Recent advances in cell-mediated nanomaterial delivery systems for photothermal therapy

Zhibin Li,\textsuperscript{a,ab} Xue-Feng Yu\textsuperscript{b,*} and Paul K. Chu\textsuperscript{a,*}

Nanomaterial-based photothermal therapy using near-infrared (NIR) light is a promising strategy for cancer treatment. However, delivery of nano-agents to specific tumor sites poses a great challenge, especially in the tumor hypoxia regions, where the hypoxia-associated drug delivery barriers prevent the effective intratumoral distribution of nanomaterials. In this respect, tumor-tropic cells including macrophages, monocytes, neural stem cells (NSCs), and mesenchymal stem cells (MSCs) have been proposed as “Trojan Horse” delivery vehicles to transport various nano-agents to overcome the drug delivery barriers, increase the tumor targeting ability of nano-agents, and enhance the efficiency of photothermal therapy. In this review, we summarize recent research activities and emerging trends in this field, describe the advantages of combining cellular therapies and nanotechnology to achieve more effective cancer treatment, and discuss the clinical prospects of photothermal therapy.

1. Introduction

Benefitting from the tremendous development of nanotechnology, various nanomaterials with unique physicochemical properties have attracted extensive attention for tumor photothermal therapy (PTT) applications in recent years.\textsuperscript{1–5} PTT is a newly-developed non-invasive cancer therapeutic strategy, in which photon-absorbing nanomaterials are first delivered to tumor sites and then photo energy is converted to heat under NIR light irradiation. PTT can selectively ablate cancer cells via the temperature increase at the irradiated tumor sites.\textsuperscript{6–8} Different from traditional chemotherapies and radiotherapies,\textsuperscript{9,10} PTT is considered to be a promising strategy due to its simple, safe, and noninvasive treatment process as well as targeted and remote-controlled properties.\textsuperscript{11}

Until now, a large number of photothermal nanomaterials have been fabricated and widely investigated for efficient photothermal therapy of cancers. These nanomaterials can be broadly categorized as inorganic and organic nanomaterials.\textsuperscript{12} Inorganic nanomaterials include mainly but not limited to gold-based nanomaterials...
(e.g., gold nanoshells (AuNSs), gold nanorods (AuNRs), gold nanocages (AuNCs)), carbon-based nanomaterials (e.g., carbon nanotubes, carbon nanodots, and graphene), nano-semiconductors (e.g., silicon nanoparticles, magnetic nanoparticles, copper sulfide nanoparticles and black phosphorus (BP) nanosheets), etc.11–15 Organic nanomaterials include primarily conjugated polymers (e.g., polyaniline and polypyrrole), branched macromolecules (e.g., dendrimers), biodegradable organic-based liposomes (e.g., photofrin encapsulated liposomes, poly(ethylene glycol)-phosphatidyl ethanolamine (PEG–PE) micelles), and natural carriers (e.g., albumin),16 as well as small molecule-loaded nanomaterials (e.g., fluorophore-spacer-receptor molecular probes).17

In the past decade, both inorganic and organic nanomaterials have been widely investigated and the encouraging efficacy of photothermal therapy has been demonstrated in preclinical animal trials.18–27 Particularly, some of the AuNP-based theranostics have already entered the clinical stage (NCT03020017, NCT01270139 NCT02755870, NCT01420588, and NCT02782026).28 However, in spite of these advantages, there are several obstacles remaining to restrict further clinical applications of these nanomaterials. Firstly, most of these nanomaterials target tumor sites passively. Generally, nanomaterials are localized in tumor sites via the enhanced permeability and retention (EPR) effect.29,30 Therefore, it is difficult for them to penetrate poorly vascularized hypoxic tumor regions and to cross the blood–brain barrier (BBB). Once nanomaterials enter the dynamic bloodstream, they tend to be non-specifically adsorbed by numerous blood proteins (e.g., albumin, globulin and serum proteins),28,31,32 which may hinder them from effectively targeting tumor sites. Moreover, they may also be captured by mononuclear phagocytic systems before reaching the tumor sites. Secondly, nanomaterials have a low physiological stability in circulation in vivo. Intravenously administered nanomaterials are prone to aggregation, which may severely block blood capillaries. Subsequently, the aggregated nanoparticles eventually accumulate in the reticuloendothelial system (RES) such as the liver and spleen to cause long-term inflammatory responses.21,23 Thirdly, the physiological barriers in the human body limit efficient delivery of nanomaterials. These physiological barriers mainly include the BBB44–48 and those existing in tumors such as growth-induced solid stress, a large transport distance in the interstitium, elevated interstitial fluid pressure, and a poor blood vessel network.12,41–43

