Stem Cells as Vehicle and Target of Nanoparticles

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Abstract

Modulation of endogenous adult stem cells niches represents a promising strategy for regeneration of tissues and to correct cell abnormalities, including cancer. Recent advances show the possibility to target endogenous stem cells or their progenies by using nanoparticles (NPs) conjugated with specific biomolecules. In addition, the targeting of stem cell niche can be accomplished by using stem cells loaded with nanoparticles. This review examines principles for the targeting of endogenous stem cells as well as factors for the modulation of stem cells.

Teaser:

Stem cell targeting is important in the context of regenerative and therapeutic medicine. Nanoparticles alone or within stem cells offer a platform to target the stem cell niche.

Research highlights:

- Nanoparticle formulations for endogenous stem cell targeting
- Factors released by nanoparticles to modulate stem cell activity
- Stem cells as nanoparticle carriers
- Stem cells loaded with nanoparticles having the tumor homing capacity

Keywords

Stem cells; nanoparticles; nanomedicine; stem cell niche; cancer

1. Introduction

Stem cells are defined as cells that have self-renew and differentiation capacity. There are many postnatal tissues containing stem cells, which are normally termed as adult stem cells (ASCs) or tissue-specific stem cells. Their role is to replenish the differentiated cells as the need arises for cell turnover or in the case of injury and stress to the tissue [1]. Numerous types of ASCs have been identified in various organs and tissues. It is believed that bone marrow is the largest stem cell pool. There are several ASCs being investigated from a fundamental and regenerative point of view: hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), neural stem cells (NSCs), adipose-derived stem cells (ADSCs), intestinal stem cells, among others. ASCs physiologically reside in tissue specific niches (stem cell niches). The stem cell niche has an anatomic location and comprises a highly organized microenvironment. Within the niche, ASCs are in contact with neighbouring cells that provide short-range signals via soluble factors and transmembrane proteins. The niche plays an important role since it influences/controls the "stemness" of ASCs, that is, their self-maintenance or differentiation [2]. Nevertheless, the niche may also induce pathologies by imposing aberrant function on ASCs or other targets. Deregulations in the biological program of stem cells may lead to the appearance of cancer stem cells [3].

Nanotechnologies are an emerging platform to control the activity of endogenous stem cells [4, 5]. Examples of nanotechnologies used to target and control the activity of stem cells are organic and inorganic nanoparticles (NPs). These NPs can be fabricated from a wide variety of components, including polymers, lipids and metals [6-8]. Because of their small size, surface chemistry for cell targeting, the possibility of remote activation, the chance of encapsulate both hydrophilic or hydrophobic molecules while protecting them during their circulation in the body, these NPs are very promising for the control of stem cell activity. These NPs may be used to replace viral vectors for gene edition and therapy, and thus preventing undesired side effects. NPs offer a simpler, less expensive, method for the transfection of stem cells, inducing a lower risk of immunogenicity,

mutagenicity or toxicity. Smart polymers have been developed featuring spatio-temporal release. The payload of the NPs may be covalent or non-covalent attached to the formulation.

This review examines the recent developments in the intersection of two different fields, nanomedicine and stem cells. In the first part of the review we examine the use of nanotechnologies to control the activity of endogenous stem cells. We review the factors that modulate the stem cell niche and we describe some examples related to the modulation of stem cells by the NPs. In the second part of the review, we examine the use of stem cells to transport NPs to stem cell niches in the setting of cancer.

2. NPs to target endogenous stem cells

2.1 NPs to overcome *in vivo* barriers

NPs are structures widely accepted as having a size between 1 and 100 nm [9], although bigger structures up to 999 nm may also be considered. Their physical and chemical properties differ from bulk material, and may result in different biological behavior, depending on the manufacturing and functionalization [10]. For example, metallic NPs possess lower melting points, higher specific surface areas, specific optical and magnetically properties and, different mechanical strengths, compared to the original materials. Several NP formulations have been already approved for clinical use in the context of therapeutic medicine. The first NP formulation approved by the FDA was in the 1980´s for the treatment of cancer [6]. Since then, several NP formulations have been approved, mostly for cancer and infection diseases treatment [6].

