), while the rates of control animals only reached 45 bpm ($p < 0.05$; (Potapova et al., 2004)). Nests of adult human MSCs were found at the site of injection, as well as evidence for gap junctional coupling between adult MSCs and myocytes.

In another study from the same group (Plotnikov et al., 2007), mHCN2 expressing MSCs were administered into the left ventricular wall of adult dogs in complete heart block and with backup electronic pacemakers to operate in “tandem” mode. After stabilization around day 10 to 12, the biological pacemaker functioned stably and with little time-dependent variation in dogs that had received at least 700,000 hMSCs. The pacemaker function was stable until the end of the study at day 42. Following Plotnikov et al., no cellular or humoral rejection, loss of function, or apoptosis was observed during this time (Plotnikov et al., 2007). A later analysis by Rosen et al. (Rosen et al., 2008) assessed the observation of loss of function and histological evidence of rejection in some of the xenotransplants. The studies show that, despite great progress in the development of MSCs as vehicles for biological pacemakers, some questions remain unanswered. Further studies are needed to investigate how reliable and durable pacemaker function can be obtained without rejection and how the cells can be maintained in the target area.

4.1.4 Clinical progress with MSCs delivering therapeutics

While many preclinical studies have shown proof of concept for the use of MSCs as targeted delivery vehicle, clinical studies are focused on unmodified human MSCs. Additional modification of the cells may help to increase levels of therapeutics generated by the cells, or induce the production of therapeutics which are not present in naïve cells. MSCs can be modified using genetic and non-genetic techniques, including the pre-differentiation of MSCs in growth factor containing media. In a first clinical study, adipose-derived insulin-producing MSCs (h-AD-MSC) were used for the treatment of type 1 diabetes mellitus (DM), an autoimmune disorder with disturbed glucose/insulin metabolism, which has no medical treatment other than life-long insulin therapy (Trivedi et al., 2008). To obtain MSCs that produce insulin, the adipose-tissue derived MSCs were cultured in differentiation medium. The DM patients received intraportal administration of h-AD-MSC together with xenogeneic-free, cultured bone marrow-derived hematopoietic stem cell transplantation. Five insulinopenic DM patients at the age of 14 to 28 years received a mean dose of 3 million h-AD-MSC. The patients showed 30% to 50% decreased insulin requirements with 4- to 26-fold increased serum c-peptide levels, at a mean follow-up of 2.9 months. No adverse side effects related to the stem cell infusion or the administration of induction therapy were reported. The study provided initial evidence of potential treatment of insulinopenic diabetics using insulin-producing h-AD-MSC in conjunction with hematopoietic stem cell transplants. It is a first step in the use of modified MSCs in clinical settings, and additional larger studies will be important to assess the durability of the response.

Another clinical study with autologous cultured mesenchymal bone marrow stromal cells secreting neurotrophic factors (MSC-NTF, NurOwn™) is planned by the Hadassah Medical Organization in Jerusalem, Israel, in collaboration with Brainstorm Cell Therapeutics Ltd. The study will evaluate the safety and therapeutic effects of MSC-NTFs injections as a treatment for patients with amyotrophic lateral sclerosis (ALS). ALS, also called Lou Gehrig's disease, is a progressive neurodegenerative disease that affects nerve cells in the brain and the spinal cord, characterized by progressive degeneration of motor neurons. Animal studies have shown that glial-derived neurotrophic factor (GDNF) can protect motor neurons from degeneration *in vitro* (Henderson et al., 1994; Suzuki et al., 2007). For this clinical study, adult bone marrow cells capable of releasing neurotrophic factors, including GDNF, will be generated and transplanted into ALS patients. In early ALS

subjects, MSC-NTF cells will be transplanted intramuscularly, while cells will be administered intrathecally in progressive ALS patients. The hypothesis is that the administration of MSCs expressing GDNF might protect motor neurons from further neurodegeneration. Results are not available yet, as the study is in its early stages.

4.2 MSCs as vehicle for nanoparticles

Nanoparticles can be used not only as a carrier for drugs, but also as a diagnostic and therapeutic tool. As nanoparticles reach their target site by passive targeting via the EPR effect, their target specificity is limited. Target specificity, however, could be improved by using MSCs as a vehicle to deliver nanoparticles specifically to sites of inflammation and tumorigenesis. At the site of interest, the nanoparticles could serve different purposes, dependent on the nanoparticle material and therapeutic aim.