Owing to the limited penetration depth of NIR light and optical scattering by biological tissues,44 it is difficult for nanomaterials to effectively convert the laser energy into heat if they do not accumulate in the tumor sites at a sufficiently high concentration. To address these issues, much effort has been made to develop advanced nanomaterial-based delivery systems for PTT.45–67 In this respect, cell-mediated nanomaterial delivery systems have emerged as a promising strategy to improve the efficiency of PTT.68–71 Because of the tumor-tropic properties of the delivery cells and high nano-agent loading ability, they can overcome the barriers to track tumor sites resulting in the effective delivery of nano-agents.76–82 Generally, cell-mediated PTT hinges on that some of the cells are nature’s own ‘delivery vehicles’ since they have evolved to perform optimal delivery functions under natural conditions.83 Multiple types of intrinsic tumor-tropic cells such as macrophages,84,85 NSCs,86–88 MSCs,89,90 human cytokine induced killers (CIK) cells,91 human induced pluripotent stem cells (iPSCs),92 and T-cells93 have hitherto shown great potential for future photothermal tumor therapies. In these cell-mediated PTT strategies, cells loaded with the nanomaterials dramatically improve the efficacy of photothermal tumor ablation by increasing the targeting and penetrating ability of the nanomaterials. As shown in Fig. 1, compared to intravenous injection of AuNRs, the cells with AuNRs serving as “Trojan Horse” vehicles overcome the physiological barriers and exhibit much improved delivery and PTT efficiency. The early work reported by Choi et al.44 established a macrophage-mediated AuNS delivery therapeutic strategy to penetrate tumor hypoxic regions for targeted ablation of breast tumors. In their subsequent work, they demonstrated that nanoparticle-laden-monocytes/macrophages efficiently penetrated brain metastasis tumor tissues by crossing the BBB after injection into the systemic circulation.95 Many novel nanomaterials have since been fabricated and several types of cells have been investigated because of their therapeutic potential for antitumor treatment.96–103 On the other hand, the nanoparticle-laden-cells have also been combined with chemo- and radiation therapies to further enhance the PTT efficacy.104–107 In this review, we summarize current research advances in this emerging field and discuss the perspectives and challenges confronting clinical applications.

2. Cell-mediated delivery of nanomaterials for PTT

2.1. Gold-based nanomaterials for cell-mediated PTT

Among the different types of inorganic nanomaterials, gold-based nanomaterials such as AuNSs, AuNRs, and AuNCs have

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become the most widely employed PTT agents. These gold-based nanomaterials have received considerable attention due to their promising properties such as the efficient light-to-heat conversion efficiency, tunable localized surface plasmon resonance (SPR) absorption band in the NIR region, and ease of synthesis and modification. Moreover, they possess outstanding biocompatibility and multi-functionalization thus boding well for biomedical applications. They localized and induced irreversible photothermal ablation of SKBr3 human breast epithelial carcinoma cells with gold–silica nanoshells. Link and EI-Sayed studied the photothermal properties of gold nanoparticles in cancer therapy. By conducting successive experiments, it was demonstrated that the gold-based nanomaterials enabled photothermal ablation of tumors by using a low-energy continuous-wave (cw) NIR laser. Gold-based nanomaterials with various shapes and sizes have been synthesized and extensively explored as PTT agents as summarized in Fig. 2 and many of them are presently in clinical trials: lung, head & neck, and prostate cancer.