NPs with different physical-chemical properties have been used to target endogenous stem cells. Typically, the NPs are synthetic and biodegradable, formed by polyesters (e.g. poly(lactic-*co*-glycolic acid (PLGA)) [11, 12], poly(α-amino ester)s [13], lipids [14, 15], poly(imines) [16] and polysaccharides [16]. Natural NPs such as exosomes have been also used [17]. In both cases, NPs with diameters between 60 and 300 nm have been used [14, 15]. The biomolecules for stem cell

regulation includes small molecules (e.g. retinoic acid (RA), curcumin, glycogen synthase kinase -3β inhibitor)[11, 15, 16, 18, 19], transcription factors (neurogenin)[13], cytokines/chemokines (IL-15, IL-21, stromal cell-derived factor-1α (SDF-1α))[15, 20] and miRNAs [12].

In case of stem cell niches spatially well defined, NPs can be administered directly into the niche. This is the case of the subventricular zone (SVZ) niche located in the brain that hosts an important niche of NSCs (**Fig. 1**). In this case, NP formulations may be administered by intracerebroventricular/intracerebral infusion [16]. This strategy increases the success of targeting, maximizing the amount of bioactive agent that reaches the stem cells. However, in most cases, the stem cell niche is not spatially well defined. For example, the HSC niche is located in the bone marrow within the human body (**Fig. 1**). In this case, the NP formulation access to this stem cell niche requires a systemic delivery administration either within or conjugated to cells [15, 21]. Alternatively, the NPs should have components (e.g. ligands or antibodies) in their surface to overcome the multiple barriers in the human body and finally target the stem cell niche [22, 23]. For a more comprehensive understanding of the biological barriers that NPs should overcome we refer readers to several recent reviews [24, 25]. In brief, NPs should avoid kidney clearance and immunological phagocytosis before reaching the targeted stem cells. Strategies for increasing NPs half-life in circulation focus mainly in surface modification with poly(ethylene glycol) or biomimetic cell membrane coatings [26, 27]. Upon arrival to the targeted tissue, NPs should succeed in exiting circulation towards stem cell niches and surpass the endothelial barrier. In case of the brain, this means to overcome the blood brain barrier (BBB) [25]. After stem cell targeting, the NPs need to overcome the endolysomal compartment and reach the cell cytoplasm.

In the last years, some factors that target stem cell populations have been reported [22, 23, 28]. Magnetic resonance imaging-traceable NP formulations conjugated with a monoclonal antibody against CD15 were very effective to target endogenous NSCs during their rostral migratory stream in adult mouse brain [22]. The same formulation has been used successfully to track NSCs in an

ischemic stroke model [29]. The anti-CD15 conjugated NPs could label activated endogenous NSCs *in situ* 7 days after ischemic stroke. This formulation offers an interesting possibility to monitor *in vivo* the dynamic process of endogenous NSC activation [29]. Others stem cells have been successful with this targeting approach. For example, magnetic resonance imaging-traceable NPs conjugated with an antibody against GD2 have been used for the successful targeting of MSCs in the human bone marrow [23]. Yet, NP formulations may target stem cells outside the stem cell niche. For example, reactive oxygen species-responsive NPs loaded with SDF-1α have been administered intravenously in mice to accelerate wound healing [30].

An attractive possibility to facilitate the targeting of the NP formulations to the stem cell niche is by the use of external stimuli. In this case, NPs respond to magnetic forces, light, or ultrasounds and release the cargo with spatio-temporal control. The use of external stimuli may improve significantly the therapeutic effect of the NPs in the stem cell niches. For example, up-conversion NPs, activated by a near-infrared laser, released efficiently kartogenin into MSCs and specifically promoted chondrogenic differentiation [31]. In addition, the NPs may open biological barriers. For example, the thermal energy generated by magnetic heating of magnetic NPs may increase BBB permeability [32].

