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Biomedical Materials

TOPICAL REVIEW

Recent advances of engineered and artificial drug delivery system towards solid tumor based on immune cells

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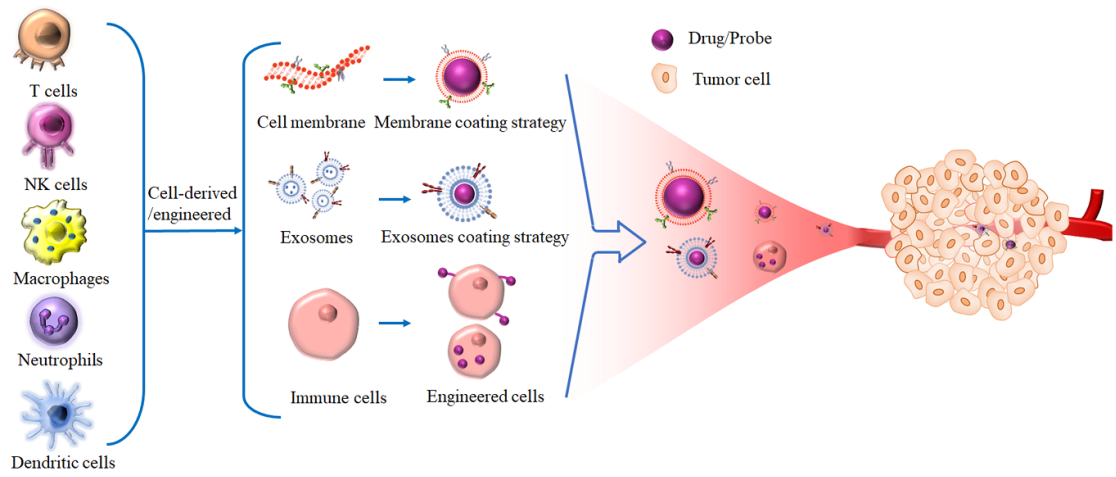
Abstract

Precise drug delivery in cancer treatment is a long-standing concern of modern medicine. Compared with traditional molecular medicines and nano-medicines, emerging cell-based biomimetic delivery strategies display numerous merits, including successive biological functions, innate biocompatibility and superior security since they originate from living organisms, providing a very promising approach. Among them, immune cells receive increasing attention because of their inherent ability in tumor resistance, pathogen elimination, and other significant physiological functions. Herein, we investigated the recent advances on immune cell-based high efficient delivery and therapeutic strategies in solid tumor treatment, mainly focus on T cells, natural killer cells and macrophages, which have been used as drug cargos directly or provided membrane/exosomes as nanoscale drug delivery systems. We also discuss the further potential applications and perspective of this innovative strategy, as well as the predictable challenges in forward exploration in this emerging area.

1. Introduction

Since accurately and efficiently delivered drugs to lesion area in a controllable manner is key point to cancer therapy, drug delivery system receives wide attentions in modern medicine. Traditional small chemotherapeutic drugs such as doxorubicin (DOX), paclitaxel (PTX) and cisplatin face low-dose accumulation in solid tumor tissues dominated by their water solubility, structural stability or pharmacokinetic distribution [1]. In recent years, targeted drug delivery systems have been promoted and investigated, and have shown great potential in cancer treatment due to their numerous advantages, such as improving drug solubility, protecting drug molecules from degradation and nonspecific adsorption, prolonging blood circulation, and manipulating drug release in a spatio-temporally controlled manner, thereby achieving enhanced bioavailability and minimal toxicity. Abraxane, Doxil, EMEND, Marqibo, Onyvide, Vyxeos, etc have even been approved by the U.S. Food and Drug Administration [2], improving the overall survival of patients in a certain. However, there are five steps drugs undergo after being administered: blood circulation, tumor accumulation, avascular tumor tissue penetration, cytosol internalization and drug release [3, 4]. Each of those steps can behave as biological barriers against drug delivery.

Recently, cell-based biomimetic delivery strategy has been raised attention since their possession of characters are desired in drug delivery system: (a) successive biological function. Biological engineered carriers often inherit the specific instinct ability from parental cells, or provide homologous targeting ability [5]. (b) Decent biocompatibility, and (c) high security *in vivo* as endogenous component originated from living organisms. The instinct abilities of different parental cells differ with various survival or physiological functional needs, mainly because of their unique shapes or specific markers presenting on the surface. To fully utilize biological features, various biomimetic drug delivery agents have been designed.



Scheme 1. Employment of immune cell-based drug delivery system against solid tumor.

