Review

Iron oxide-loaded nanotheranostics: Major obstacles to in vivo studies and clinical translation

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ABSTRACT

A major issue in current cancer therapies is the lack of selectivity, which leads to damage in healthy tissues. Therefore, researchers have focused on numerous innovative targeting strategies to address this problem with the goal of increasing selectivity to avoid or minimize accumulation in healthy tissues. These strategies include (i) passive targeting, (ii) active targeting and (iii) stimuli-mediated targeting. Moreover, due to the high intra- and inter-variability found in tumors, nanotheranostics, which is the combination of a therapeutic and an imaging agent in a single vector, have emerged as indispensable tools for personalized therapy. Superparamagnetic iron oxide (SPIO) are MRI contrast agents that produce predominant T2 relaxation effects with excellent sensitivity compared with other MRI agents. Therefore, they have received increased interest in the field of theranostics during the past decade. However, few studies have been successfully conducted in vivo. This review aims to provide an overview of the targeted SPIO-based nanotheranostics recently used in pre-clinical studies and the major obstacles to in vivo studies and clinical translation. In the first section, we discuss personalized therapy as a biomedical application of theranostics. Then, we summarize the different imaging agents that have been used for theranostic purposes, with a focus on SPIO. In the third section, we detail recent advances in targeted SPIO-based nanotheranostics that have been used in pre-clinical studies. In the final sections, we discuss the limitations for in vivo studies, clinical translation and the clinical perspectives of SPIO-based nanotheranostics.

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1. Introduction

According to the American Cancer Society, approximately 1.6 million new cancer cases are expected to be diagnosed in 2014 [1]. Early detection and effective cancer treatments are the most important factors for saving lives [2]. Anti-cancer drugs suffer from poor pharmacokinetics and inappropriate biodistribution properties. Consequently, many agents that are shown to be highly effective in vitro become relatively ineffective when administered in vivo and produce significant toxicity [3]. Numerous drug delivery systems have been designed to address these problems, specifically, nanomedicines, which refer to nanoscaled therapeutics that have been extensively studied. In particular, liposomes, nanoparticles, micelles and antibodies have been demonstrated to be clinically relevant for cancer therapy [4–6]. The first rationale for the use of nanomedicines for cancer therapy is the preferential delivery of nano-vectorized drugs to solid tumors due to enhanced permeability and retention (EPR) effect [7]. Tumor vessels surrounding the tumors are leaky due to abnormal basement membranes and incomplete endothelial linings, allowing nanomedicines to reach the tumor passively through the leaky vasculature. Therefore, the EPR effect enables higher local drug concentrations at the tumor site when the drug is delivered in a nanovector. The efficacy of passive targeting depends primarily on (i) the degree of tumor vascularization and angiogenesis, which is reliant on the tumor type and anatomical site and (ii) the high interstitial fluid pressure and heterogeneous blood flow, which limit drug uptake and homogenous distribution in the tumor [8,9]. Targeting can be improved by grafting ligands to the surface of the nanomedicines, allowing active targeting by binding to receptors that are overexpressed by cancer cells or angiogenic endothelial cells [8,10]. One potential advantage of targeted delivery may be altered intracellular distribution. Both targeted and non-targeted systems arrive at the tumor via the EPR effect. Subsequently, the tumor cell internalization mechanism is enhanced by the presence of surface ligands [11,12]. It has been demonstrated that ligand-grafted nanoparticles enter cells via receptor-mediated endocytosis [13,14]. Nanomedicines can also enhance drug-circulation times, control drug-release kinetics and enable superior dose scheduling [10]. Nanomedicines avoid formulations that contain toxic excipients, which contribute to side effects for many conventional chemotherapeutics. Nanomedicines also may potentially overcome the development of tumor resistance to conventional chemotherapeutics [13]. Finally, nanomedicines allow the delivery of more than one therapeutic agent, facilitating combination therapies.

This review aims to provide an overview of the targeted SPIO-based nanotheranostics recently used in pre-clinical studies and the major obstacles to in vivo studies and clinical translation. In the first section of this review, we discuss personalized therapy as a biomedical application of theranostics. Then, we summarize the different imaging agents used for theranostic purposes, with a focus on SPIO. In the third section, we detail the recent advances in targeted SPIO-based nanotheranostics that have been used in pre-clinical studies. In the final sections, we discuss their limitations for in vivo studies, clinical translation and analyze the clinical perspectives of SPIO-based nanotheranostics.

2. Theranostic nanomedicine

In addition to therapeutic applications, passively and actively targeted nanomedicines are also increasingly being used for diagnostic purposes. Antibodies, liposomes, nanoparticles and micelles may deliver a contrast agent, such as radionuclides and MR imaging probes, to detect cancer as well as visualize the drug delivery process [3]. In addition to nanomedicines that are designed for therapeutic or diagnostic purposes, diagnostic (contrast agent) and therapeutic (anti-cancer drug) agents can be combined within a single multifunctional nanomedicine, known as “theranostic nanomedicine or nanotheranostics” (Fig. 1A). Based on the advances in and close collaboration between several different scientific disciplines, such as chemistry, biology, pharmacy, nanotechnology, medicine and imaging (Fig. 1B), numerous reports have shown that nanomedicine is one the most promising methods for theranostics, although theranostic development is still at an early stage [3,14,15]. Theranostic design requires broad knowledge and a solid understanding of the detection and therapeutic mechanisms. This knowledge includes understanding the molecular mechanisms, diagnostic strategies, therapeutic efficiency, toxicity, side-effects of materials and nanomedicine preparation techniques for the dual purposes of diagnosis and therapy [14].

The ideal theranostic nanoparticle should possess several advantageous properties: (i) the ability for selective tumor accumulation, (ii) the capacity to deliver therapeutic doses of anti-cancer drugs and to detect tumors at their earliest stage and (iii) the nanovector must be biocompatible and biodegradable [2].

Nanotheranostics can be used for various applications: (i) non-invasive assessment of biodistribution and target site accumulation, (ii) drug release monitoring, (iii) the enhancement of therapeutic efficacy via triggered drug release and (iv) prediction of therapeutic response [3]. Additionally, nanotheranostics can be used to preselect patients, leading to their significant potential for personalized nanomedicine (chemo-) therapeutic interventions. Theranostic strategies include situations where patients are pre-selected based on data from initial target site accumulation studies.

It is important to note that theranostics take their place in its broadest sense. Many publications claim that theranostics can be used for simultaneous diagnosis and treatment. Actually, their suitability for real diagnoses is questionable. The diagnostic purpose of theranostics should not refer to the identification, localization and/or staging of tumors, but to the pre-selection of patients, prediction of potential therapeutic responses, and/or longitudinal monitoring of treatment efficacy [17]. This critical point has been addressed in an interesting review written by Rizzo et al. [17]. As exemplified in Fig. 2, the first step toward personalized nanomedicine treatment is to pre-select patients on the basis of non-invasive imaging data examining target site accumulation. If the tumor accumulation is sufficient, patients can be treated with the nanotheranostics, whereas other patients will receive conventional chemotherapy or another intervention. A second personalization step consists of non-invasive monitoring of the response to the first 1 to 3 treatment cycles. During this monitoring, the treatment should be adapted or switched to other therapeutic interventions, if necessary [17,18].

3. Contrast agents and SPIO

3.1. Imaging techniques and contrast agents for theranostics

The diagnostic aspect of theranostics primarily revolves around imaging mechanisms using different contrast agents. The most commonly used imaging techniques are positron emission tomography (PET), single photon emission computed tomography (SPECT), magnetic resonance imaging (MRI) and different optical imaging techniques (bioluminescence and fluorescence) that have high sensitivity [19].
Among these techniques, MRI is the most commonly studied technique and a considerable amount of research has been devoted to the use of magnetic particles as contrast agents. Particles of gadolinium, iron oxides, gold, silver and other metals are currently being investigated. The imaging aspect of theranostics can also be exemplified by manganese oxide, quantum dots, microbubbles, radionuclides and silica nanoparticles[14]. Non-exhaustive examples of theranostics using different contrast agents are presented in Table 1. Since biomedical applications of theranostics have been extensively reviewed[14,16], in this review, we only focus on iron oxides as contrast agents.