2.2 Factors to modulate in the stem cell niche

In the adult tissues, the stem cell niche is composed by a variety of cell types that contribute for the self-renewal of the stem cell population. As a title of example, the NSC niche, at the SVZ, is formed by ependymal cells, quiescent NSCs (type B cells), type C transit-amplifying progenitor cells, neuroblasts (type A cells) and glia, while the HSC niche is composed by osteoblasts, vascular cells neural cells, megakaryocytes, macrophages and immune cells [33] (**Fig. 1**). In both stem cell niches, the stem cells interact with the other cells of the niche by direct (physical contact) or indirect (factors secreted by cells).

Multiple signaling pathways regulate the activity of stem cells. So far, in the context of endogenous stem cell regulation, Wnt [11, 15], RA [16, 18], Notch [34] and SAPK/JNK [12] signaling pathways have been explored. Wnt proteins regulate the renewal and differentiation of stem cells [35]. In general, Wnt proteins act as short-range intercellular signals. When Wnt signals bind to their receptors there is an accumulation of ß-catenin in the nucleus that regulates gene expression and directs cell proliferation, polarity and fate determination in the niche. NPs containing activators of Wnt signaling enhanced the proliferation and engraftment of HSCs and leads to the proliferation of NSCs in the hippocampus and SVZ regions of adult animals [11, 15]. Like Wnt, Notch signaling pathway is responsible for the homeostasis of the stem cell niche. Notch signaling is activated by direct cell-cell contact while Wnt signaling does not require cell-cell contact [33]. Ligand and receptor are trans-membrane proteins that, upon binding, induces a proteolytic cleavage of the Notch intracellular domain that subsequently enters the nucleus to regulate gene transcription. Notch pathway regulates stem cell function in niches as the skin, muscle and bone marrow [33]. NPs containing Notch inhibitors have been shown to promote muscle stem cell differentiation [34]. Another important signaling pathway in stem cell regulation is the RA. RA is a diffusible signaling factor that is transported to the nucleus of stem cells by proteins, regulating gene expression after binding to the RA receptors. The role of RA is firmly confirmed in neural, heart and male germ progenitor cell differentiation [36]. RA-loaded NPs have been explored to regulate the activity of NSCs [16, 18].

2.3 Applications of NPs to modulate stem cells

2.3.1 NSCs

In the adult mammalian brain two main neurogenic regions have been identified: the SVZ on the lateral walls of ventricles and the subgranular zone (SGZ) on the hippocampus [37] (**Fig. 1**). Modulation of the neurogenic niches has been pointed as a therapeutic strategy for brain repair for

almost 20 years [38], even so, no therapy was yet successful to completely restore brain function [5]. Recently, some of us have developed RA-loaded polymeric NPs (RA-NPs) able to induce differentiation of NSCs into functional neurons both *in vitro* and *in vivo* [16, 18]. *In vivo*, the RA-NPs were administered by intracerebroventricular infusion. RA-NPs induced the expression of *Ngn1* and *Mash1* pro-neurogenic genes at lower doses of RA (approximately ~2500-fold lower than soluble RA). Because, brain vascular cells are important for the self-renewal of NSCs, in a separate study it was investigated the effect of RA-NPs in endothelial cells. RA-NPs mediated the proliferation and survival under ischemia of endothelial cells. Conditional medium from NP-treated endothelial cells after hypoxia culture, showed an additional capacity to modulate proliferation and differentiation of NSCs [39].

NPs may also regulate NSCs in animal models having ischemic or neurodegenerative diseases. NPs containing SDF-1α enhanced neurogenesis and angiogenesis in animals after stroke [40]. Moreover, intraventricular administration of miR124-loaded PLGA NPs induces neurogenesis in a mouse model of Parkinson's disease. This strategy offered a partial reversion of the disease motor phenotype [12]. In an independent study, PLGA NPs encapsulating curcumin induced NSC proliferation and neuronal differentiation in a rat model of Alzheimer's disease. The NPs reversed Aβ-mediated inhibition effects on hippocampal neurogenesis and restored cognitive ability [11].

2.3.2 HSCs

The targeting of NPs to the HSC niche in the bone marrow may be achieved by conjugating the NPs with specific ligands. For example, PLGA NPs were surface modified with bisphosphonate to make them with bone-binding capacity [41]. The bisphosphonates are calcium ion-chelating molecules that after systemic administration deposit in bone tissue, particularly at the sites of high bone remodeling.