An essential requirement for the development of nanoparticle-carrying MSCs as a diagnostic or therapeutic tool is the successful uptake of nanoparticles by the cells and low toxicity of the nanoparticles within the cell. Studies have shown that, among other factors, nanoparticle size, shape and surface charge play an important role in the uptake of nanoparticles in cells (Chithrani & Chan, 2007; Chithrani et al., 2006; Jo et al., 2010; Patra et al., 2010). Chithrani et al. investigated the intracellular uptake of different sized and shaped colloidal gold nanoparticles in HeLa cells and other mammalian cell lines (Chithrani & Chan, 2007; Chithrani et al., 2006). Gold nanoparticles were used as a model nanoparticle system, as their size and shape can be easily controlled during synthesis, and quantification of the nanoparticles is possible in biological samples. Among spherical nanoparticles with diameters between 14 nm and 100 nm, the maximum uptake by a cell occurred at a nanoparticle size of 50 nm. Similar findings were made by other research groups (Malugin & Ghandehari, 2010). The uptake of rod-shaped gold nanoparticles was lower, and the fraction of exocytosed rod-shaped nanoparticles higher, than that of their spherical counterpart. In general, exocytosis occurred at a higher rate and higher percentage in smaller compared to larger nanoparticles. Thus, both, uptake and removal of nanoparticles were highly dependent upon the size of the nanoparticles, but the trends were different (Chithrani & Chan, 2007). Besides nanoparticle size and form, the type of ligand coating the cells also influenced the cellular uptake. The number of transferrin-coated gold nanoparticles that entered the cells was about three times less than that of the citrate-stabilized gold nanoparticles (Chithrani et al., 2006). The uptake of gold nanoparticles was also investigated in MSCs (Koshevoy et al., 2010; Yamada et al., 2009). In-depth knowledge of nanoparticle properties crucial for cellular uptake will help to accelerate the development of nanoparticle candidates for targeted cell therapies.

4.2.1 Nanoparticle labeled cells as diagnostic tool

The development of nanoparticle-loaded MSCs in the recent past has concentrated on diagnostic applications. The nanoparticles of choice for such cell labeling studies in MSCs were superparamagnetic iron oxide (SPIO) particles which can be used to track the biodistribution and migration of transplanted cells by MRI and other imaging methods (Arbab et al., 2004; Bulte et al., 2005; Chen et al., 2010a; Jo et al., 2010; Kostura et al., 2004; Loebinger et al., 2009b; Reddy et al., 2010; Walczak et al., 2005). Tracking labeled MSCs after transplantation will enable a better understanding of the dynamics of cell-tissue interactions and help improve the design of stem cell therapies by optimizing cell manufacturing and

cell delivery protocols (Bulte et al., 2005; Srinivas et al., 2010). The impact of nanoparticle uptake on cell viability and cell functionality was analyzed in *in vitro* studies. Bulte et al. injected poly-L-lysine coated ferumoxide (PLL-Feridex) labeled canine MSCs via MR fluoroscopy in a canine myocardial infarction model, and followed their biodistribution (Bulte et al., 2005). The cells could be serially tracked by MRI for at least eight weeks following implantation. Feridex-labeling did not affect cell proliferation, adipogenesis, or osteogenesis, but markedly diminished the cells' ability to undergo chondrogenesis (Bulte et al., 2005; Kostura et al., 2004). This could also be shown for human MSCs labeled with SPIO nanoparticles coated with carboxydextran (Resovist) by Reddy et al. (Reddy et al., 2010), while Schäfer et al. (Schäfer et al., 2010) did not observe such effect. In contrast, human MSCs labeled with chitosan-coated superparamagnetic iron oxide did not exhibit any significant alterations in the surface marker expression or adipo /osteo /chondrogenic differentiation potential when compared to unlabeled control cells (Reddy et al., 2010).

These studies show that careful selection of the MR contrast agent and modification protocol are required to retain full functionality of the cells after modification. Extensive *in vitro* and *in vivo* analyses of nanoparticle-loaded cells have to be conducted to ensure safety and effectiveness of the cell based diagnostic tool. As mammalian cells are well adapted to regulation of iron homeostasis, the use of ferumoxides appears clinically safe. So far, the iron oxide-based contrast agents Feridex and Resovist have been approved by the FDA. Despite technical challenges that still have to be addressed, the studies show that MSCs can be successfully loaded with the MR contrast agents and tracked via MRI. Besides MRI, other non-optical cell tracking methods are available, like positron emission tomography (PET) and single photon emission computed tomography (SPECT). An overview of stem cell tracking methods addressing their pros and cons can be found in reviews by Reagan and Kaplan (Reagan & Kaplan, 2011) and Srinivas et al. (Srinivas et al., 2010).