3.2. Iron oxides

Superparamagnetic iron oxide (SPIO) nanoparticles are contrast agents that have a variety of applications in molecular and cellular MRI. Superparamagnetic nanoparticles are composed of iron oxides, i.e., magnetite (Fe₃O₄), maghemite (Fe₂O₃) or other ferrites. Their given name originates from their ability to exhibit “superparamagnetism”. This property allows SPIO to be magnetized upon external magnetic field application without exhibiting any residual magnetic interactions after magnetic field removal[40]. SPIO are prepared using several different methods, including micro-emulsion, thermal decomposition and co-precipitation. Among these methods, the classical iron salt co-precipitation technique is the most commonly used procedure. This method is based on the precipitation of ferrous (Fe²⁺) and ferric (Fe³⁺) salt mixtures in a basic medium to form Fe₃O₄[41–43]. To avoid aggregation and prevent surface oxidation, which are crucial for their clinical use, it is necessary to coat the synthetized SPIOs. SPIO have been coated with a wide variety of materials. Among the coating agents usually used, the most common are biocompatible polymers (dextran and its derivatives, siloxane, poly(ethylene glycol), poly(lactic acid), poly(ε-caprolactone).

Fig. 1. Theranostics. (A) Schematic representation of a theranostic multifunctional nanomedicine. (B) Schematic representation of the interdisciplinary field of theranostics. Adapted from [16].

Fig. 2. Schematic representation of the rationale for image-guided and personalized nanomedicine. Adapted from [18].
chitosan), inorganic materials (gold and silica) and monomeric stabilizers (carboxylates, phosphates) [41,42,44].

SPIO are primarily used because of their negative enhancement effect on T2 and T2*–weighted sequences [40]. However, their effects on T1 relaxation time are also employed. Different classes of superparamagnetic nanoparticles have been investigated and are classified by their particle size: SPIO (>50 nm), ultra-small superparamagnetic iron oxide (USPIO) (<50 nm) and micron size iron oxide particles (MPIO) (>1000 nm) [41]. SPIO provide significant advantages over traditional contrast agents: (i) high magnetic signal strength, (ii) relatively low cytotoxicity, (iii) longer lasting contrast enhancement, (iv) improved tumor margin delineation and (v) low sensitivity to the number of surrounding water molecules. Additionally, studies have shown that the released iron from SPIO is metabolized by the body, reducing the potential for long-term cytotoxicity [41,42,45].

After intravenous injection, numerous opsonin proteins, cells, and salts bind to the surface of the nanoparticles, known as the opsonization process responsible for easier recognition by the mononuclear phagocytic system (MPS) leading to rapid clearance from the circulation. Nanoparticles are usually taken up by macrophages in the liver (Kupffer cells ~ 80–90%), spleen (~5–8%) and bone marrow (~1–2%) [46]. After uptake in specialized macrophages, SPIO are degraded lysosomally. The core material is supplied to the iron storage pool of the body (total body iron approximately 4–5 g) and deposited in the liver in the form of ferritin and/or hemosiderin whereas the coating material is eliminated via other decomposition and elimination pathways, e.g., the coating of ferumoxtran-10 (Sinerem®) is degraded via intracellular dextranases and is eliminated primarily renally (89% in 8 weeks). The natural decomposition of these iron reserves is only possible via hematopoiesis (Fe2+ as central atom in the hemoglobin) and the exfoliation of epithe-lial cells of the skin and bowel [47]. Limiting phagocytosis in the liver and spleen decreases the uptake rates for some nanoparticles, resulting in longer blood half-lives and facilitating their clinical application as T2 contrast agents. SPIO coating shell via physical interactions, leading to more intense signals in MRI.

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Table 2 provides a non-exhaustive list of recent examples of passively targeted SPIO-based theranostics in pre-clinical studies. These examples were chosen from the literature providing information on both the therapeutic and imaging properties of these SPIO-based theranostics in vitro and/or in vivo preclinical tests.

For example, Schleich et al. developed dual paclitaxel (PTX)/SPIO loaded PLGA-based nanoparticles for cancer therapy and imaging [56]. They demonstrated the efficiency of the nanoparticles for cellular uptake and MRI contrast enhancement in vitro and in vivo as an anti-cancer treatment in CT-26 bearing mice. Similarly, Ling et al. developed dual docetaxel/SPIO-loaded PLGA-based nanoparticles and demonstrated their efficient in vitro cellular uptake, drug release and MRI contrast enhancement in a prostate cancer cell line [57]. The same group developed temozolomide/SPIO-loaded PLGA nanoparticles for the imaging and treatment of malignant gliomas and demonstrated their in vitro efficacy for cellular uptake, cytotoxicity and MRI contrast enhancement [58]. Another example of passively targeted SPIO-based nanotheranostics is provided by Chen et al. [59]. They developed inorganic silica spheres loaded with both doxorubicin (DOX) and a single SPIO nanocrystal core. These nanoparticles demonstrated DOX-induced toxicity in vitro and in vivo efficacy as an MRI contrast agent [60]. In addition to SPIO-loaded nanotheranostics, Kohler et al. developed a methotrexate–SPIO conjugate (MTT–SPIO), which demonstrated higher in vitro anti-tumor efficacy compared with free MT and significant in vitro MRI contrast enhancement in 9L cultured glioma cells [61]. Finally, Park et al. developed a gene delivery-based therapy using a SPIO polyplex in HeLa human cervical tumor cells [62]. This in vitro study demonstrated that MRI could be a sensitive tool for assessing gene delivery via DNA release from the SPIO-Polyethylene imine (PEI) polyplex, based on the “T2-magnetic relaxation switch effect” (Fig. 4A). Cell viability after 24 h incubation with polymers at various concentrations was determined by MTS assay (Fig. 4B). Conjugation of PEI to USPIO resulted in reduced toxicity maybe by restricting polymer mobility and thus limiting cellular interaction. Thus, conjugation of PEI to USPIO resulted in minimal change to gene delivery efficiency and significantly reduced cytotoxicity. When packaged with DNA, the particles had an increased T2 relaxation time, whereas a decrease in T2 relaxation time was observed when the DNA was released (Fig. 4C). This phenomenon may be exploited to monitor DNA release using MR imaging.

Although most of the currently developed nanoparticul systems rely on passive targeting via the EPR effect, this strategy has recently been questioned. Because passive targeting is highly dependent on tumor vascularization and this vascularization is highly heterogeneous depending on the tumor type, the patient (inter-variability) and the disease stage as well as the tumor area (intra-variability), the EPR effect is a highly heterogeneous phenomenon. Some tumor types have been shown to exhibit practically no EPR effect (e.g., metastatic liver, pancreatic and prostate cancer) [63,64]. Moreover, the EPR effect has been shown to be more important in animal models compared with humans. This is due to the faster growth of animal tumors compared with human tumors.