<table>
<thead>
<tr>
<th>AuNPs</th>
<th>Unique features</th>
<th>Size</th>
<th>Theranostic applications</th>
<th>Stage of development</th>
</tr>
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<tbody>
<tr>
<td>Nanoshells</td>
<td>Core: shell structure</td>
<td>~150 nm diameter</td>
<td>• Cell imaging (visible lights)</td>
<td>Clinical trials: lung, head &amp; neck, and prostate cancer</td>
</tr>
<tr>
<td>Nanorods</td>
<td>Two resonance peaks</td>
<td>~10 nm x 40 nm</td>
<td>• PTT (enhanced nonradiative property; NIR)</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Nanogels</td>
<td>Drug loading capabilities</td>
<td>~40-60 nm length</td>
<td>• Delivery of therapeutic cargos</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Nanostars</td>
<td>Large surface area for bioconjugation</td>
<td>~45-120 nm</td>
<td>• Tumor imaging (enhanced radiative property; NIR)</td>
<td>Preclinical</td>
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Fig. 1  Schematic diagram comparing the EPR effect of AuNRs with and without macrophage delivery. Penetration of AuNRs is limited due to the physiological barriers such as dense tumor stoma and hypoxic tumor cores. In contrast, the macrophage-mediated delivery vehicles overcome these barriers and transport the AuNRs to penetrate the tumor hypoxic regions to improve the delivery efficiency.

Fig. 2  Description of common AuNPs with theranostic applications and clinical development stages. Copyright 2017 Wiley Periodicals, Inc.
phase III human clinical studies. However, the low physiological stability, non-biodegradability, and potential long-term toxicity of gold nanomaterials still pose significant challenges for clinical practice. A common strategy to improve the tumor-targeting ability of gold nanomaterials is to conjugate the nanomaterials with molecules including antibodies, carbohydrates, small molecules, and peptides. However, surface conjugation of nanomaterials may affect their NIR absorption properties. For example, negatively-charged antibodies can attach to positively-charged gold nanoparticles by electrostatic interactions and may cause aggregation of the nanoparticles due to neutralization of the surface charge of the gold nanoparticles further resulting in the redshift and decrease of the NIR SP band. In view of these limitations, Choi et al. showed that AuNSs could be easily engulfed and delivered by monocytes/macrophages. In this cell-mediated treatment scenario, the Au-nanoshell-laden monocytes/macrophages served as “Trojan Horse” delivery vectors which efficiently penetrated the hypoxic regions and transported AuNSs into the tumor sites.

Besides AuNSs, AuNRs with unique plasmonic properties in the NIR region have promising prospects for cell-mediated PTT of cancer. Owing to SPR oscillations along the two directions of the rods, AuNRs possess two absorption bands and have a larger NIR absorption cross section than other Au nanostructures. By increasing the aspect ratio (length/width) of AuNRs, the maximum absorption of the longitudinal SPR band shifts gradually from the visible to NIR regions with maximum light penetration because most human tissues show minimal light absorption between 750 and 1300 nm. El-Sayed et al. first used AuNRs as a PTT agent in 2006. Since then, AuNRs have been widely studied in and in vivo PTT. However, most of the AuNRs commonly produced by the seed-mediated technique are 14–15 nm in diameter and more than 50 nm in length, and so they cannot be effectively excreted from the body through urinary excretion consequently raising concerns about long-term toxicity. In our previous work, we established a seedless synthetic strategy to prepare AuNRs with a diameter and length as small as 7 and 30 nm, respectively (designated as sAuNRs). Compared to the common AuNRs with a diameter of 14 nm, the sAuNRs had similar SPR and photothermal properties but could be exerted efficiently to lower the cytotoxicity and improve the cell uptake efficiency. Studies on the biodistribution and clearance have demonstrated that AuNRs are mainly distributed in the liver and spleen and can be rapidly cleared by renal clearance. Based on these merits, a new delivery system of sAuNR-laden-macrophages has been developed as a PTT agent for cancer therapy (Fig. 3). In this system, the sAuNRs possess higher cellular loading capability and negligible cytotoxicity. Moreover, the macrophage mediated sAuNR delivery system penetrates tumor tissues efficiently and enhances the intratumoral distribution of the sAuNRs. In in vivo photothermal cancer therapy, the sAuNR-laden-macrophages significantly improved photothermal conversion efficiency and have a smaller tumor recurrence rate compared to bare sAuNRs. These results demonstrate that AuNRs with a smaller size are promising for cell-mediated cancer therapeutics and diagnostics.