By now, the reported endogenous cells include stem cells [6], red blood cells [7, 8], platelets [9], immune cells [10], cancer cells [11], etc. For example, red blood cells circulate in blood for prolonged period of time with the task of delivering oxygen, so red blood cell membrane coated nanoparticles (NPs) are capable of long circulation and evasion from capture by mononuclear macrophages [12]. So are platelet membrane camouflaged particles, with additional thrombus adherence ability [13]. As to tumor cells, they have instinct ability in evading immune systems and managing to survive, so their bio-mimicking NPs are often involved in stimulating anti-tumor immune responses or homologous targeting [14–16].

As for immune cells, they express various bioactive molecules with specific functions and they have shown unique obligations in both tumor recognition and resistance, as well as other physiological functions. Besides, immune cells do not depend on random diffusion, but on complex cellular machinery to navigate into tumor microenvironment (TME) [17]. Therefore, utilities of immune cells to design advanced drug delivery system provide promising therapeutic potential for cancer studies in the future. As agents delivery strategy using cells like red blood cells, platelets, stem cells, etc have been sufficiently discussed in pervious reviews [18–20], herein, we focus on the drug delivery system based on immune cells, to classify and display recent advances in this promising and revolutionary area (scheme 1).

2. T cells

T cells, as known as T-lymphocytes, possess about 60% portion of total lymphocytes. Naive T cells are mature recirculating T cells that have not yet encountered their specific antigens. In order to participate in an adaptive immune response, naive T cells must encounter their peptide-presented specific antigens: major histocompatibility complexs (MHC) on the surface of an antigen-presenting cell, followed by proliferating and differentiating into effector T cells that contribute to removal of antigens. Unlike naive T cells, effector T cells perform their functions once they encounter their specific antigens on other cells, without further differentiate [21]. They can manifest three broad classes of activity: (a) helper T cells provide signals to activate the functions of other cells, like render B cells produce antibodies and obligate macrophages kill engulfed pathogens. Those signals are often shown in the form of specific cytokines; (b) cytotoxic T cells (CTLs) kill other cells that are infected with viruses or other antigen-bearing intracellular pathogens. They are capable of being spontaneously triggered by tumor-specific peptides, leading to the destruction of tumor cells presenting target antigens [22, 23]; (c) regulatory T cells suppress the functions of other lymphocytes and contribute to restrict the possible damage of immune responses, which also act as a suppressive immune cell in TME.

Based on those inherent ability, using artificially modified T cell-related platform as a precise arsenal to treat cancer has undertaken increasing expectation in recent years. Herein, we discuss several novel and potential therapies by employing engineered T cells or T cell-derived membrane/exosomes.

2.1. Engineered T cells strategy

Engineered T cell can act as a living arsenal to target tumor and enhance tumor treatment efficiency. Zhang *et al* designed an alternative strategy to delivery drugs by T cell-surface anchor-engineering technology (figure 1(A)). They first linked functional tetrazine (Tre) groups onto T cell membrane by lipid insertion. Next, they used click reaction to link liposome Avasimible (Ava) containing bicyclo-[6.1.0]-nonyne (BCN) groups onto cell surface. The liposomal Ava stayed tight on the surface of T cells during both circulation and extravasation,

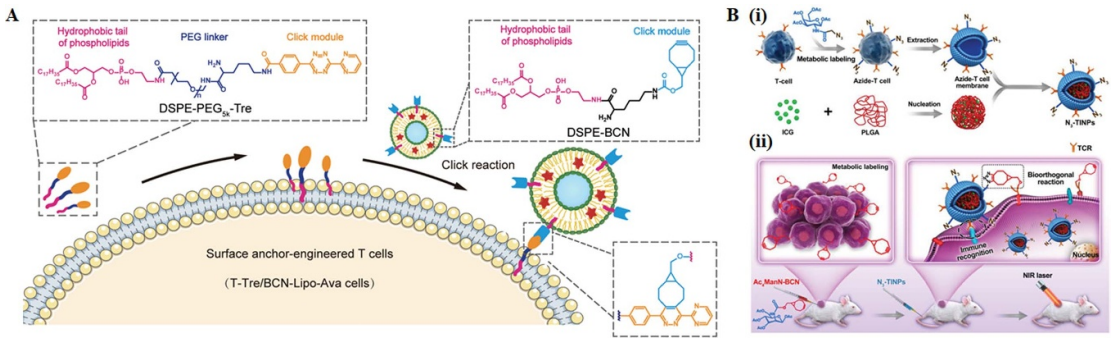


Figure 1. (A) Schematic diagram of assemble process of T cells engineered with cell surface anchored. Reprinted with permission from [24] AAAS. (B) Schematic diagram of N_3-labeled T cell membrane mimicking nanoparticles (N_3-TINPs) for tumor specific targeting and therapy. (i) Synthetic progress of N_3-TINPs. (ii) *In vivo* application process and mechanism. [26] John Wiley & Sons.