**Table 2** Non-exhaustive examples of passively targeted SPIO-based theranostics in pre-clinical studies.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Drug</th>
<th>Cell line</th>
<th>In vitro/in vivo MRI</th>
<th>Results</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLGA NP</td>
<td>PTX</td>
<td>CT-26</td>
<td>In vitro</td>
<td>High tumor regrowth delay, high relaxivity</td>
<td>[56]</td>
</tr>
<tr>
<td>PLGA NP</td>
<td>DTXL</td>
<td>C3 glioma</td>
<td>In vitro</td>
<td>Effective cellular uptake, good relaxivity</td>
<td>[57]</td>
</tr>
<tr>
<td>PLGA NP</td>
<td>TMZ</td>
<td>C6 glioma</td>
<td>In vitro</td>
<td>Good antiproliferative activity and relaxivity</td>
<td>[58]</td>
</tr>
<tr>
<td>Silica sphere</td>
<td>curcumin</td>
<td>HL-60</td>
<td>In vitro</td>
<td>Time-dependent induction of apoptosis</td>
<td>[59]</td>
</tr>
<tr>
<td>Silica sphere</td>
<td>DOX</td>
<td>MCF-7</td>
<td>In vitro</td>
<td>In vitro cytotoxicity and in vivo MRI</td>
<td>[60]</td>
</tr>
<tr>
<td>SPIO-conjugate</td>
<td>MTT</td>
<td>9 L glioma</td>
<td>In vitro</td>
<td>In vitro cytotoxicity, in vitro T2 contrast enhancement MRI</td>
<td>[61]</td>
</tr>
<tr>
<td>Polyplex DNA</td>
<td>DNA</td>
<td>HeLa</td>
<td>In vitro</td>
<td>Monitoring of DNA release with MRI</td>
<td>[62]</td>
</tr>
</tbody>
</table>

**Abbreviations**: DOX = doxorubicin, DTXL = docetaxel, MTT = methotrexate, NP = nanoparticles, PLGA = poly(lactic-co-glycolide), PT = passive targeting, PTX = paclitaxel, TMZ = temozolomide.
patients. This speed increase results in increased leaky vasculature and, consequently, a larger EPR effect [18]. Secondly, due to the high interstitial pressure and dense extracellular matrix, carrier penetration is quite limited [65]. Interestingly, some pharmacological treatments that interfere with the extracellular matrix (matrix degrading enzymes, fibrosis inhibitors) or enhance extravasation (inflammatory mediators, such as TNF-α) have been shown to promote tumor penetration when co-administered [66,67].

4.2. Active targeting

Passive targeting has several disadvantages. Specifically, the accumulation resulting from the EPR effect may not be sufficient to induce high intracellular uptake. Therefore, ligand-mediated drug delivery appears to be a powerful tool for overcoming this problem. Passively targeted nanomedicines primarily rely on macropinocytosis for cellular uptake. Conversely, the molecular recognition of a ligand coupled to the

Table 3
Non-exhaustive examples of actively targeted SPIO-based theranostics in pre-clinical studies.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Drug</th>
<th>Functional molecules</th>
<th>Cell line</th>
<th>In vitro/in vivo</th>
<th>Results</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEG-PCL micelles</td>
<td>DOX</td>
<td>FA</td>
<td>Bel7402</td>
<td>In vitro</td>
<td>2.5× higher intracellular accumulation</td>
<td>[71]</td>
</tr>
<tr>
<td>Wormlike polymer vesicles</td>
<td>DOX</td>
<td>FA</td>
<td>HeLa</td>
<td>In vitro</td>
<td>Higher relaxivities compared to Feridex</td>
<td>[72]</td>
</tr>
<tr>
<td>Silica sphere</td>
<td>PTX/CMPT</td>
<td>FA</td>
<td>PANC-1 and BxPC3 (pancreatic)</td>
<td>In vitro</td>
<td>Increased cellular uptake, high relaxivities in vitro</td>
<td>[73]</td>
</tr>
<tr>
<td>SPIO-conjugate</td>
<td>MTT</td>
<td>FA</td>
<td>MCF-7, HeLa</td>
<td></td>
<td>In vitro anti-cancer efficacy, Higher internalization compared to the non-targeted SPIO</td>
<td>[74]</td>
</tr>
<tr>
<td>Polymeric micelles</td>
<td>DOX</td>
<td>cRGD</td>
<td>SLK (Kaposi’s sarcoma)</td>
<td>In vitro</td>
<td>Increased cytotoxicity, cellular uptake, relaxivity</td>
<td>[75]</td>
</tr>
<tr>
<td>Polymeric NP</td>
<td>Fumagillin</td>
<td>cRGD, peptidomimetics</td>
<td>MDA-435</td>
<td>In vitro</td>
<td>Effective angiogenesis MRI, good anti-angiogenic efficacy</td>
<td>[76]</td>
</tr>
<tr>
<td>Polymeric micelles</td>
<td>DOX</td>
<td>LCP</td>
<td>H-2009</td>
<td>In vitro</td>
<td>3× increased cellular uptake, high relaxivity</td>
<td>[77]</td>
</tr>
<tr>
<td>SPIO conjugates</td>
<td>DOX, ADOX</td>
<td>HuCC49ΔCH2 (anti-TAG-72 antibody)</td>
<td>LS174T, A375</td>
<td>In vitro</td>
<td>Increased cancer cell targeting, decreased T2 values in in vitro MRI, lower IC50 compared to non-specific targeted SPIO</td>
<td>[49]</td>
</tr>
</tbody>
</table>

Abbreviations: ADOX = azido-doxorubicin, AT = active targeting, CMPT = camptothecin, DOX = doxorubicin, FA = folate, LCP = lung cancer targeting peptide, MTT = methotrexate, PCL = poly(ε-caprolactone), PEG = poly(ethylene glycol), TAG-72 = tumor-associated glycoprotein-72.
nanomedicine surface promotes cellular entry via receptor mediated endocytosis, resulting in enhanced nanovector cellular uptake [52,68]. Therefore, the introduction of targeting ligands should increase internalization of both drug and contrast agent, resulting in improved anti-tumor activity, reduced toxicity and increased target-to-background contrast during imaging [69]. In addition to targeting tumor cells directly, nanomedicine can be directed against neoangiogenic endothelial cells, resulting in subsequent tumor cell death due to oxygen and nutrient deprivation [19,70]. Table 3 provides an overview of selected recent examples of actively targeted SPIO-based theranostics in pre-clinical studies.

A popular targeting ligand for cancer cells is folate (FA). The folate receptor is a high-affinity, glycosylphosphatidylinositol-anchored protein. Because FA is vital for rapid proliferation, the folate receptor is overexpressed in many tumor types and is highly restricted in healthy tissues. Therefore, FA-conjugates and FA-grafted nanocarriers have been intensively investigated for both drug delivery and imaging [78]. For example, Hong et al. reported the development of SPIO/DOX-loaded PEG-PCL micelles functionalized with folate on their surface (attached to the PEG chain) [71]. In vitro intracellular accumulation studies demonstrated a 2.5 higher concentration of folate-functionalized micelles compared with non-functionalized micelles in Bel 7402 cells (human hepatic carcinoma cells). Similarly, Yang et al. developed SPIO/DOX-loaded wormlike polymer vesicles grafted with FA and tested their magnetic properties in HeLa cells (Fig. 5) [72]. These theranostic particles displayed higher relaxivities compared with the commercial form Feridex® (Fig. 5C) and improved antitumor efficacy compared with non-functionalized vesicles in vitro (Fig. 5B). However, their theranostic system was not as effective as free DOX. Additionally, Liong et al. developed multifunctional inorganic nanoparticles for combined imaging and drug delivery comprised of mesoporous silica nanoparticles containing SPIO and PTX or camptothecin (CMPT) that were functionalized with FA on their surface [73]. Finally, Kohler et al. developed MTT–SPIO conjugates as a simultaneous MRI contrast agent and anti-cancer therapy [74]. The conjugate was comprised of iron oxide nanoparticles covalently bound to MTT, a chemotherapeutic drug that can target multiple cancer cells that overexpress folate receptors. The nanoparticles were first surface-modified with (3-aminopropyl) trimethoxysilane to form a self-assembled monolayer and were subsequently conjugated with MTT via amidation between the carboxylic acid end groups on MTT and the amine groups on the particle surface. The authors demonstrated the nanocomposite in vitro anti-cancer efficacy using cellular viability studies in human breast cancer cells (MCF-7) and human cervical cancer cells (HeLa). However, they did not assess the imaging ability of the developed theranostic.