2.2. Two-dimensional nanomaterials for cell-mediated PTT

As a new class of ultrathin nanomaterials, two-dimensional (2D) layered nanomaterials with anisotropy and chemical functionalities with relatively high biocompatibility and biosafety have been explored for a variety of biomedical applications such as drug delivery, imaging, diagnostics, and thermal/photodynamic therapy. Moreover, 2D nanomaterials are the thinnest materials known to date and the low-dimensional nanostructures possess very large specific surface areas allowing them to respond to light rapidly. Graphene oxide (GO) and reduced GO (rGO) are the first explored materials for PTT applications. Liu et al. reported that polyethylene glycol (PEG) functionalized reduced nano-graphene oxide (nRGO-PEG) had high NIR absorbance and photothermal conversion efficiency and delivered better photothermal performance than AuNSs. Although GO nanosheets show no noticeable toxicity against different types of cells, the non-biodegradability and possible long-term safety concerns of GO nanosheets in vivo impose challenges concerning future clinical translation. Therefore, graphene analogues such as MoS2, WS2, MoSe2, Bi2Se3, and BP have been investigated as new-generation of PTT agents (Fig. 4). In 2013, Chou et al. reported the optical properties of MoS2 nanosheets and their potential as NIR absorbing photothermal agents in killing cancer cells in vitro. Liu et al. synthesized ultra-small MoS2 nanodots as bio-clearable PTT agents using a one-step facile bottom-up solvothermal approach. After modification with glutathione (GSH), the MoS2 nanodots exhibited high tumor tropic and rapid body clearance capacities for in vivo PTT. In addition to MoS2 nanosheets, transition-metal dichalcogenides (TMDCs) such as WS2, WSe2, MoSe2, and Bi2Se3 have been studied due to their fascinating physical properties. Particularly, Bi2Se3 nanosheets as PTT agents possess excellent biocompatibility and metabolizability because Bi is an environmentally friendly therapeutic element and Se is an essential trace element. Based on these unique properties, a cell-mediated therapy involving transportation of 2D Bi2Se3 nanosheets in macrophage vehicles was established by our group (Fig. 5). Compared to the bare Bi2Se3 nanosheets, the Bi2Se3-laden-macrophages showed prolonged blood circulation and negligible cytotoxicity. After intravenous injection, the cells overcame the hypoxia-associated drug delivery barriers and selectively targeted tumor sites to dramatically enhance the efficiency of photothermal cancer therapy. Most of the Bi2Se3 nanosheets were excreted from the body within 25 days. Hence, this Bi2Se3-laden-macrohage system not only constitutes an efficient delivery system to improve the PTT efficiency, but also provides a biodegradable PTT system. Besides Bi2Se3 nanosheets, BP nanosheets have attracted enormous interest for PTT due to the excellent biocompatibility and biodegradability. Both BP nanosheets and quantum dots exhibit broad absorption spanning the visible and NIR regions and possess a high photothermal conversion efficiency rendering them ideal for PTT. The human body contains about...
650 grams of P which plays a critical role in many key biological processes such as cell signaling and bone metabolism.\textsuperscript{156,157} Furthermore, BP nanosheets are biodegradable in aqueous media and the final degradation products are nontoxic phosphates and phosphonates. On the heels of experiments conducted by our and other research groups\textsuperscript{156–163} BP nanosheets and quantum dots have been demonstrated as biocompatible and NIR absorbing agents for PTT. In addition, the biodegradability can be controlled by surface modification or deposition of a polymer coating.\textsuperscript{156–159} As a result, a robust biocompatible cell-mediated delivery platform with BP nano-agents has large potential for PTT.

In summary, Au/Bi\textsubscript{2}Se\textsubscript{3}-based nanomaterials have shown great potential for cell-mediated PTT and received tremendous attention. However, the work is still in the initial stage. In the past several years, several multifunctional photothermal nanoagents have been fabricated and explored for PTT, for example, multifunctional imaging-guided nanocomposites \textit{(e.g., \textit{NaDyF}_4:50\%Lu@PB, PPy@BSA-Ce6 nanoparticles)},\textsuperscript{164–166} pH-responsive nanoagents \textit{(e.g., ultrasmall \textit{NaDyF}_4:10\%Nd-Fe-GA)},\textsuperscript{167} thermoresponsive nano-platforms \textit{(e.g., Nanogel + phenylethynesulfonamide (PES))},\textsuperscript{168,169} and biodegradable nanocarriers \textit{(e.g., PANI-PSiNPs)},\textsuperscript{170,171} and so on. Although these photothermal nanoagents have not been reported for the cell-mediated PTT, their fascinating physical properties and multifunctionalities provide good opportunities to design the functional cell-mediated delivery systems for PTT.