NPs may interfere with the paracrine activity of HSCs. We demonstrated recently that protamine sulfate-coated PLGA NPs encapsulating a magnetic resonance imaging agent have the capacity to downregulate the immune response of HSCs [42]. The NPs decreased the secretion of pro-inflammatory cytokines such as IFN-γ, IL-8, MCP-1, MIP-1β and TNF-α. We further showed that the decrease was mediated by an attenuation in the activity of toll-like receptors 6 and 7.

NPs may also regulate the engraftment of HSCs in the bone marrow. NPs containing a Wnt activator have been chemically conjugated to the membrane of HSCs enabling continuous pseudo-autocrine stimulation of the transplanted HSCs [15]. This induced a ≈ 6 fold increase in the HSC engraftment without affecting HSC multilineage differentiation potential.

NP-mediated modulation of HSCs has been also explored for the treatment of blood and genetic disorders including anemia, leukemia, lymphoma and thalassemia. For example, PLGA NPs containing triplex-forming peptide nucleic acids (PNAs) mediated gene editing in HSCs of a β-thalassemic mouse model [43]. PNAs bind DNA in a site-specific manner to recruit endogenous DNA repair. The 6.9% frequency of NP-mediated gene editing in HSCs was sufficient to achieving an improvement in the phenotype of a mouse transgenic model with a β-globin carrying a thalassemia-associated mutation. NPs may be also used to mobilize cells to the HSC niche. For example, SDF-1α-loaded chitosan NPs were able to induce bone marrow MSCs migration *in vitro* [44].

3. Stem cells as NP carriers

Current therapy against cancer relies mainly on surgery and chemo- and radiotherapy. However, it has become evident that this approach is not effective enough to fight some types of cancer. Indeed, mortality rates are still very high because the lack of efficient treatment. Through surgery is not always possible to remove the entire tumor and cancer drugs often display low targeting efficacy and short half-lives. Also, effective drugs available in the market are often not able to cross barriers, like the BBB in brain cancer, and are not able to penetrate the tumor. Cancer stem cells also

present a setback since they are resistant to current standard therapies and have proven to play an important role in tumor recurrence [45].

NPs can be delivered to tumors through passive targeting, using the enhanced permeability and retention effect, or through active targeting, functionalizing their surface and enabling them to cross barriers and to enhance targeted delivery [46]. A high number of therapeutic agents can be loaded per nanocarrier enabling a better accumulation of drug at tumor site. NPs protect the therapeutic agent from premature degradation and allow its controlled release, decreasing toxicity to normal tissues [45, 47]. However, NPs present several disadvantages. NPs can be engulfed by the phagocyte system which decreases the amount of drug that actually reaches tumor site. Also, NPs only benefit from the enhanced permeability and retention effect when tumors are highly vascularized. As a result, NPs may present an uneven intratumoral distribution and may not be able to effectively target infiltrative areas [45, 48, 49].

Since nanotechnology has not achieved the expected results in improving drug targeting, the use of stem cells as a vehicle to transport NPs towards tumor areas may offer an alternative strategy (**Fig. 2**). Some stem cells have tropism for injury and tumor sites. They can be loaded with NPs without losing this capacity. They can spread through existing migratory pathways and non-typical routes and are able to cross barriers, inclusive the BBB, providing an increased intratumoral distribution [45, 50]. The tropism of stem cells towards tumors might be explained by the SDF-1α/CXCR4 axis. Tumor cells express SDF-1α and the SDF-1α receptor CXCR4 is expressed on stem cells. Basically, stem cells can migrate to the tumor site following the concentration gradient of SDF-1α, but other mechanisms might also be involved in the migration of stem cells [51] (**Fig. 3**).

3.1 Leukemia

Acute myeloid leukemia is characterized with high relapse rates following chemotherapy. This relapse results from quiescent cells within the hematopoietic niche termed leukemia stem cells [52].