Another interesting target in cancer therapy is the well-known $\alpha_v$ integrin family. In the integrin family, $\alpha_v\beta_3$ is the most extensively studied because of its strong implication in angiogenesis regulation. Interestingly, integrin $\alpha_v\beta_3$ has been shown to be overexpressed on both neoangiogenic endothelial cells and on the surface of many tumor cell types [19,51]. Therefore, targeting $\alpha_v\beta_3$ with SPIO-based theranostics may provide interesting insights into angiogenesis using MRI as well as effective tumor cell killing by both indirect (inducing tumor cell death as a consequence of nutrient and oxygen deprivation by killing neoangiogenic endothelial cells) and direct killing of tumor cells expressing $\alpha_v\beta_3$. For this purpose, Nasongkla et al. developed multifunctional polymeric micelles loaded with DOX and SPIO functionalized with cRGD [75]. The RGD tripeptide sequence has been shown to efficiently bind to $\alpha_v\beta_3$ integrin [19,79]. Similarly, Schmieder et al. demonstrated the ability of RGD functionalized fumagillin- (an anti-angiogenic agent) loaded SPIO nanoparticles to improve drug delivery and angiogenesis imaging in MDA-435 cells [76]. Guthi et al. reported LCP (lung cancer targeting peptide) functionalized multifunctional micelles for targeting $\alpha_v\beta_5$ [77]. These DOX/SPIO-loaded micelles displayed high

![Fig. 5.](image-url)
T₂ relaxivity, increased cytotoxicity and MRI capacity in vitro. Finally, Zou et al. developed antibody- and fluorescence–SPIO conjugates for MRI and fluorescence cancer cell imaging. DOX and azido-DOX were entrapped into an oleic acid shell coating [49]. These conjugates had increased cancer cell targeting, lower IC₅₀ compared with non-specific targeted SPIOs and decreased T₂ values in in vitro MRI.

In its first conception, as imagined by Paul Ehrlich in 1908 as a “magic bullet”, ligand-mediated targeting was thought to improve both biodistribution of the targeted nanomedicine and tumor accumulation, resulting in enhanced efficacy. However, because targeted nanomedicines have no means of self-propulsion, reaching the target site and subsequently accumulating in the tumor tissue, relies on EPR-mediated extravasation, which has several disadvantages as outlined above [53,80]. Therefore, the advantage of functionalizing nanoparticles is the higher chance of internalization. The mechanisms of tumor internalization for non-targeted nanoparticles mostly involve macrophagocytosis [81,82]. Conversely, functionalized nanoparticles are internalized via receptor-mediated endocytosis. Consequently, functionalized nanoparticle intratumor distribution shifts from the extracellular compartment to the tumor cell intracellular compartment, which improves anti-cancer efficacy [11]. Targeting neo-angiogenic endothelial cells may provide an interesting alternative because nanomedicines do not need to enter the interstitium to reach their target; therefore, they do not rely on EPR-mediated extravasation to exert their activity. This targeting can be easily achieved using RGD ligand-grafted nanomedicines. Another important point that needs to be considered is the fact that despite the rather easy in vitro demonstration of receptor-mediated targeting, in vivo efficacy is more difficult to show. Moreover, even if a better internalization is demonstrated in vivo, the entire amount of drug localized in the tumor is often still lower than 0.01% of the injected dose [13]. Indeed, before reaching their specific receptor expressed on tumor cells, targeted-nanomedicine face different challenges including escaping target receptor present on normal tissue, heterogeneity in receptor expression by tumor cells or according to the disease stage and a possible receptor saturation limiting targeting efficiency [83]. Furthermore, since PEGylation is a common technique improving nanocarriers stealth to avoid capture by the MPS, ligand grafting, by covering PEG corona, lowers the amount of available PEG and, thereby, alters pharmacokinetic and biodistribution profile [84]. Hence, it is of great importance for novel nanomedicines to verify the efficacy of targeting strategy in vivo and to document the dose- and time-dependency of targeting to guide protocol design in a view of a translational approach [83]. To date, many receptors have been explored in tumor targeting. Knowledge of basic cell biology, tumor biology, immunology, and cancer biology would be required to develop smart nanocarriers and to guide researchers in the choice of the proper ligand to attach. This choice should take several knowledge into account including tumor-specific receptors allowing endocytosis, tumor-specific biomarkers, tissue-specific and tumor-specific homing proteins, and tumor-specific enzymes allowing selective uptake into cells or accumulation in tumor microenvironments [85].

4.3. Stimuli-mediated targeting

Stimuli-mediated targeting is a commonly used term to refer to systems designed to release their contents or react upon exposure to internal or external stimuli. The stimuli can either be internal (change in pH or temperature in certain tissues or disease stages) or external (magnetic, electric fields, light, ultrasound, etc.) [52,86]. Table 4 presents a non-exhaustive list of SPIO-based nanotheranostics using stimuli-mediated targeting in pre-clinical studies.

4.3.1. Acid triggered drug release

Acid triggered drug release is based upon the particular microenvironment in tumor tissues characterized by a slightly more acidic pH compared with physiological pH (pH 6.5 compared to 7.3 in physiological conditions) [95]. Compared to the conventional drug delivery systems, the pH-sensitive nanocarriers have been shown to offer a better control in terms of adjustment of the location of drug release, internalization in the tumor cells and their microenvironment by responding to local stimuli [96]. However, due to the small difference between physiological pH and tumoral pH, early uncontrolled release of the drug payload may occur before nanocarriers reach their target site. To overcome this phenomenon, researchers increased the stability of their nanocarriers targeting the lower pH found in endosome (5.5) and lysosomes (below 5.5). Several research groups have used this characteristic to develop a labile link between the drug and carrier or labile self-assembling micelles to provide tumor intracellular drug release. For example, Feng et al. recently developed polyethylene glycol (PEG), poly (N- (N’, N’-diisopropylaminoethyl) aspartamide) (P(Asp-DIP)) and poly (lysine-cholic acid) (P(Lys-Ca)) (PEALCa) micelles loaded with SPIO and PTX for cancer therapy [87]. In physiological conditions, the polymers organize themselves to form micelles with the hydrophobic core, electric or temperature in certain tissues or disease stages) or external (magnetics, electric fields, light, ultrasound, etc.) [52,86]. Table 4 presents a non-exhaustive list of SPIO-based nanotheranostics using stimuli-mediated targeting in pre-clinical studies.