and locally released to increase cholesterol in the T cell membrane, whose duty was promoting rapid T cell receptor (TCR) clustering and sustained T cell activation. Such an engineered T cell strategy showed superior antitumor efficacy with no obvious systemic side effects [24]. Mitchell *et al* took advantage of T cell instinct ability in crossing blood brain barrier (BBB), investigating if T cells modified with RNA could deliver macromolecules to brain tumor. They utilized electroporation, a technology developed for genetically modified T cells and recently completed clinical trials, to turn T cells into biological carriers of RNA encoding for granulocyte macrophage colony-stimulating factor (GM-CSF), which had been applied as adjuvants in cancer immunotherapy and been used to overcome the immunosuppressive TME. It had been proven that the GM-CSF RNA-modified T cells enhanced GM-CSF levels in brain TME without increasing systemic concentrations, leading to extremely low toxicity in mice. By i.v. injection of modified T cells, it showed significantly improved survival in murine brain tumor model [25]. Dong *et al* put forward a feasible approach by clicking NPs (biodegradable photobleaching-resistant fluorescent polymer-polylactide copolymers, shorten as BPLP-PLAs NPs) onto primary human T cells, successfully employing T cells as NPs carriers. This can be confirmed by the SEM images of T cells and different binding conditions of NPs, as T cells clicked with BPLP-PLA-NPs clearly have many attachments on the surface [27].

Besides, T cells can be engineered as biological arsenals themselves. In adoptive immunotherapy, chimeric antigen receptor (CAR) T cell therapy have attracted great attention in both tumor targeting and treatment. CAR T cell immunotherapy enables T cells to express specific CARs on their surface, then return these T cells to patients to kill the corresponding tumor cells. Numerous CAR T cell therapies are under clinical investigation, which show promising results in treating multiple myeloma by targeting a variety of antigens [28]. Till now, CAR T cell immunotherapy has showed applaudable therapeutic effect when facing against hematologic malignancies [29–32]. These significant cases encourage researchers to employ CAR T cell immunotherapy in the treatment of solid tumors. However, unlike hematologic tumors, the therapeutic effects of CAR T cell immunotherapy in solid tumors are far from impressive [33, 34], which have been attributed to the complex biological characteristics such as absence of specific targets, immunosuppression of TME, tumor antigen heterogeneity, trafficking and infiltration into tumor tissue, etc [35, 36], leaving a great upside potential in treating solid tumors.

To engineer T cells into arsenals themselves, some scientists come up creative ideas. They use NPs to act on cells *in vivo*. Stephan *et al* designed a DNA-carrying NP to introduce leukemia-specific CAR genes into T cells nuclei in the bodies efficiently and selectively [37]. Lai *et al* also combined a bispecific antibody and a lentivirus together to engineer T cells *in vivo* [38].

2.2. T cell-derived membrane coating strategy

After altered, enabled mature T cells have the instinct to target specific nonlymphoid tissues. This migration relies on chemokines receptors expressed on T cells and chemokines on tumor. Chemokines can attract T cells to TME and the composition of local chemokines is influenced by tumor intrinsic characters [39, 40]. TME is composed of resident components and non-resident components. The prior part includes carcinoma-associated fibroblasts, epithelial cells, cancerous cells, cancer stem cells, all of those can secrete specific cytokines or chemokines, which endow a natural and high tumor affinity of T cells [41]. Taking advantage of the immunological recognition properties of T cell, T cell membrane-coated NPs (TNPs) recently emerged as a unique biomimetic platform to target tumor for drug delivery, which shows extraordinary capacity in evading immune clearance, achieving long circulation (same as other endogenous cell membrane coated particles),

and in particular, cytotoxic functions as well. As reported, activated $CD8^+$ T cell derived nanovesicles maintain their antitumoral activity even in the immunosuppressive TME compared to T cells [42].