NPs able to target leukemia stem cells in the bone marrow niche have been proposed as a therapeutic solution for the relapsing of acute myeloid leukemia. Thus in the last years, several NP formulations have been developed to target LSCs based in NPs conjugated with E-selectin thioaptamer [53], CD45.2 antibody [54] or CD117 antibody [55]. However, this strategy has limitations because the NPs although showing bone marrow targeting they still accumulate in other regions of the body and thus may lead to toxic effects. We have tested recently an alternative strategy based in the use of leukemic cells to transport light-triggerable RA-containing NPs to the leukemic stem cell niche [21]. These NPs had the capacity to differentiate efficiently *in vitro* human leukemia cells isolated from bone marrow aspirates of patients with acute myeloid leukemia. Leukemia cells transfected with the NPs were able to home in the bone marrow and recognize engrafted leukemia cells. When the NPs were activated by a blue laser, the disassembly of the NPs promoted a biological differentiation program in the transporting cells which in turn resulted in the secretion of vesicles containing RA that had differentiation properties in the HSC niche (**Fig. 3**).

3.2 Glioblastoma

The glioblastoma (GB) is the most common and malignant subtype of malignant glioma [56]. Despite aggressive treatment using surgical resection, radio- and chemotherapy, the prognosis of this disease remains very poor and GB remains an extremely lethal tumor [56, 57]. This tumor is characterized by aggressive invasion and diffuse infiltration of tumor cells into the white matter tracts which makes complete surgical resection impossible [45, 56].

Stem cells may act as vehicles of NPs to reach the tumor sites. It has already been proven that they are able to migrate within glioma tissue, which allows for an increased intratumoral distribution of the NPs and dispersion into infiltrative tumor areas [45]. Both MSCs [47] and ADSCs [58] have been used as vehicles. MSCs have been used efficiently for the transport of poly(lactic acid) NPs and lipid nanocapsules [47]. They could efficiently internalize the NPs, being the uptake concentration

and time-dependent, and accumulate them for at least 7 days. Cells loaded with the NPs presented a similar migration pattern as compared to unloaded cells. Seven days after intratumoral cells injection, cells were largely distributed at the border zone between tumour and normal parenchyma. In a separate study, ADSCs were used as vehicles of NPs [58]. In this case, the ADSCs were transfected with superparamagnetic iron oxide nanoparticles containing paclitaxel. Loaded ADSCs showed selective delivery across the BBB toward brain glioma. The NPs within the cells were then activated with a high frequency magnetic field for drug release. Importantly, glioma-bearing mice treated with this cell-based therapy had a significantly prolonged survival time as compared with the control and did not show pathological lesions in major organs, particularly in the liver.

3.3 Breast cancer

Stem cells, in particular MSCs, have been used as vehicles of NPs to reach breast tumors. In this case, MSCs were loaded with silica NPs containing purpurin-18, a hydrophobic photosensitizer, for photodynamic therapy [59]. After light activation, the NPs produce cytotoxic reactive oxygen species to induce necrosis and/or apoptosis in cancer cells. MSCs loaded with the NPs were capable of producing reactive oxygen species to kill cancer cells. Importantly, stem cells loaded with NPs, and then irradiated, significantly reduce the breast tumor size and weight. In a separate study, MSCs have been loaded with mesoporous silica NPs containing doxorubicin [46]. The NPs could accumulate for 5 days in the cytoplasm of the cells. The studies showed that the MSCs retained their *in vivo* homing capacity when carrying NPs and could transport them do the tumor.

3.4 Lung cancer

Lung cancer is a major death cause in developed countries and its incidence is increasing in developing countries [60]. The lungs are a frequent site of metastasis, being metastasis the primary cause of cancer-associated death [61]. Metastatic cancers have a systemic nature, present resistance

to existing therapeutics and have low targeting efficiency agents what makes them mostly incurable [61-63]. Stem cells have been also used to target lung metastasis [48]. In this case, MSCs were loaded with PLGA NPs containing doxorubicin. Cells with or without the NPs maintained the same tropism toward the tumor. Importantly, *in vivo* studies showed that the administration of MSCs loaded with NPs lead to their accumulation in the lungs for at least 24 h, whereas bare NPs (without MSCs) were unable to reach the lungs. Finally, mice treated with MSCs loaded with NPs had a significant reduction of lung metastasis.