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Examples of stimuli-responsive SPIO-based nanotheranostics in pre-clinical studies.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formulation</td>
<td>Targeting type</td>
</tr>
<tr>
<td>PEALCa micelles</td>
<td>pH-triggered</td>
</tr>
<tr>
<td>Polymeric micelles</td>
<td>pH-triggered</td>
</tr>
<tr>
<td>(\gamma)-cyclodextrin-pluronic coated SPIO NP</td>
<td>HT</td>
</tr>
<tr>
<td>PLGA-PEG NP</td>
<td>HT + AT</td>
</tr>
<tr>
<td>CHC microbubbles</td>
<td>US</td>
</tr>
<tr>
<td>Squalene based nanoplatinum SPIO conjugates</td>
<td>MT</td>
</tr>
<tr>
<td>SPIO conjugates</td>
<td>MT</td>
</tr>
<tr>
<td>SPIO conjugates</td>
<td>MT</td>
</tr>
</tbody>
</table>

Abbreviations: CHC = carboxymethyl hexanoyl chitosan, CPT = camptothecin, DOX = doxorubicin, EPI = epirubicin, FA = folate, (F)US = (focused) ultrasound, GEM = gemcitabine, HT = hyperthermia, i.a. = intra-arterial, MT = magnetic targeting, MTX = mitoxantrone.
effectively delivered the micelles for MRI in vivo. Similarly, Li et al. studied acid-triggered core cross-linked nanomicelles loaded with DOX and SPIO for drug delivery and magnetic resonance imaging in liver cancer cells [88]. These micelles were comprised of folate-poly(ethylene glycol)–b–poly[N-(N,N-diisopropylaminoethyl) (DIP) glutamic acid (GA)] (folate-PEG-P[GA-DIP]) amphiphilic block copolymers. DIP was grafted to ~50% of the poly(L-glutamic acid) (PGA)’s carboxyl groups for nanomicelle stability. Micelle stability is ensured by the ionic interaction between the electronegative carboxylic function of non-grafted PGA (COO−) and the positively charged, namely N,N-diisopropyl, tertiary amine group. In acidic conditions (e.g., endosome, lysosome), the carboxylic groups are protonated, resulting in a weaker interaction between the two polymers and drug release (Fig. 6A). The authors demonstrated the micelle efficacy as a contrast agent for MRI in vitro and assessed in vitro cellular uptake (Fig. 6B–C).

Nevertheless, it should be noted that such an intracellular-targeting could not be specific to cancer cells and is thereby considered as a tumor targeting strategy as such. Therefore, these intracellular-targeting strategies are often combined with other targeting strategies in order to optimize drug delivery. Hence, an interesting combination should be the association of active targeting and pH-triggered drug release. This strategy is often chosen by researchers and will be discussed later.

4.3.2. Hyperthermia

The majority or cancer tissues have been shown to have higher heat-sensitivity compared with normal tissue. Therefore, hyperthermia can be used in cancer therapies to destroy pathological cells. Moreover, SPIOs have been shown to react after the application of an alternating magnetic field (AMF) by absorbing this energy and converting it into heat. This generated heat can subsequently be used to destroy cancer cells [41, 68]. Multiple encouraging preclinical data led to the first clinical trials in 2007, which were conducted by two groups, Johansen and colleagues and Maier-Hauff and colleagues, using aminosilane-coated SPIO in patients with locally recurrent prostate cancer and glioblastoma multiforme, respectively [97,98]. After those clinical trials, numerous SPIO-loaded theranostics have been used in hyperthermia-based pre-clinical studies.

This strategy has been successfully applied by Yallapu et al. using β-cyclodextrin-pluronic coated SPIO nanoparticles [89]. Magnetic nanoparticles loaded with curcumin had promising results for hyperthermia and MRI in ovarian, breast and prostate cancer cells. More recently, an elegant study performed by Baldi et al. demonstrated the efficacy of anti-human epidermal growth factor receptor (EGFR)-targeted and 99mTc functionalized PLGA-PEG nanoparticles loaded with SPIO (Fig. 7A) for both hyperthermia tumor regression (Fig. 7B) and in vivo imaging capacity using PET (Fig. 7C) [90]. The tumor site was clearly visible in the PET images, indicating nanosystem accumulation. However,

![Fig. 6.](image-url) Acid-triggered self-assembly of folate-PEG-P(GA-DIP) nanomicelles loaded with DOX and SPIO. (A) Schematic representation of nanomicelle assembly following medium acidification. (B) In vitro cellular uptake. Bel-7402 cells were incubated with different samples. Arrow: clear DOX fluorescence in targeted group. (A) PBS control; (B) targeted group: folate-PEG-P(GA-DIP); (C) non-targeted group: PEG-P(GA-DIP); (D) the competitive inhibition group: folate-PEG-P(GA-DIP) and 1 mM free folic acid. (C) in vitro MRI. T2-weighted (A), T2-mapping (B), and colored (C) T2-mapping images. (1) Gelatin contrast of cells not incubated with micelles; (2) targeted group: folate-PEG-P(GA-DIP); (3) non-targeted group: PEG-P(GA-DIP); (4) competitive inhibition group: folate-PEG-P(GA-DIP) micelles with 1 mM free folic acid. Arrow: clear decreased signal in targeted group. Abbreviations: DOX, doxorubicin; DMSO, dimethyl sulfoxide; folate-PEG-P(GA-DIP), folate-poly(ethylene glycol)-b–poly[N-(N,N-diisopropylaminoethyl) glutamine]; SIONs, superparamagnetic iron oxide nanoparticles; THF, tetrahydrofuran.

Adapted from [88].
substantial liver accumulation was observed (Fig. 7C). A substantial decrease in tumor size was correlated with an increase in nanoparticle accumulation resulting from the EGFR-targeting moieties and local temperature.

Although this strategy has shown promising results in clinical trials, some limitations exist and further efforts by the researchers will be needed in order to overcome these problems. Indeed, challenges associated with hyperthermia-based therapy are to induce and sustain temperatures above the systemic temperature (37.5 °C) in a defined target volume and also to achieve homogenous heat distributions within the target organ [99].

4.3.3. Ultrasound

Ultrasound (US, acoustic sound with frequencies >20 kHz) can produce different effects, which are classified into thermal and non-thermal effects. Thermal effects are commonly used for thermal ablation of diseased tissue as a non-invasive alternative to surgery. Experimental clinical trials have shown promising results for various tumor types, including prostate and pancreatic cancer [100]. US non-thermal effects, mainly cavitation, induce a variety of biological responses, resulting in increased drug or carrier extravasation, blood–brain barrier (BBB) disruption and membrane permeabilization to otherwise cell-impermeable compounds. Meanwhile, thermal effects are primarily used to trigger thermostimulated drug delivery and tumor tissue thermal ablation [101,102]. For example, Yang et al. developed carboxymethyl hexanoyl chitosan (CHC)-based microbubbles, encapsulating both SPIO and CPT along with sulfur hexafluoride (SF6) gas, for cancer therapy and imaging (Fig. 8) [103]. They used US to combine US-triggered release with US-enhanced cellular uptake. The authors demonstrated the efficacy of their system for in vivo MRI (Fig. 8C) showing effective contrast enhancement after injection, US imaging and in vitro cytotoxicity in MDA-MB-231 human breast cancer cells (Fig. 8B). However, few studies have researched simple US-triggered targeting. The majority of researchers use combined targeting delivery systems for SPIO-based theranostics, which will be discussed below.

Ultrasound therapy also has several limitations. The challenges for ultrasound-enhanced drug delivery are the same as for the other targeting strategies, namely formulation challenges. There is a crucial need to develop a new generation of delivery vehicles that have sizes smaller than 200 nm, which are tailor-made for cavitation-enhanced drug delivery and may profit from high extravasation into tumor tissues [104,105].