Zhang and Spector recently used TNPs to act as decoy for viral attack and neutralize HIV. Generally, infecting HIV leads to the depletion of immune cells, then induces acquired immunodeficiency syndrome. In particular, $CD4^+$ T helper cells can be depleted by HIV virus in various ways, such as direct killing of infected cells and being killed by HIV-specific CTLs. The entry and fusion of virus initiates from the reaction between viral envelope glycoproteins and cluster of CD4 receptor. Based on that, they came up with the idea of deriving cell membrane of natural $CD4^+$ T cells and coating it onto polymeric cores, successfully mimicking parent T cells to target and bind with the viruses [43]. Cai and Li constructed indocyanine green NPs (INPs) which were coated with N_3-labeled T cell membrane (N_3-TINPs) to specifically target tumor (figure 1(B)). First, they pretreated activated T cell with the azido sugar Ac_4GalNAz to label N_3 group on cell membrane via glycometabolism. Then they co-extracted labeled-T cell membrane with photosensitizer (indocyanine green, ICG)-loaded poly (lactic-coglycolic acid) (ICG-PLGA polymeric core), resulting in N_3-labeled-T cell membrane-coated ICG-PLGA NPs (N_3-TINPs). This novel NP was designed to target both neoplastic natural antigens and BCN artificial receptors through immune recognition and bioorthogonal reaction. The combined mechanism provided an alternative artificial targeting strategy for photothermal therapy (PTT), increasing the drug accumulation in tumor. *In vivo* experiments showed that the N_3-TINPs presented applaudable photothermal therapeutic effect and excellent anchoring effect on tumor cells by dual-targeting mechanism [26].

2.3. T cell-derived exosomes coating strategy

Exosomes are membrane vesicles of endosomes that cells release into the extracellular environment. Those extracellular vesicles (EVs) play important role in intercellular communication by acting as carriers for cell membrane and cytoplasmic proteins, lipids, and RNA, which are transported between cells [44]. Exosomes derived from T cells have been proven to directly attack tumor cells by carrying perforin, granzymes and other effector molecules, in the meanwhile, some of exosomes manifest indirectly capabilities of inhibiting tumor development and progression by enhancing T cell-mediated antitumor activities [45]. Those biological properties of exosomes inspire their further utilities as targeting agents in treatment. In order to reduce choroidal neovascularization, Wei and Yu engineered regulatory T cell-derived exosomes and connected them with anti-vascular endothelial growth factor (anti-VEGF) antibodies by a peptide linker, which can be cleaved by matrix metalloproteinases in inflammatory lesions [46].

As for engineered T cells, like CAR T cells, exosomes originated from them show abilities to successively express all surface membrane molecules from parental cells like CARs, CD8 and TCRs. For instance, Jie and Yang found that exosomes, which were derived from mesothelin (MSLN)-targeted CAR T cells, maintaining most characteristics of the parental T cells like surface expression of the CARs and CD3. They further found that those exosomes significantly inhibited the growth of both endogenous and exogenous MSLN-positive triple-negative breast cancer cells without obvious side effects [47]. Hu *et al* reported that exosomes released from CAR T cells carrying CAR on the surface could express a high level of cytotoxic molecules and inhibit tumor growth. Human CAR T cells may be inactivated when treating solid tumors via multiple mechanisms such as programmed death (PD)-1 pathway. However, similar results were not observed on CAR exosomes *in vitro* and *in vivo*. Adding recombinant PD-L1 to CAR exosomes did not cause significant loss of cytolytic activity. The cause may be the absence of PD-1 on CAR exosomes. So recombinant PD-L1 treatment could not weaken antitumor effect of CAR exosomes. Based on the result in preclinical *in vivo* model of cytokine release syndrome, they further suggested that exosomes could be used as biomimetic nanovesicles against tumors in the future [48].

3. Natural killer (NK) cells

NK cells are lymphocyte-like cells that arise from the common lymphoid progenitor in the bone marrow. They play an important role in the early innate response to viral infections with the absence of the antigen-specific receptors of the adaptive immune system cells. However, they express members of various families of innate receptors, and with distinctive granular cytoplasm possessed, they have the ability to recognize and kill cells infected with viruses and certain tumor cells [49]. NK cells can produce some of the cytokines [50] same as those produced by effector T cells, and exhibit the cytotoxic capacity of T cells. It appears that NK cells are the innate homolog of CTLs. They keep certain viral infections in check before CTLs of the adaptive immune system become functional. Compared with T cells, immunosurveillance of NK cells is theoretically tougher for tumor to escape, even if engineered targeting antigen have been downregulated in tumor. That's because tumor or viral infected cells often suppress or even lose their human leukocyte antigen (HLA) molecules as an