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**Fig. 3** Macrophage-mediated sAuNR delivery system and its use in PTT to enhance the tumor coverage and photothermal conversion efficiency. (a) TEM images of sAuNRs. (b) Diagram of sAuNR-laden-macrophages. (c) Infrared thermal images and (d) time-dependent temperature increase of HepG2 tumor-bearing nude mice under an 808 nm light irradiation after 48 h post intratumoral injection of PBS, free macrophages, free BSA-coated sAuNRs, and BSA-coated sAuNR-laden-macrophages. The color bar refers to the relative temperature values. (e) Diagram highlighting the difference between the treatment of free BSA-coated sAuNRs and BSA-coated sAuNR-laden-macrophages. Copyright 2015 Elsevier Ltd.
3. “Trojan Horse” for PTT

3.1. Macrophage as a promising delivery vehicle for PTT

Macrophages are myeloid immune cells derived from myeloid progenitor cells and are observed in virtually all tissues, where they display remarkable plastic characteristics.\(^{172}\) For instance, they switch phenotypes/functions dynamically to respond to micro-environment signals and show great functional diversity in the host defense, tissue remodeling, and immunity. Since the phagocytosis of macrophages was discovered by Ilya (Elie) Metchnikoff,\(^{172}\) immunologists have considered macrophages to be important immune effector cells for tumor growth and development. Early evidence reveals that macrophages perform tumoricidal functions and induce the anti-tumor activity of T cells. However, there is emerging evidence suggesting that tumor-associated macrophages promote tumor growth under induction of IL-4 cytokines in the tumor micro-environment. The biofunctions of macrophages depend on their phenotypes which are influenced by the local micro-environment.

Generally, as their name “the big eaters” in Greek implies,\(^{37}\) the innate phagocytic functions of macrophages are ingestion and degradation of dead cells or foreign materials. Their innate phagocytic capability allows them to combine with therapeutic nanoparticles. On the other hand, macrophages are highly abundant and easily obtained as patient specific cells from the peripheral blood of patients and meet the need of a typical human dose.\(^{173}\) After intravenous administration of nanoparticle-laden macrophages, the cells overcome the tumor hypoxia barrier to deliver nanoparticles to the tumor sites. Tumor recruitment of macrophages is complex and multifaceted because macrophages can be polarized and have different properties under induction of different tumor micro-environmental chemokines.\(^{174}\) Some evidence suggests that the tumor micro-environmental chemokines (e.g., monocyte chemotactic protein-1 (MCP-1)), the macrophage colony stimulating factor (M-CSF), the vascular endothelial growth factor (VEGF),\(^{175}\) and IgG85-88 may work synergistically in the recruitment of macrophages to the tumor sites.\(^{174}\) Besides, the hypoxia, apoptotic cells, and hyaluronan fragments of the tumor micro-environment make great contributions to tumor recruitment of macrophages. It has also been found that macrophages are composed of a high density of cells in the tumor sites. Approximately 70% of the cells in the breast carcinoma mass are macrophages and about 30–40% are in the gliomas.\(^{124}\) Particularly, high densities of macrophages are found from both the vascularized tumor stromal areas and avascular hypoxic necrotic areas. Therefore, the inherent properties of macrophages open up the possibility of their use as vectors to overcome physiological barriers in delivery of nano-agents to tumor sites.\(^{85}\) The feasibility of macrophage-mediated delivery was first tested by Baek \textit{et al.} with macrophages combined with AuNSs \textit{for in vitro} and \textit{in vivo} PTT.\(^{176,177}\) When the AuNSs were incubated with macrophages, they were phagocytosed by macrophages in vacuoles and dispersed throughout the cytoplasm (Fig. 6).\(^{124}\) The AuNS-laden-macrophages exhibited excellent NIR photothermal performance and negligible cytotoxicity. Subsequently, Choi \textit{et al.}\(^{94}\) examined the delivery ability of macrophage mediated PTT \textit{in vivo}. After intravenous injection and comparison with bare AuNSs, the AuNS-laden-macrophages penetrated the tumor sites and accumulated gradually in the hypoxic rim around the central necrosis. After irradiation with the NIR laser, sufficient hyperthermia was generated to suppress tumor growth. An envisaged clinical protocol based on AuNS-laden-macrophages was reported by Hirschberg \textit{et al.} (Fig. 7)\(^{178}\) Clinically patients are usually treated with tumor surgical resection first, followed by cell-mediated therapies to eliminate the infiltrating tumor cells that remain in the margins of the resection cavity instead of treating the un-resected tumors. Macrophages are frequently observed from and around glioblastomas of biopsies and similar results have been observed for AuNS-laden-macrophage mediated PTT. After injection of AuNS-laden-macrophages, the cells migrate to and are enriched around the tumor borders and the brain adjacent-to-tumor (BAT) region drastically enhancing the targeting capacity and improving the efficacy of PTT. Although the protocol has not yet been implemented clinically and how to acquire capable macrophages for effective clinical PTT is unanswered,
Fig. 5 Bi$_2$Se$_3$-laden-macrophages for efficient cancer targeting and photothermal cancer therapy. (a) Schematic representation of macrophage loaded Bi$_2$Se$_3$ nanosheets in vitro and these loaded cells overcome the hypoxia-associated drug delivery barriers significantly improving the tumor coverage of nanosheets and PTT efficiency; (b and c) blood circulation curves of (b) Bi and (c) Se at different time points post-injection; (d) residual Bi (up) and Se (down) biodistributions in different organs at different time points post-injection. Copyright 2017 Elsevier Ltd.
macrophage-mediated PTT is promising for tumor treatment. Furthermore, besides PTT, macrophages can be implemented in various diagnostic and therapeutic applications such as delivery of chemical drugs and gene products.