4.3.4. Magnetic targeting

Due to their superparamagnetic properties, an external magnetic field can be used to guide SPIO-based theranostics directly to the

![Fig. 7. (A) Schematic representation of the synthesis of anti-human epidermal growth factor receptor (EGFR)-targeted and 99mTc functionalized PLGA-PEG nanoparticles loaded with SPIO (B) Mouse tumor volume on day 12 through day 24, showing a decrease in tumor volume. (C) In vivo scintigraphic image of hybrid radiolabeled Fe3O4-1-PNP-hEGFR-99mTc in an A431 tumor-bearing SCID mouse model. Abbreviations: hEGFR = human epidermal growth factor receptor; PNP = polymeric nanoparticles; SCID = severe combined immunodeficiency. Adapted from [90].](attachment:fig7.png)
When a permanent magnet is applied externally near the tumor region, the magnetic gradient produced exerts attractive forces on magnetic nanoparticles delivered via the circulation [43]. Lübbe et al. were the pioneers in this field, using an external magnetic field to guide a treatment [106]. Subsequently, numerous studies have used this method, employing magnetic field strengths ranging from 0.4 to 4 T. For example, Arias et al., reported a magnetically responsive squalene-based nanoplatform for theranostic purposes [92]. This concept was based on the inclusion of SPIO nanocrystals into nanoparticles constructed by self-assembling molecules of a squalenoyl gemcitabine (SQgem) bioconjugate. This nanoplatform simultaneously allows (i) the targeting of a gemcitabine prodrug to an experimental solid tumor under the influence of a magnetic field produced by an external permanent magnet placed on the tumor surface and (ii) the imaging of the targeted tumor nodule. Yang et al. developed epirubicin (EPI) conjugated SPIO for magnetically targeted cancer therapy [93]. This EPI–SPIO conjugate increased accumulation 10-fold in tumors following intra-arterial injection and magnetic targeting using a 0.4 T neodyme permanent magnet and displayed in vivo MRI contrast enhancement. Finally, Alexiou et al., examined a mitoxantrone (MTX)–SPIO conjugate (Fig. 9A) that accumulated in the tumor tissue of VX-2 squamous carcinoma-bearing rabbits using a 1.7 T electromagnet [94]. Complete remission of the rabbits was induced after treatment with the magnetically targeted MTX–SPIO conjugate (Fig. 9B) and MRI was used for the in vivo visualization of particle accumulation (Fig. 9C).

Magnetic targeting has received increasing interest in the last decade. Many research groups have demonstrated its potential for increasing tumor accumulation using magnetic attraction [92–94]. However, the success of this targeting strategy has been limited by inadequate magnetic gradients, i.e., the distance between the magnets and the target site. To facilitate magnetic targeting, magnetic forces should exceed the linear blood flow rates in the arteries (10 cm s⁻¹) or capillaries (0.05 cm s⁻¹). Because the magnetic gradient decreases as a function of the distance from the magnetic source, magnetic targeting should be more effective in regions closer to the magnets, limiting its application to superficial tumors [41,94]. Nevertheless, many strategies to overcome this problem are being investigated. Generally, the magnetic field gradient is generated by a strong permanent magnet, such as Nd–Fe–B. This type of magnet is known to enhance the depth of magnetic field by up to 10–15 cm [42]. However, this distance does not
allow the magnetic targeting of tumors that are localized deeper in the body. For such tumors, the particles could be focused to the tumor region using rotating magnetic fields [107]. Another proposed strategy is to improve the quality of the source (e.g., using a MRI system magnet as a source [108]). Finally, another solution could involve magnetic implants near the tumor region [109].

### 4.3.5. Combined targeting strategies

Considering the different disadvantages of each simple targeting strategy, numerous efforts have been made to develop multifunctional platforms that combine targeting strategies (Table 5).

The classical approach for combining targeting strategies consists of combining the active and acid-triggered drug release strategies. Yang

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**Fig. 9.** (A) Schematic representation of mitoxantrone-conjugated SPIO nanoparticles. (B) Effect of i.a. injection of MTX-SPIO conjugates after magnetic drug targeting (●) compared with control group (no treatment △). (C) MRI of tumor hind limbs of rabbits after i.a. injection and magnetic drug targeting (1 h exposure). The “f” arrow points to the accumulation of SPIO (adapted from [94]).

**Table 5**

Nonexhaustive Examples of combined-targeted multifunctional SPIO-based nanotheranostics in pre-clinical studies.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Targeting type</th>
<th>Drug</th>
<th>Functional molecules</th>
<th>Cell line</th>
<th>In vitro/in vivo MRI</th>
<th>Results</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPIO conjugates</td>
<td>AT + pH triggered</td>
<td>DOX</td>
<td>cRGDfC</td>
<td>U87MG cells</td>
<td>In vitro</td>
<td>In vitro MRI contrast enhancement, enhanced tumor accumulation (PET)</td>
<td>[23]</td>
</tr>
<tr>
<td>Liposomes</td>
<td>MT + HT + AT</td>
<td>DOX</td>
<td>FA</td>
<td>KB and HeLa cells</td>
<td>In vitro</td>
<td>Improved cellular uptake and cytotoxicity</td>
<td>[110]</td>
</tr>
<tr>
<td>Phospholipid-based microbubbles</td>
<td>MT + FUS</td>
<td>DOX</td>
<td></td>
<td>GS glioma cells</td>
<td>In vitro</td>
<td>BBB opening and highly effective drug delivery</td>
<td>[111]</td>
</tr>
<tr>
<td>PEG-PLGA-PLC NP</td>
<td>MT + AT</td>
<td>PTX</td>
<td>GRGDS</td>
<td>CT-26 cells</td>
<td>In vitro/in vivo</td>
<td>High tumor accumulation, drastic tumor growth delay, strong contrast enhancement</td>
<td>[112]</td>
</tr>
<tr>
<td>PEG-PAA NP</td>
<td>PDT + AT</td>
<td></td>
<td>F3</td>
<td>9L glioma cells</td>
<td>In vitro</td>
<td>Significant improvement in survival rate, in vivo MRI</td>
<td>[21]</td>
</tr>
<tr>
<td>PEGylated SPIO nanoclusters</td>
<td>PDT + MT</td>
<td></td>
<td></td>
<td>4T1 (mammary carcinoma)</td>
<td>In vitro</td>
<td>Dramatically delayed tumor growth and MF-enhanced PDT</td>
<td>[113]</td>
</tr>
<tr>
<td>SPIO conjugates Gold nanoshell</td>
<td>PTT + AT</td>
<td></td>
<td>C225</td>
<td>A431 cells FaDu, O5C19, and HNS</td>
<td>In vitro</td>
<td>Thermal ablation, in vitro MRI contrast enhancement</td>
<td>[114].</td>
</tr>
</tbody>
</table>

Abbreviations: AT = active targeting, BBB = blood brain barrier, DOX = doxorubicin, FA = folate, FUS = focused ultrasound, HT = hyperthermia, MF = magnetic field, MT = magnetic targeting, PAA = poly(acrylamide), PDT = photodynamic therapy, PTT = photothermal therapy.
et al. used this strategy and developed $^{64}\text{Cu}$ labeled SPIO–DOX conjugates linked by an acid labile bond that was further functionalized with cRGD [23]. This system exhibited high in vitro MRI contrast enhancement and tumor accumulation using PET.

Another strategy combines magnetic targeting (MT) with SPIO induced hyperthermia. For example, Pradhan et al. combined these two strategies along with active targeting for theranostic purposes [110]. They developed folate functionalized SPIO/DOX-loaded liposomes and tested their efficacy for improving cellular uptake and cytotoxicity in vitro.

More recently, Fan et al. developed a US/MRI dual contrast agent using SPIO-labeled phospholipid based microbubbles that incorporated DOX (Fig. 10A) [111]. They combined magnetic targeting (for tumor accumulation) along with focused US to induce BBB opening and therapeutic agent delivery in a glioma-bearing rat. This study demonstrated enhanced anti-tumor efficacy mediated by the DOX-loaded microbubbles combined with focused US in an in vitro cytotoxicity experiment (Fig. 10B) and contrast enhancement after focused US and 40 min of magnetic targeting (Fig. 10C).