Clinical studies tracking radiolabeled or SPIO labeled cells have been conducted with different cell types, including dendritic cells, neural stem cells, hematopoietic stem cells, and cadaveric islet cells (Bulte, 2009; Srinivas et al., 2010). With increasing clinical use of MSCs, the *in vivo* tracking of MSCs has become useful because it can help to evaluate and optimize MSC therapies. One phase I/II clinical study conducted in Jerusalem, Israel involved the administration of autologous Feridex-labeled MSCs in patients with multiple sclerosis and amyotrophic lateral sclerosis in an effort to prevent further neurodegeneration (Karussis et al., 2010). The Feridex-tag was added to allow cell tracking via MRI and evaluate migration of the transplanted cells. Intrathecal and intravenous administration were combined to maximize the potential therapeutic benefit by accessing the central nervous system through the cerebrospinal fluid and the systemic circulation. Of the 34 patients enrolled, nine patients received SMIO labeled (Feridex) MSCs. MRIs of the brain and whole spine performed at different time points after MSC injection indicated possible dissemination of the MSCs from the lumbar site of inoculation to the occipital horns, meninges, spinal roots, and spinal cord parenchyma. The results, however, need to be interpreted with caution, as the number of patients in the study is small and it is not ruled out that macrophages phagocytized the iron oxide magnetic resonance contrast agent and migrated to the inflammatory lesions.

The ability of MSCs to migrate to sites of tumorigenesis might also allow for the development of labeled MSCs as a clinical tool for cancer detection. In support of this idea, Loebinger et al. showed that intravenously injected iron-labeled MSCs could be tracked *in*

in vivo to multiple lung metastases using MRI (Loebinger et al., 2009b). Human MSCs were labeled with starch-coated FluidMAG iron nanoparticles and injected via the lateral tail vein into mice with metastatic lung tumors. MRI and immunohistological staining confirmed the localization of SPIO-loaded MSCs to lung metastases one hour after injection. Preceding experiments with mice carrying subcutaneous MDA-MB-231 tumors showed that as few as 1,000 Feridex-labeled MSCs could be visualized in tumors using MRI (Loebinger et al., 2009b). The ability to track MSCs homing to primary tumors and metastases using a noninvasive scanning method could be of great benefit for future diagnostic applications.

4.2.2 Nanoparticle-loaded cells as therapeutic tool

Besides diagnostic applications, nanoparticle-carrying cells are also interesting for therapeutic use. High Z-elements like gold can be utilized for radiotherapy enhancement (Butterworth et al., 2010; Chang et al., 2008; Chithrani et al., 2010; Hainfeld et al., 2004, 2008; Herold et al., 2000; Kong et al., 2008; Liu et al., 2010; Rahman et al., 2009; Rose et al., 1999), photothermal ablation of cancer cells by heating with near-infrared lasers (Atkinson et al., 2010; Chen et al., 2010b; Cherukuri et al., 2010; Diagaradjane et al., 2008; Gobin et al., 2010; Kennedy et al., 2011), and thermal destruction of cancer cells by radiofrequency field-induced heating (Gannon et al., 2008). Radiotherapy is one of the most commonly used methods in cancer therapy. Gold nanoparticles were shown to increase radiotherapy efficiency when accumulated in tumors due to their high absorption of X-rays (Hainfeld et al., 2008). However, injections of gold did not result in the delivery of gold nanoparticles to the tumor sites only. Although some nanoparticles were transported to the tumor via the EPR effect, others were detected in the blood, liver, spleen, and muscle, before renal clearance (Hainfeld et al., 2006). MSCs have been shown to home to sites of tumorigenesis and represent a potential vehicle for the targeted delivery of radiotherapy enhancers to tumor sites. Delivering a curative dose of radiation to tumor tissues, while sparing normal tissues, would help to reduce side effects of radiotherapy treatment and increase radiotherapy efficiency.

First steps towards the development of cells carrying nanoparticles for radiotherapy enhancement have been made. Radiation enhancement was quantified in HELA cells by irradiating the cells with 220 kVp X-rays in the absence and presence of different sized internalized gold nanoparticles. Radiosensitization was dependent on the number of gold nanoparticles internalized in the cells, with gold nanoparticles of 50 nm diameter showing the highest radiosensitization enhancement factor (1.43 at 220 kVp) among gold nanoparticles ranging from 14 to 74 nm diameter. Radiation sensitization in HELA cells carrying 50 nm gold nanoparticles also depended on the energy of the radiation source (Chithrani et al., 2010).