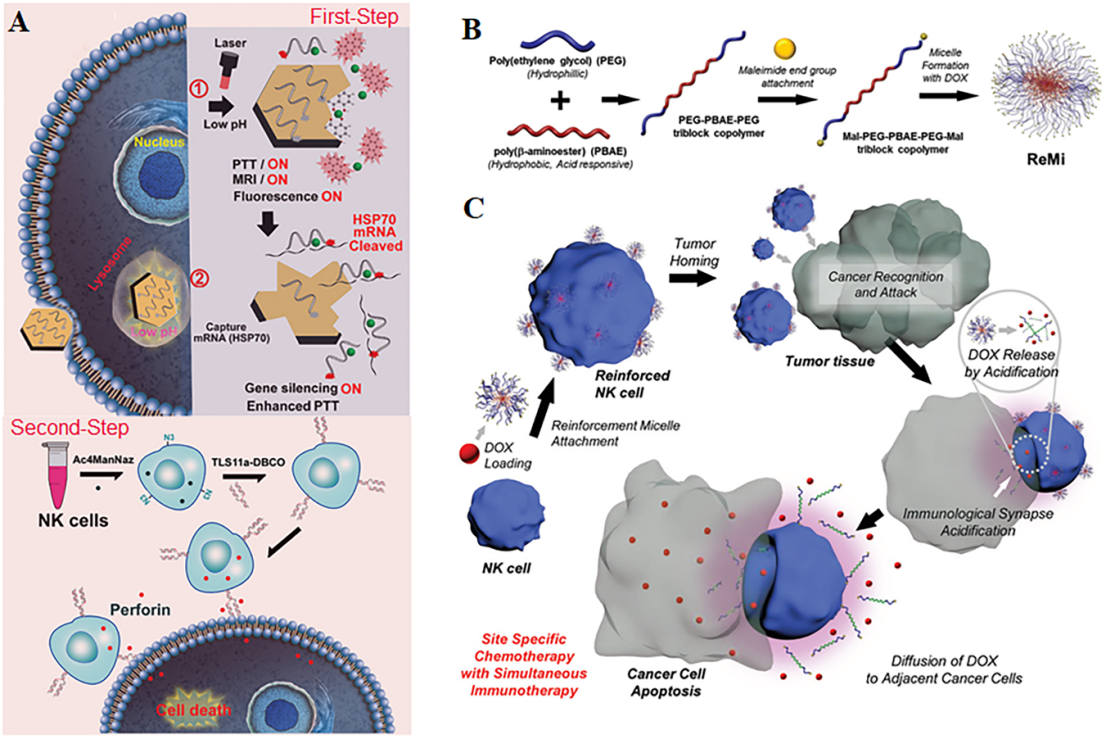


Figure 2. (A) Schematic diagram of artificial engineered NK cells combined with anti-hyperthermia endurance agents strategy for improving the therapeutic efficiency of PTT. [54] John Wiley & Sons. (B) Schematic diagram of the synthesis of maleimide-functionalized triblock copolymer, and the formation of IS environment sensitive micelles. (C) Schematic diagram of the reinforced NK cells system and the illustration of antitumor effect when encounter tumor cells. [55] John Wiley & Sons.

escape mechanism against T cells, while NK cells express germline-encoded receptors that only show either activating or inhibitory signal, and healthy cells are recognized by 'self' HLA molecules on their surface to be prevented from NK mediated lysis. So the loss of HLA class I expression makes those villain without inhibitory signal susceptible to lysis by the NK cells [51, 52]. Taking advantage of those superiorities, several novel strategies employing NK cells have been put up against tumor.

3.1. Engineered NK cells strategy

NK cells may have inherent ability against pathogens and tumor cells. However, many tumor cells have evolved immunoescape mechanisms to run away from administration and elimination of NK cells. Besides, although engineered T cells strategies, especially CAR T cell immunotherapy, have been regarded as promising strategies against hematologic cancers in clinical, they still face some shortcomings and clinical obstacles. For instance, individual-patient basis requires high investment, and substantial toxic effects involves treatment in specialized care units [53]. Therefore, engineered NK cells came into our sight, and emerged some therapies include engineered NK cells.

Liu *et al* built up an artificial engineered NK cells decorated by hepatocellular carcinoma (HCC) specific targeting TLS11a-aptamer, and combined it with photothermal therapeutic agents and DNAzymes (used as anti-hyperthermia endurance agents) to fight against HCC (figure 2(A)). They selected IL-2 activated NK cells with the expression pattern of CD3-CD56^{+}, and fed those NK cells by Ac4ManNAz. Then they used click chemistry reaction to decorate Cy3 labeled TLS11a-dibenzoazacyclooctyne (DBCO) on the surface of activated NK cells (TLS11a-DBCO can specifically target to HCC). This tumor targeting NK cell could remarkably improve the efficiency of PTT [54]. Kim *et al* took advantages of NK cells instinct ability to spontaneously release lytic granules to induce cell death after identifying tumor cells abnormal, and modified NK cells with an immunological synapse (IS) environment sensitive micellar system, which embedded therapeutic cargo (figure 2(B)). Combination between NK cells and the micelles was based on maleimide-thiol coupling chemistry, in which the free thiol groups were provided on the surface of NK cells. The local acidification of the IS cleft could break the balance of hydrophilic and hydrophobic blocks in IS responsive micelle, then rendered rapid disassembly of the micelle (figure 2(C)). Therapeutic cargo loaded in the micelle was DOX, which was prepared by solvent exchange by dialyzing DOX dissolved in the initial organic solvent against water. During the