3.2. Neural stem cell-based delivery vehicles for PTT

The BBB is a major hurdle for efficient brain tumor drug delivery. Early studies of animal glioma models have provided some evidence that macrophages can penetrate brain metastases even though the BBB is intact.\(^{165}\) Another therapeutic strategy using NSCs has been developed for brain tumors. The tumor-tropic properties of NSCs enable them to traverse the BBB obstacle to improve the nanoparticle delivery to brain tumors.\(^{179,180}\) In general, NSCs are generated in the early embryonic brains and located in the subventricular zone and hippocampal dentate gyri. They are present in the developing brain and adult brain and exhibit self-renewal and multipotentiality.\(^{181,182}\) Since NSCs play key roles in neural regeneration, NSC-based cell therapy is a promising treatment modality for neurodegenerative diseases such as Amyotrophic lateral sclerosis (ALS), Parkinson’s disease (PD), and Alzheimer’s disease (AD). Moreover, \textit{in vivo} and \textit{in vitro} studies demonstrate the human NSCs that over-expression of BMP4 can trace and suppress glioma cell growth.\(^{183}\) Aboody \textit{et al.}\(^{184}\) first reported that NSCs were able to migrate throughout the tumor as delivery vehicles for gene therapy of brain tumors. NSCs have since been widely used as “Trojan horse” vehicles to carry therapeutic agents into glioblastomas including genes, antibodies, chemotherapy drugs, and replication-competent viruses, into glioblastomas.\(^{173}\) Nevertheless, the cellular and molecular mechanisms of NSC targeting gliomas are still not completely understood. In an early study, Aboody \textit{et al.} found that the
glioma hypoxic environment resulting in the remarkable up-regulation of NSCs expressed stromal cell-derived factor 1 (SDF1) and its receptor CXC-chemokine receptor 4 (CXCR4). Moreover, a variety of chemokine receptors such as urokinase-type plasminogen activator receptor 2 (uPAR), vascular endothelial growth factor receptor 2 (VEGFR2), and c-Met receptors have been reported to play major roles in association with tumor tropism. These tumor-released receptors induce NSCs to migrate and penetrate glioma. Kendall et al. verified that NSCs targeting of glioma depended on phosphoinositede 3-kinase (PI3K) signal pathway regulation. They observed that short hairpin RNA-mediated ablation of c-Met attenuated the NSC tropism to glioma and inhibited PI3K signaling pathway activation, which seriously impaired NSC migration to the hepatocyte growth factor (HGF) and other growth factors. In addition, activation of Ras is a critical step for PI3K activation, which directly regulates the cytoskeletal rearrangement and cell motility. These results provide a possible signal regulation pathway for NSC migration to target tumor sites. As shown by recent studies, human NSCs selectively migrate to and inhibit brain tumors by expressing rabbit carboxyl esterase (F3.CE). The over-expressed F3.CE activates CPT-11 to inhibit the growth of human lung adenocarcinoma A549 cells. Mooney et al. investigated tumor growth inhibition with intratumoral injection of AuNR-laden-NSCs followed by NIR irradiation. Compared to bare AuNRs injection, the AuNR-laden-NSCs improved the intratumoral distribution of AuNRs (Fig. 8). After irradiation with the NIR laser (2 W cm$^{-2}$ for 5 min), the NSC-mediated AuNRs delivery enhanced the PTT efficiency and reduced the tumor recurrence rates (Fig. 9).