3.5 Gastric cancer

Tumors in the digestive tract are among the most common and deadliest. Gastric cancer, alongside with colorectal and hepatic cancers, present the highest mortality rates. Late diagnosis and lack of efficient therapeutics make these tumors hard to treat. Standard therapy resides in surgery associated with chemotherapeutics that normally fails to eliminate subpopulations of highly aggressive cancer cells that are resistant to chemotherapy and are capable of developing metastasis [64]. Stem cells have been recently used in combination with nanomedicine to target gastric cancer cells. Proliferation-arrested human induced pluripotent stem cells have been used to transport gold NPs to gastric tumors [51]. The tropism of induced pluripotent stem cells to tumors is not completely understood [50, 51]. After targeting the tumor, the tumor site was irradiated with a near infrared laser to heat the gold NPs and kill the cancer cells distributed in the tumor.

4. Conclusions and future perspectives

The use of nanotherapeutics to control the activity of endogenous stem cells had some limitations, the most important one related to the targeting. In many cases, the NPs were administered in the stem cell niche or in its proximity. This is the case of intracerebral administration to target the NSC niche [12, 14, 16]. Few studies have administered the NPs systemically [11, 53]. In this case, the

biodistribution of the NPs has not been characterized and thus it is possible that the nanotherapeutics will affect several regions in the body besides the targeted stem cell niche. The use of stem cells or progenitor cells carrying the nanotherapeutics to the stem cell niche is a promising strategy. In this case, the cells may transport the NPs at the cell surface [15] or within the cell cytoplasm [21]. In both cases, the NPs should not interfere with the homing of the cells, which has already been demonstrated in several studies. To maximize the effect of the drug in the transporting cell or in the other cells of the niche, the release of the biomolecules should occur at the niche. In most formulations developed so far, the formulation releases the biomolecule until reaches the niche. Few formulations have the capacity to release at the niche [21]. Thus, further advances are necessary to develop NP formulations that are triggered remotely. In addition, it is important that the NP formulations reach the cytoplasm and thus escape the endolysosomal compartment during the intracellular trafficking. Finally, the production of the NPs should be optimized to increase their reproducibility, cost-effectiveness and biocompatibility.

The use of stem cells as vehicles for NPs requires further developments in the expansion of these cells in the laboratory under controlled conditions. Moreover, studies should be performed to determine the safety of the cells with and without the NPs, in relevant animal models. In case of stem cells for the transport of NPs to tumors, further studies are necessary to clarify whether the transporting cells may or not contribute for the vascularization of the tumors [65]. It will be important to determine their function in the tumor microenvironment.