collapse of micelle, about 90% of DOX loaded was released, with a burst release in the first 1 h. *In vivo* experiment showed that the effect of tumor inhibit of decorated NK cells was better than ordinary NK cells [55].

Apart from particles loaded-decorated NK cells, variable CAR NK cells have been constructed in recent years [56–58]. Same as CAR T cells, this strategy makes NK cells themselves more powerful arsenals. Proven by years of reports, NK cells can be safely transferred while avert the risk of graft-versus-host-disease (GVHD), and can even prevent T cell-mediated GVHD development [59]. Besides, due to the relatively limited life-span of mature NK cells, the probability of long-term adverse events of NK cell therapy is reduced, permitting a safe antitumor activity in application [60]. Moreover, the splendid immunosurveillance ability of NK cells discussed above is retained by CAR NK cells. As a result, allogeneic NK cells offer the potential for immunotherapy that conquer some current shortage of CAR T cells therapy in many regards [61, 62]. Xu *et al* constructed NKG2D CAR mRNA NK cells as a promising therapeutic potential to treat metastatic colorectal cancer. The CAR NK cells were engineered by linking NKG2D, an extracellular domain of NK cell receptor, to DAP12, and using RNA electroporation approach to provide transient expression of CAR [63].

3.2. NK cell-derived exosomes coating strategy

Exosomes contain variable proteins depending on their parent cells, the biological functions of them also differ following the physiological conditions, while exosomes still share certain characteristic protein compositions. NK cells release exosomes in a constitutive manner, which means the exosomes are secreted under both activated and resting conditions. NK cell derived exosomes express typical NK markers and killer proteins, and also possess antitumor and immune homeostatic ability, indicating that exosomes secreted from NK cells participate in command of the immune response with no need for specific stimuli. Being released in resting conditions, NK cell-derived exosomes contain Fas ligand and perforin (both are killer proteins), otherwise, NK cell-derived exosomes function on the control of immune cell only after being activated via cell-intrinsic or cell-extrinsic stimuli [64]. Ahn *et al* explored the potential of NK cell-derived exosomes against aggressive melanoma *in vitro* and *in vivo*. They isolated exosomes from NK-92MI cells by ultracentrifugation and density gradient ultracentrifugation, characterizing the quality by electron microscopy and western blotting, and naming it NK-92 Exo. In the meanwhile, the cancer xenograft model was built up via transfection of B16F10 cells with enhanced firefly luciferase and thy1.1 genes. NK-92 Exo was injected into tumors. The results demonstrated that NK-92 Exo has antitumor effect against B16F10/efflux cells *in vitro*, and *in vivo* experiments. Tumors in NK-92 Exo-treated group were significantly smaller than the control group. The expression of exosomes has been verified. NK cell-derived exosomes expressed typical exosome proteins namely CD63 and ALIX, as well as two functional NK proteins which were perforin and FasL, the latter were also expressed on membrane. In the meanwhile, NK-92 Exo was proven to secrete tumor necrosis factor-α (TNF-α) [65].

Recent researches demonstrated that NK cell derived exosomes have the ability of restoring miR-186 levels in neuroblastoma, leading to reduction of tumor burden and higher survival [66]. Fabbri *et al* discussed the neuroblastoma growth and immune escape inhibiting phenomenon, and the role that NK cell-derived exosome could play in it. It was shown that miR-186 was present in NK-derived exosomes. They further proved evidence that in high risking neuroblastoma, targeting delivery of miR-186 was feasible and able to inhibit tumor growth, spreading and evading from common immune. Since NK cell-derived exosomes retain killing capacity and toxicity in immunosuppressive microenvironment, there leaves notable potential to employ NK cell exosomes as coating materials in tumor immunotherapy treatment [67].