3.3. Other types of cell-mediated delivery vehicles

In cell-mediated therapeutic agent delivery applications of NSCs, multiple types of stem cells have been demonstrated to possess extensive tropism to the glioma properties. The tumors’ migratory capacity of marrow MSCs was first reported by Nakamura et al. in 2004. In the 9L glioma-bearing rat model, MSCs possessed excellent tumor migration and penetration ability. Meanwhile, intratumoral injection of MSCs inhibited tumor growth and prolonged the survival of glioma-bearing rats. Studeny et al. further demonstrated that MSCs selectively migrated to tumor sites by expressing the beta interferon (IFN-$\beta$). The forced expression of IFN-$\beta$ in the tumor micro-environment by MSCs further inhibited the growth of tumors by regulating the proliferation of cancer cells. In fact, MSCs not only selectively migrate to solid tumors, but also actively track metastases far from the primary tumor. By taking advantage of the MSCs tumor tropism properties, Seokyung et al.
Fig. 9  Schematic illustration showing the PTT effect of intratumoral injection of bare AuNRs vs. AuNR-laden-NSCs. The NSCs-mediated AuNRs delivery system dramatically improves the AuNRs intratumoral distribution to enhance the PTT efficiency and reduce the tumor recurrence rates. Copyright 2014 American Chemical Society.

Fig. 10  Schematic representation of the mechanisms of the improved tumor-targeting photothermal therapeutic efficiency by employing pH-sensitive AuNP-laden MSCs. Compared to cAuNPs, pH-insensitive cAuNPs’ surface ligands cause AuNPs aggregation via electrostatic interactions, thus the AuNPs are easily exocytosed by MSCs and exhibited high tumor-targeting photothermal therapy efficiency. Copyright 2015 American Chemical Society.
demonstrated that MSCs were easily isolated from adult bone marrow and could be amplified in vitro. They fabricated a pH-sensitive gold nanoparticle (PSAuNP)-laden-MSCs delivery system that reduced exocytosis of AuNPs via aggregation of PSAuNPs in the MSCs. Meanwhile, the PSAuNP-laden-MSCs exhibited a high tumor-targeting capability (5.6% ID) which enhanced the photothermal efficiency of AuNPs as shown in Fig. 10.

Besides MSCs, IPSCs are another class of tumor-tropic stem cells. Generally, IPSCs are generated through reprogramming of somatic cells using different transcription factors and exhibit intrinsic tumor tropism characteristics. In biomedical applications, IPSCs can replace embryonic stem cells (ESCs) and avoid various ethical issues. Furthermore, they can be easily isolated, purified, cultured, and expanded in vitro due to the self-renewal and differentiation ability. Their patient-specific properties can eliminate the chance of immune rejection. An IPSCs mediated AuNRs delivery system was established by Cui et al. In their study, AuNRs@SiO$_2$@CXCR4 were uptaken by IPSCs through endocytosis. After loading with the AuNRs, IPSCs from the injection site migrated to the tumor sites and showed high tumor targeting ability and low cytotoxicity. Their results also showed that IPSCs prolonged the retention time of the AuNRs and improved the intratumoral spatial distribution of nanoparticles. In the IPSC mediated delivery process, the G-protein coupled seven-span transmembrane receptor CXCR4 played a key role in promoting the IPSC uptake of AuNRs and activating IPSC migration to the tumor sites.