Long visible and NIR lights can be used to generate reactive oxygen species (ROS) after the activation of a compound presented by the drug carrier or the drug itself. After delivery of a photosensitizer-containing drug to the tumor cell, light radiation activates the drug, generating “killer” ROS, which induce apoptosis and enhance drug release. This technique is called photodynamic therapy (PDT) [69,94, 115]. PDT has been investigated for several decades, but light-associated toxicity limits its clinical application. Therefore, the classical strategy combines PDT and active targeting to increase specificity and limit potential damage to healthy tissue [116]. For example, in a study conducted by Reddy et al., vascular targeted PEGylated Photofrin/ SPIO-loaded poly(acrylamide) nanoparticles were developed for the treatment of glioma (Fig. 12A) [21]. The vascular targeting capacity was accomplished by the functionalization of the particles using the F3-peptide. In vivo MRI was used to assess biodistribution and the efficacy of the active targeting prior to PDT (Fig. 12B). Data displayed that the administration of F3-targeted Photofrin-encapsulated nanoparticles resulted in the most significant increase in mean tumor apparent diffusion coefficient values (Fig. 12B g) as shown in the images acquired at day 8. There was a significant improvement in the survival rate of 9L glioma-bearing rats treated with this combined targeting therapy compared with the group treated with PDT alone (Fig. 12C).

Our group recently developed another strategy that combined magnetic and active targeting. We developed RGD-functionalized PTX/SPIO-loaded PLGA-based nanoparticles and separately compared the double active and magnetic targeting strategy to passive, active and magnetic targeting. These SPIO-based multifunctional theranostics displayed a

![Fig. 10.](image_url)
high tumor accumulation using the combined strategy (RGD + MT, Fig. 11B), as demonstrated by the ex vivo biodistribution that corresponds to a strong contrast enhancement of the tumor region by in vivo MRI. This high tumor accumulation was correlated to drastically enhanced survival time and long-term survival in mice (Fig. 11C) [112].

A recent example of combined PDT-magnetic targeting is provided by Li et al., who developed PEGylated iron oxide nanoclusters loaded with chlorin e6, a photosensitizer, for targeted, NIR light induced, photodynamic therapy [113]. SPIO were used for both their MRI contrast agent properties and the magnetic targeting potential. To further improve tumor accumulation, an external magnet was placed, allowing magnetic targeting.

Another light-mediated strategy uses NIR light to induce local heating and, consequently, hyperthermia-induced cell death, known as photothermal therapy (PTT) [69,94,115]. Melancon et al. developed a SPIO-coated gold nanoshell functionalized with C225, an anti-EGFR monoclonal antibody, for head and neck cancer treatment [114]. They used these C225-SPIO@Au nanoshells to image guide thermal ablation (using in vivo MRI). They used the PTT thermic effect by applying an NIR laser to induce local hyperthermia and destroy the tumor tissue.

PDT also has limitations in terms of light penetration. The singlet oxygen produced by the photochemical reaction between the sensitizer and light can only diffuse 0.02 μm, limiting tissue damage to the penetration depth of the light used to activate the photosensitizer [117]. Although this strategy allows more selective and irreversible destruction of tumor cells than conventional therapies, there are several challenges that need to be addressed for better clinical acceptance. First, the efficiency of this strategy is limited by light penetration depth, restricting the use of PDT relatively superficial lesions [118]. Second, using PDT requires specialized equipment and training [119]. Finally, the most important challenge that needs to be addressed is the high concentration of photosensitizer (PS) needed at the target site for clinical efficiency. Poor cancer cell uptake and inefficient tumor delivery of PS agents are the two main limiting factors for the current application of PDT in cancer therapy [113].

5. Discussion: major obstacles for in vivo and clinical studies

SPIO have been extensively used during the last decade because of their (i) high surface functionalization for forming SPIO-conjugates, (ii) high efficiency as MRI contrast agents, (iii) high potential for multi-modal imaging and (iv) high potential for theranostic purposes. To further improve functionality, release kinetics (controlled release) and drug protection against degradation, nanoparticles encapsulating SPIO have recently received particular attention. These nanoparticulate systems are interesting candidates that provide multifunctional platforms for theranostic applications [41,120].
The concept of theranostics has received increasing interest in recent years. Nanotheranostics can be used for various biomedical applications including (i) non-invasive assessment of biodistribution and target site accumulation, (ii) monitoring of drug release, (iii) enhancement of therapeutic efficacy via triggered drug release and (iv) prediction of therapeutic response [3]. Furthermore, the potential of theranostic platforms to promote personalized therapy has become clear. Because of the potential major adverse effects of current available anti-cancer therapies and patient inter- and intra-variability, it is important to pre-select patients, based on drug biodistribution, that will likely respond to therapies [66]. Patients that have good tumor accumulation and less undesirable accumulation in healthy tissues are more likely to be treatment-responsive and have minimal adverse effects.

Despite these exciting potential biomedical applications and as seen in the tables presented above, only a few in vivo MRI studies of nanotheranostics have been successfully conducted in pre-clinical models. Furthermore, the majority of nanotheranostics designed for personalized therapy have only demonstrated in vitro efficacy. Moreover, if researchers describe in vivo data, that data generally only assesses one of the two purposes of theranostics, either therapeutic efficacy or imaging efficacy [121]. Studies that have shown in vitro and in vivo efficacy for therapeutic and imaging purposes are mostly represented by studies combining multiple strategies. This observation raises the question: what are the obstacles responsible for so few successful in vivo MRI studies? The answer to this question is that SPIO-loaded theranostics have some limitations that lead to challenging in vivo and clinical translations.

5.1. Impaired biodistribution

As previously discussed, following i.v. injection, a significant proportion of SPIO-based nanoparticles are sequestered by the liver and spleen [41,42,65,80]. This phenomenon can be explained by their capture by the MPS (i.e., Kupffer cells) due to the size of the particles (often larger than 100 nm) and their interaction with plasma proteins (opsonins). It is noteworthy that only a small proportion of the injected dose actually reaches the tumor [42,43]. This phenomenon has led the researchers to usually use SPIO-loaded nanoparticles as a contrast agent for liver imaging [122]. Various physico-chemical parameters have been shown to modulate this biodistribution, including particle size, surface charge and surface modifications (PEGylation) (see Fig. 13). Another strategy for improving tumor accumulation is to use an external magnetic field to guide the therapy to the tumor site and maintain its position using magnetic force, known as magnetic targeting [42,43]. Although this strategy has drastically improved nanoparticle accumulation in tumor tissues, a large fraction of the injected dose still accumulates in the liver [65,112]. This phenomenon raises the question of the toxicity of liver accumulation. An analysis of the literature shows conflicting results on SPIO toxicity. Some studies have shown that SPIO may generate ROS production in liver cells, which could lead to toxicity [123].
Furthermore, a major challenge for theranostic therapies is the opposing time lines and concentrations of imaging and therapeutic agents. Although the primary goal of imaging is to deliver the smallest amount of imaging agent for a short time to ensure a strong signal-to-noise ratio, the amount of drug required to induce a strong anti-cancer effect is the highest possible dose (maximum tolerated dose) [129]. Consequently, there is a formulation challenge to address to ensure maximum drug delivery and minimum imaging agent delivery. This challenge is more easily addressed by nanocarrier-based theranostics compared with SPIO-conjugates because nanocarriers encapsulate various amounts of drug and imaging agent. Moreover, this issue may be easily solved by maintaining the original two-step process where the same nanocarrier is used to encapsulate, first, the imaging agent to visualize biodistribution and tumor accumulation and, thereafter, the anticancer drug to treat the patient [129].

Finally, another formulation challenge lies in the effective co-encapsulation of both therapeutic and imaging agent in a same nanoparticle. Literature lacks information on this issue. Moreover, it is not clear whether the drug and the imaging agent must be in the same nanoparticle. In theory, if the encapsulated moieties do not change the surface properties and the size of the nanocarriers, coencapsulation is not mandatory provided that they are delivered together in an equilibrated ratio. However, since it is well known that the encapsulated drug can influence the physicochemical properties, to avoid any bias, a coencapsulation would be recommended. Again, the proof that both agents are included in the same nanoparticles is usually not provided. Electron microscopy of SPIO-loaded nanocarriers usually shows a homogenous staining. The problem is less relevant for SPIO-conjugates even if again the homogeneity of drug loading/conjugation to SPIO is rarely discussed.