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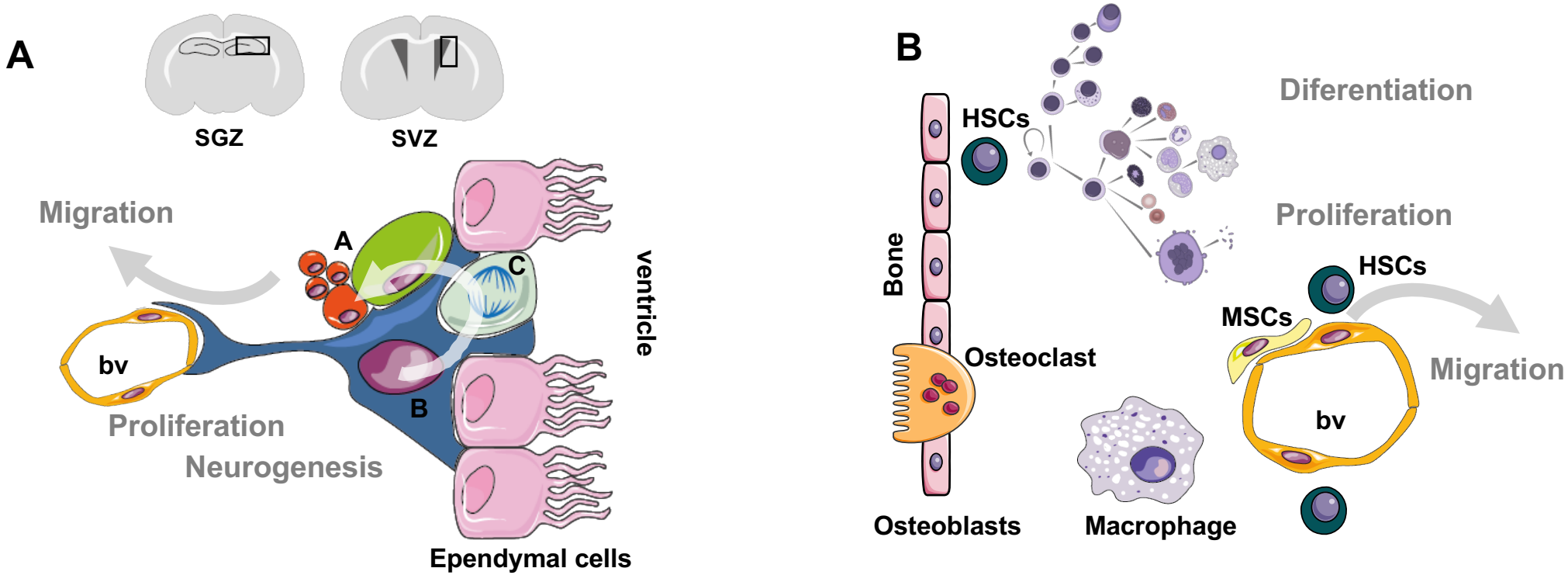
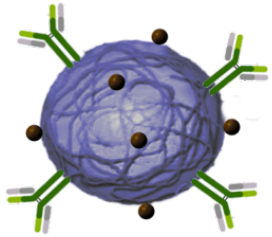
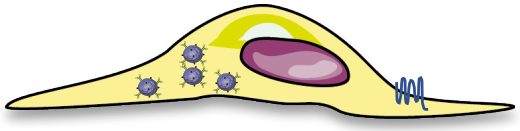


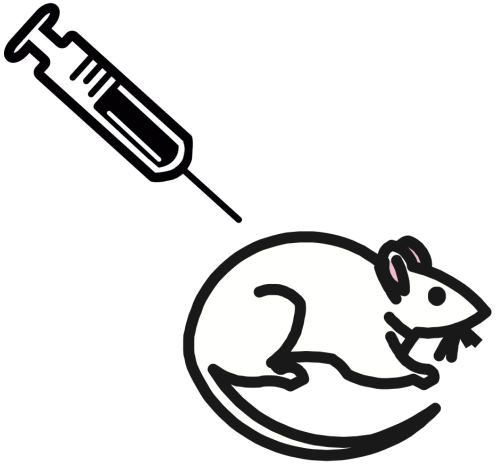
Figure 1 – Complexity of endogenous neural and hematopoietic niches and opportunities for stem cell modulation. (A) NSCs appear in the SVZ and SGZ in the adult mammalian brain. NSCs in the SVZ (type B cells) give rise to transit amplifying progenitors (type C cells) that further differentiate into neuroblasts (type A cells). (B) HSCs reside in the bone marrow niche and interact with other cell types. In A and B, NPs may mediate several biological processes which are highlighted in grey. BV means blood vessel.



Does the formulation releases passively the drug? What is the release profile of the biomolecule?



What is the internalization level of NPs?
For how long the NPs accumulate in the cell?



Does the stem cells loaded with NPs home to the stem cell niche ?
Is any biological effect in the stem cell niche homeostasis?

Figure 2- Questions that need be to addressed in the use of stem cells as transport vehicles of NPs.