4. Macrophages

Almost all tissues possess macrophages. Many tissue-resident macrophages arise when embryonic developed, while some macrophages that arise in the adult animals from the bone marrow are the mature form of monocytes, which will participate in blood circulation and then migrate into tissues, where they differentiate into macrophages or dendritic cells (DCs). Life-span of macrophages is relatively long. They can survive for months, much longer than granulocytes, who can only survive for only a few days. Macrophages play essential roles in pervading innate and subsequent adaptive immune response: (a) devouring and killing microorganisms invaded, which also provides first defense in innate immunity. (b) Disposing pathogens and infected cells targeted by an adaptive immune response. (c) Orchestrating immune responses by inducing inflammation, which is a prerequisite element in an immune response, and producing various inflammatory mediators. Other immune cells can be activated by those inflammatory mediators, and being recruited into the immune response. Macrophages are cellular species that characterized by diversity and plasticity. With various signals stimulating, macrophages undergo classical M1 activation or alternative M2 activation. The M1 phenotype, stimulated by toll-like receptor (TLR) ligands and interferon (IFN)-γ, expresses high level of proinflammatory cytokines, reactive nitrogen and oxygen intermediates, and promoting Th1 response, with strong microbicidal and antitumor capacity.

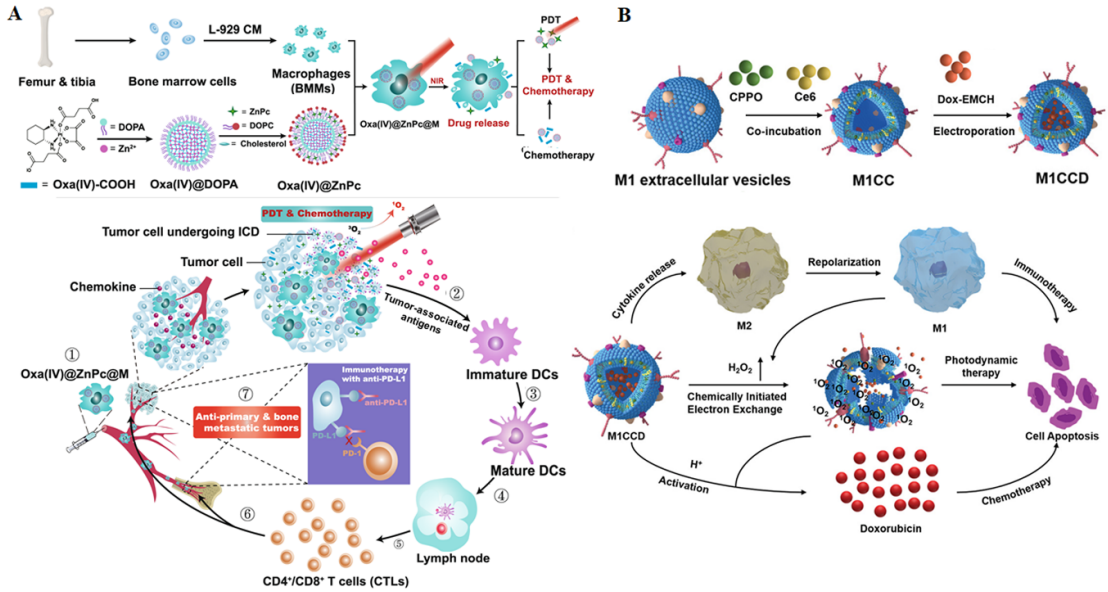


Figure 3. (A) Schematic diagram of the preparation and the mechanism of Oxa(IV)@ZnPc@M mediated chemo-photodynamic therapy. ① I.v. injection and tumor accumulation of Oxa(IV)@ZnPc@M, ② PDT and chemotherapy can induce ICD and the release of tumor-associated antigens, ③, ④ DCs maturation and antigen presentation, ⑤, ⑥ CTLs migrate to tumor site, and ⑦ simultaneously inhibit primary and bone metastatic tumor combined with anti-PD-L1. Reprinted by permission from [71] Springer Nature. (B) Schematic diagram of the construction of M1CCD and the antitumor mechanism by employing M1CCD. [72] John Wiley & Sons.

However, M2 phenotype, stimulated by IL-4 or IL-13, is skilled in parasite containment, promotion of tissue remodeling, tumor progression and immune regulation [68]. In summary, M1 phenotype presents capability of tumor eliminating, while tumor-associated M2 macrophages create an immunosuppressive microenvironment [69].

In tumor treatment and drug delivery, macrophages are often engineered, or directly served as carriers. Besides, macrophage-derived exosomes and membrane coated NPs also show extraordinary effect in tumor targeting and be widely used in drug delivery [70].