5.3. Combined strategies

Due to the individual limitations of each targeting strategy, there is a crucial need to merge advanced targeted delivery systems to further improve the selectivity of the currently available treatments. Researchers generally encounter poor tumor accumulation, which leads to in vivo anti-tumor efficacy or in vivo imaging efficacy difficulties. The percentage of the injected dose that reaches the tumor is rarely above 1%. Moreover, drug accumulation in healthy tissue, such as the liver and spleen, is often larger than 35%. In this context, there is a rationale for combining different strategies in the same nanomedicine to overcome the limitations of each separate strategy, which leads to the creation of multimodal nanotheranostics. This strategy is more frequently being employed by scientists, as seen in this review, with reports of promising results. For example, our group demonstrated, using ESR spectroscopy, a 3- and 2-fold increase in tumor accumulation of SPIO using a combination of magnetic and active targeting compared with the separate use of active targeting and magnetic targeting [112]. Similar results were obtained for the combination of PDT and active targeting [21]. This combination resulted in drastically improved survival rates for 9L glioma-bearing rats compared with PDT alone. Finally, the efficacy of combined strategies was also confirmed in a study conducted by Fan et al. that focused on US and magnetic targeting as a unique targeting strategy, which produced drastic contrast enhancement in susceptibility weighted images compared with focused ultrasound [111].

6. Clinical perspectives and opinion

Few clinical trials using nanotheranostics have provided proof-of-principle for personalized nanomedicine. PK2, galactosamine-modified pHIMA-GFLA-GFLG-doxorubicin convincingly showed efficient target site localization [130]. 111labeled PEGylated liposomes accumulated highly efficiently in the primary tumor mass of a Kaposi’s sarcoma patient, as well as in a number of secondary metastatic lesions. This observation explains why patients suffering from Kaposi’s sarcoma that are...
characterized by a dense, highly leaky, EPR-vascular network generally respond well to Doxil® treatment [18,131]. VEGF-R2-targeted microm bubbles are also a clinically relevant example of theranostics and are currently used in early-stage trials for prostate cancer staging and monitoring by ultrasound imaging [132].

To our knowledge, no clinical trial has been performed with SPIO-based theranostics. Nevertheless, advances in the development of SPIO-based magnetic nanoparticles (without anti-cancer drug) have demonstrated that it is a promising method for cancer treatment by hyperthermia. The advantage of these nanoparticles is their ease of tumor targeting and fewer side effects than chemotherapy and radiotherapy, as proven by the results of current/ongoing clinical trials. These trials have evaluated both tumor accumulation with magnetic targeting and anti-cancer efficacy combined with radiotherapy or via hyperthermia [41,98]. These trials are encouraging for the development of SPIO-based nanotheranostics in the future.

In our opinion, scientists, clinicians and pharmaceutical companies researching nanomedicines should investigate the image guidance of their nanomedicines during the initial phase of clinical testing. Because of the easy co-encapsulation of contrast agents and anti-cancer drugs, theranostics should be considered a tool for earliest phases of clinical trials. This would allow the collection of important information about their nanomedicine: (i) the initial biodistribution and target site accumulation of the nanomedicine and (ii) during follow-up, the response monitoring of the nanomedicine. These two results could then be correlated [18]. However, an essential question remains unanswered: why do so many papers describe nanomedicines while only a few nanomedicines are commercialized? This question has been discussed in this review. To summarize, one major reason is the EPR effect is undoubtedly larger in animal model tumors than in patients [13]. Although there appears to be a clear EPR effect in clinical tumors, practical information on the extent of the EPR effect in most solid tumors is needed for the development of nanomedicine. In this context, SPIO-based theranostics, via their non-invasive biodistribution visualization and the importance of the EPR, should address this shortcoming. Assuming that there will be a clear correlation between tumor concentration and therapeutic efficacy, it will be highly important to properly differentiate between low and high levels of target site accumulation. These studies should also guide the specific use of nanomedicines in clinical applications.

Personalized medicine as an evidence-based individualized medicine able to deliver the right treatment to the right patient at the right time, could both improve disease outcomes and reduce overall healthcare costs [133]. In our opinion, future clinical oncology needs to take advantage of these theranostic tools to adapt the treatment to each specific case and find the best treatment for each patient. Personalized medicine is expected to be the main goal in biomedical research in the next 5 years [85]. Since cancer is highly heterogeneous with both intra- and inter-variability, molecular medicine, an active strategy identifying variations in the expression of a specific target, should be a guideline in order to improve further development of theranostics. Indeed, actively-targeted nanotheranostics should offer interesting tools to verify the expression of a cancer biomarker in a non-invasive way before including the patient into a specific chemotherapy treatment bypassing genetic testing. Therefore, researchers need to keep on looking for new cancer biomarkers and develop new targeted theranostics. To date, many ligands have been widely investigated including folate, transferrin, RGD and multiple antibodies (anti-HER2, anti-EGFR, anti-VEGF, etc.). Choosing a proper ligand is often complicated. However, some criteria’s should guide researchers in their receptor choice. First, the targeted-receptor should be chosen regarding the type of tumor intended to be treated. Indeed, the expression of specific receptor varies from one tumor type to another, but also depending of the tumor stage [83]. A second important point that needs to be considered is that a receptor overexpressed by both tumor and neoangiogenic endothelial cells should be more effective. Indeed, it could provide a double targeting by killing tumor cells in both a direct (targeting tumor cells) and indirect manner (depriving tumor cells in oxygen and nutrients by killing endothelial cells). Noteworthy, some ligands are very specific (like antibodies) while others are more ubiquitous (like RGD) and can target different kind of cells. Hence, RGD peptide is an effective ligand for tumor targeting since it has been shown that integrin αvβ3 is overexpressed not only on tumoral endothelium but also on cancer cells, for a lot of cancer cell lines, while other ligands such as folate or transferrin are only expressed by tumor cells [134]. Thirdly, an ideal target receptor should be overexpressed at the surface of tumor cells or neoangiogenic endothelial cells while poorly even absent in resting cells. Conversely, transferrin is expressed at elevated levels on cancer cells but also on brain capillaries, endocrine pancreas, Kupffer cells of the liver, etc. [135,136]. Another interesting characteristic for ligands is to display an anti-angiogenic effect. Anti-angiogenic treatments have been shown to induce a potential normalization of tumor blood vessels, which leads to an increased drug delivery to tumor [137]. Finally, in order to be transposed from bench to bedside, the production of these ligands should demonstrate a low cost production, good stability, large-scale synthesis capabilities, easy manipulation, and reduced immune reaction. For this purpose, small molecules and peptides (folate, RGD, etc.) seem to be better candidates in comparison to proteins (antibodies, transferrin, etc.) [138].

The efficacy of SPIO-based theranostic systems will depend on their ability to improve selectivity. Hence, there is an increasing need for improving treatment accumulation in the target-site. This means improving physico-chemical characteristics of the SPIO-based nanotheranostics (reduced size to improve biodistribution, relaxivity of the magnetic core to improve particles reactivity to an external magnetic field, etc.), as well as combining multiple targeting strategies. Future directions should involve interesting combination of targeting strategies in multifunctional nanoplatforms. A promising example should be the combination of magnetic targeting to improve tumor accumulation, receptor-mediated targeting by grafting a specific ligand at the surface of the nanocarrier to induce cellular uptake, pH-triggered drug release in the endosome and magnetic hyperthermia to enhance tumor cell killing. Above all, researchers need to further investigate toxicology and pharmacokinetics of their SPIO-based nanotheranostics in order to meet the regulatory requirements for clinical approval.

References


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