In addition to these monocyte/stem cell types, other types of cells such as endothelial progenitor cells (EPCs) and CIK cells have been explored as delivery vehicles for tumor therapeutics. EPCs were isolated from peripheral blood CD34, VEGFR-2, or AC133 (CD133) antigen-positive cells in 1997 and the tumor tropism capability was demonstrated in vitro and in vivo. In particular, several tumor released endothelial progenitor cell (EPC)-mobilizing chemokines such as the vascular endothelial growth factor (VEGF), granu-locyte-colony stimulating factor (G-CSF), basic fibroblast growth factor (bFGF), stromal cell-derived growth factor-1 (SDF-1) as well as placental growth factor (PGF) could down-regulate the interactions between EPCs and the bone marrow micro-environment to induce EPC migration from the bone marrow to tumor sites. Varma et al. have used EPCs to deliver therapeutic genes to human glioma in a rat model and their results suggest that EPCs are promising nanomaterial delivery vehicles for PTT.

CIK cells were first discovered in 1991 and observed to have high proliferation and relatively low cytotoxicity. Since then, research and clinical work has suggested that CIK cells can regulate and generally enhance the patient’s immune functions. In general, CIK cells are generated from peripheral blood lymphocytes via stimulation with IFN-$\gamma$, IL-2, and anti-CD3 cytokines. CIK cells are also able to target tumor sites through vascular perfusion. The use of CIK cells in nanoparticle delivery was first reported by Zhang et al. who found that human CIK cells uptook PEGylated gold nanoparticles with a high efficiency and targeted tumor sites to improve the efficiency of tumor immunotherapy and PTT. The chemokine receptors’ CCL19/CCR7 and CXCL12/CXCR4 axis loops played important roles in CIK in vivo targeting of gastric cancer. Nowadays, CIK cells are combined with various tumor therapies such as chemotherapy, radiation therapy and immunotherapy. Preclinical data and clinical studies suggest that CIK cell-mediated therapies have superior clinical benefits and thus CIK cells have large potential for PTT of cancer.

4. Conclusion and prospects

In the past few years, various cell-based nanocomposites have been extensively explored for photothermal cancer therapy. The unique tumor tropic properties of certain types of cells not only offer the specific tumor-targeting ability, but also overcome the human body physiological barriers to deliver a myriad of nanomaterials to tumor sites. Rapid development of nanotechnology has spurred the development of many multifunctional nanomaterials suitable for cell-mediated cancer therapeutics. In this review, we summarize recent advances in this field and focus on cell-mediated “Trojan Horse” nanomaterial delivery systems for PTT of cancer. Encouragingly, cell-mediated therapeutics have made tremendous progress in the last decade and some clinical therapeutic strategies have been tested. We believe that future clinical applications will benefit patients.

Gold-based nanomaterials have been widely employed as a cell-mediated PTT agent due to their intrinsic physical and chemical properties. However, the low physiological stability and non-biodegradability are still the major limitations for further applications. Therefore, various nanomaterials including SAuNRs and Bi$_2$Se$_3$ nanosheets have been applied to improve the therapeutic effects of cell-mediated PTT. By taking advantage of the ultrasmall diameter of SAuNRs and biodegradability of Bi$_2$Se$_3$ nanosheets, these nanomaterials can be eliminated swiftly from the body and it is thus attractive to clinical applications. Currently, although gold nanoparticle-mediated PTT has reached the stage of clinical trials, the clinical potential of cell-mediated nanomaterial delivery systems for tumor PTT is still uncertain and future clinical translation of these tumor therapeutic strategies still faces challenges since several hurdles must still be overcome. First of all, in order to meet the requirements of U.S. Food and Drug Administration (FDA), the system must possess good biocompatibility and can be cleared completely in a reasonable time span. Furthermore, it is essential to verify that internalization of the nanomaterials does not affect the innate functions of delivery cells. Another potential challenge is that as clinical grade delivery materials does not affect the innate functions of delivery cells, more rigorous definition of fate cells is critical. For example, the diversity of phenotypes of macrophages depends on the involvement in the tumor micro-environment which affects the use of macrophages with contrasting roles in tumor progress. Additionally, future clinical treatments will require a large number of delivery cells and the relatively complicated and costly therapies may represent a challenge for clinical translation. Therefore, it is desirable to develop more effective...
ways to generate these cells and improve the tumor targeted delivery ability. Nevertheless, by overcoming these hurdles, cell-mediated nanomaterial delivery systems for tumor PTT are destined to have a bright future in the treatment of tumors.

Conflicts of interest
There are no conflicts to declare.

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