The enhancement of radiation effects by gold nanoparticles was also studied in bovine aortic endothelial (BAEC) cells (Rahman et al., 2009). Tumor growth and survival are critically linked to the proliferation of endothelial cells comprising the tumor blood vessel network (Sieman, 2006). Targeting the blood vessel network of a tumor with radiation aims to impair the nutritional support system of the tumor. Rahman et al. exposed BAEC cells carrying 1.9 nm gold nanoparticles to kilovoltage X-ray radiation therapy and megavoltage electron radiation therapy. Dose enhancement in cells irradiated with superficial X-ray reached a dose enhancement factor of 24.6 with X-ray beams of 80 kVp and correlated with the concentration of internalized gold nanoparticles. Dose enhancement in cells irradiated with

electron beams reached a factor of 4.1. The study showed that gold nanoparticles can be used to enhance the effect of radiation doses from kilovoltage X-ray radiation therapy and megavoltage electron radiation therapy beams. As lower radiation doses destroy the same fraction of cells when gold nanoparticles are present as do larger radiation doses without radiosensitizers, the use of gold nanoparticles in radiotherapy might help to reduce radiation doses in the future (Rahman et al., 2009).

4.3 Outlook on MSCs as targeted delivery tools

Safety and ready availability of MSCs, together with their ability to home to sites of injury and tumorigenesis, and their low immunogenicity profile, makes them an attractive delivery vehicle for diagnostic and therapeutic purposes. Still, there are hurdles to be overcome before drug-loaded MSCs will be ready for clinical use. One of the challenges to be mastered is the effective loading of the vehicle cell with the therapeutic. MSCs must be loaded with drugs or modified to produce high enough concentrations of agent to reach therapeutic effectiveness without compromising cell viability and properties necessary for effective drug delivery (e.g. homing or migration potential). Several animal models have shown that therapeutically effective, modified MSCs can be generated (as discussed in section 4.1), and clinical trials will show whether those results can be translated into human use. Another necessity is the development of effective drug release mechanisms. Such mechanisms to initiate the release of the therapeutic from the MSCs to gain access to or enter the target cell remain to be found.

Furthermore, for access to target sites, biological barriers, like the blood-brain barrier (Gabathuler, 2010; Patel et al., 2009), have to be crossed for the cells to be able to reach their target, which makes the route of administration another important factor to consider. In one study following intravenous injection of MSCs into rats after resuscitation from cardiac arrest, MSCs were detected in the brain. The number of detected cells was low, however neurologic recovery of the animals appeared to improve (Wang et al., 2008). Future studies have to demonstrate whether the number of MSCs crossing the blood-brain barrier is sufficient for therapeutically effective drug delivery. Alternatively, other routes of drug delivery could be chosen. Intranasally administered MSCs were shown to bypass the blood-brain barrier by migrating from the nasal mucosa through the cribriform plate along the olfactory neural pathway into the brain and cerebrospinal fluid (Danielyan et al., 2009). Also local injections of drug-loaded MSCs might be considered to gain access to diseased tissue that is difficult to target. In this case, MSCs would be responsible for the “micro-targeting” of the drug very specifically within the target area.

In addition to biological complications, there will also be technical hurdles in the development of drug-carrying MSCs. The successful transfer of MSC modification processes at the research scale to manufacturing levels could be challenging. Large-scale production of drug-loaded MSCs, whether it involves genetic modifications, culture-induced modification of cells, or loading of cells with nanoparticles, can be very expensive and time-consuming. Results from small-scale experiments cannot always easily be translated into large-scale production without significant additional efforts. Overall, MSCs are a very promising drug delivery tool. However, this new technology will only support widespread clinical use if effective drug loading and successful drug release at the site of interest can be ensured, and robust manufacturing processes are developed.

5. References

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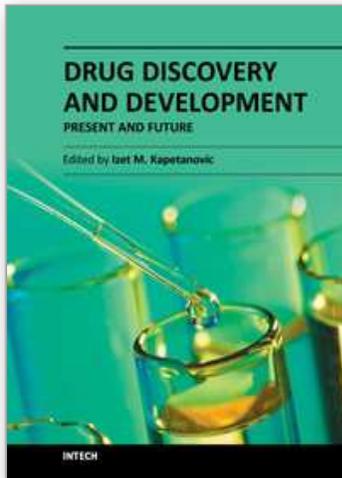
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Drug discovery and development process aims to make available medications that are safe and effective in improving the length and quality of life and relieving pain and suffering. However, the process is very complex, time consuming, resource intensive, requiring multi-disciplinary expertise and innovative approaches. There is a growing urgency to identify and develop more effective, efficient, and expedient ways to bring safe and effective products to the market. The drug discovery and development process relies on the utilization of relevant and robust tools, methods, models, and validated biomarkers that are predictive of clinical effects in terms of diagnosis, prevention, therapy, and prognosis. There is a growing emphasis on translational research, a bidirectional bench to the bedside approach, in an effort to improve the process efficiency and the need for further innovations. The authors in the book discuss the current and evolving state of drug discovery and development.

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