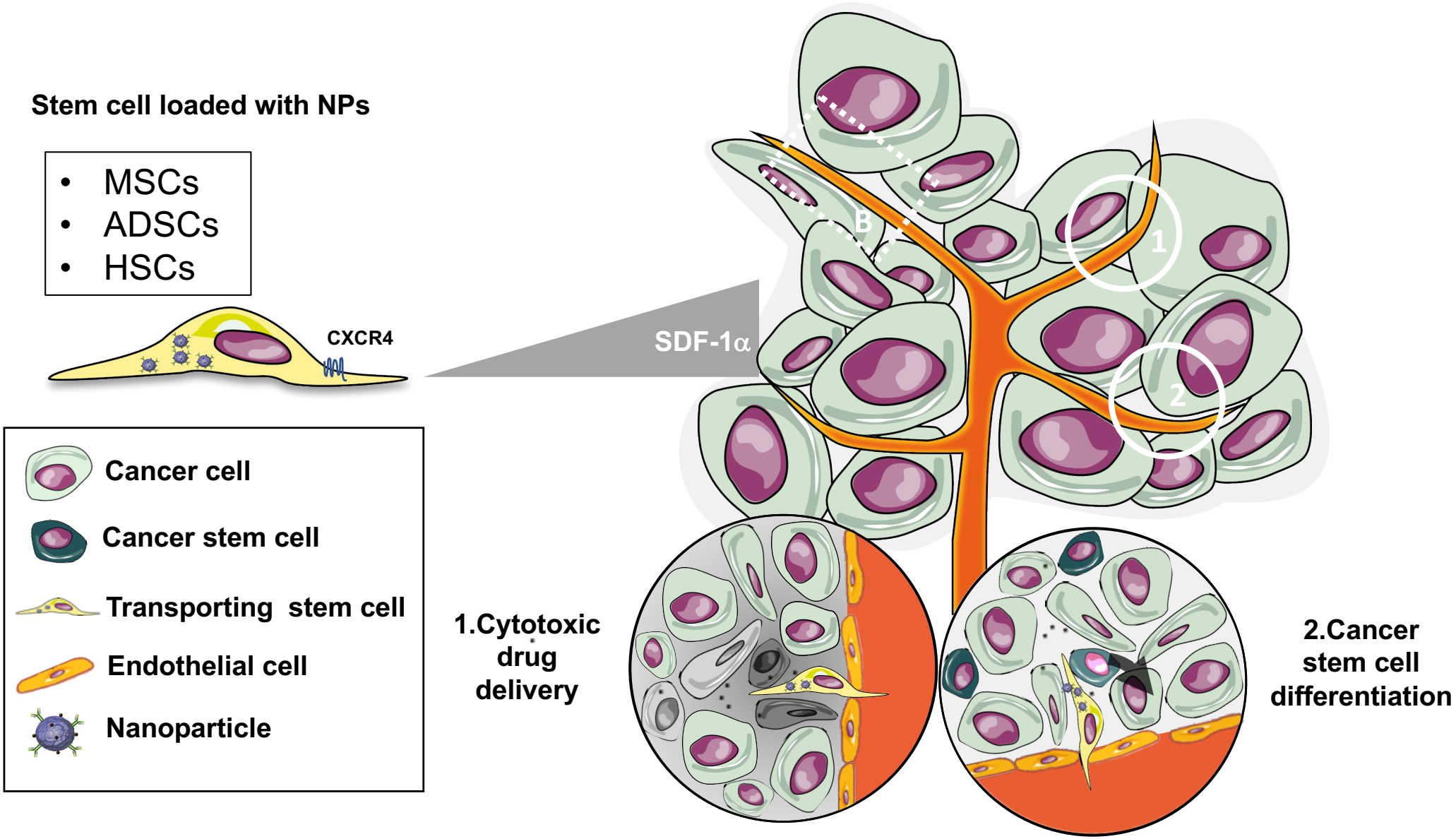

Stem cell loaded with NPs
MSCs
ADSCs
HSCs
CXCR4
SDF-1α
B
1
2
Cancer cell
Cancer stem cell
Transporting stem cell
Endothelial cell
Nanoparticle
1.Cytotoxic drug delivery
2.Cancer stem cell differentiation

Figure 3 - **Stem cells loaded with NPs to reach cancer stem cell niches.** MSCs, ADSCs and HSCs may act as vehicles of NPs to target tumors. Homing of stem cells to the tumor is explained by the SDF-1α/CXCR4 axis. Stem cells migrate to the tumor following a concentration gradient of SDF-1α. The stem cells may be then induce the differentiation of stem cells in the niche (e.g. paracrine effect of the factors released by the NPs) (1) or induce their death (2) (e.g. by the radicals created by the NPs within the transporting stem cells).