4.1. Engineered macrophages strategy

Since proinflammatory M1 macrophages demonstrate professional competence in targeting tumors and internalizing large particles like debris, apoptotic cells, etc, they can act as eligible drug carriers. To achieve better treatment effect, Zhao *et al* constructed an all-in-one macrophage using M1 phenotype polarization to combine chemotherapy, photodynamic therapy (PDT) and immunotherapy together to fight against primary and bone metastatic breast cancer. The NP engulfed by macrophages, namely Oxa(IV)@ZnPc, was composed by a core and outer layer. The core of NP was prepared first by cross-link reaction between Zn^{2+} and the carboxyl groups of Oxa(IV)-COOH, then added DOPA to stabilize via Zn-phosphate interactions. The layer was further coating on the core by hydrophobic interactions to form core–shell NPs with photosensitizer ZnPc encapsulated in it. They employed marrow-derived macrophages as carriers. After optimized, NPs could be loaded in macrophages with high loading efficiency in a short time and low cytotoxicity to carrier cells, and would be released under near-infrared (NIR) laser irradiation. This engineered macrophages is named Oxa(IV)@ZnPc@M (figure 3(A)), and cytotoxicity experiment *in vitro* indicated that it shows strikingly increased cytotoxicity after irradiation, which is attributed to the combination effect of PDT by ZnPc and chemotherapy by released platinum. In the meanwhile, immunogenic cell death (ICD) is induced in the dark by platinum, and enhanced under NIR irradiation because of the combined effect of chemotherapy and PDT. *In vivo* pharmacokinetic studies manifested that it could effectively home to the primary and bone metastatic tumors. Based on those encouraging outcomes, *in vivo* antitumor efficiency was evaluated, and anti-PD-L1 was chosen as potential checkpoint blockade therapy. As a result, in 4T1 primary tumor model, bone metastasis and related lung metastasis, Oxa(IV)@ZnPc@M combined with anti-PD-L1 accommodated a robust antitumor capacity [71].

To enhance targeting efficiency, He *et al* designed a 'dual-guide' strategy by loading NPs in macrophages. After been resected, tumor would release a burst of inflammatory factors in the resection wound, leading to recruitment of macrophages rapidly. Both under the effect of resection wound and the tropism of monocyte chemoattractant protein, lots of drug-loaded macrophages would be recruited

to surgical recurrence. The embedded NP contained PTX and resveratrol (Res) with different pharmacological mechanisms, coated by R8-modified PEGylated liposomes. PTX and Res inside liposomes in macrophages were delivered into tumor cells through a 'two-way delivery' mechanism: cell membrane fusion and cell penetrating. This mechanism was activated by both inflammation and tumor attraction. Cell membrane fusion could transfer NPs into adjacent tumor cells rapidly and directly without other complex progresses because tumor cells have natural affinity to macrophages. On the other hand, responded to the inflammatory stimuli of phorbol myristate acetate (PMA), particle-loaded macrophages could release liposomes and those just-free particles would reach distant tumor cells. In the end, *in vivo* experiments demonstrated that macrophage-based carriers exhibited remarkable tumor-targeting ability [73]. Besides, CAR macrophages (CAR Ms) have also been regarded as a promising immunotherapy against tumor [74]. On the basis of preclinical studies, CAR Ms are expected to enhance phagocytosis, polarize M2 to M1 phenotype, stimulate T cell anti-tumor activity [75]. Recent advance also shows that CAR Ms possess the ability to infiltrate solid tumors, ingest malignant tissue and stimulate adaptive immunity in mouse models. Gill *et al* found that chimeric adenoviral vector surpass the genetic manipulation of primary human macrophages, and imparting a sustained pro-inflammatory (M1) phenotype [74]. They were ready to initiate a phase I trial to assess CAR Ms in metastatic HER2-overexpressing tumors treatment. These results add CAR Macrophages as another platform to the increasing number of cell therapy modalities to treat tumors. There are also some strategies using NPs to act on macrophages. Kim and Park *in vivo* injected nanocomplexes of macrophage targeting nanocarriers and CAR-interferon-γ-encoding plasmid DNA. The nanocomplexes internalized by macrophages, turning them into CAR M1 macrophages, which are capable of CAR-mediated cancer phagocytosis, antitumor immunomodulation and inhibition of solid tumor growth [76].

4.2. Macrophage-derived membrane coating strategy

Macrophage membrane has several advantages over other immune cells, such as prolonged blood circulation, enhanced targeting ability leaded by outstanding antigens recognition, better cellular interactions, gradual drug release, and reduced toxicity *in vivo* [77, 78]. By now, macrophage membrane-camouflaged NPs have been designed and widely used in treatment of tumors, like melanoma [79], cervical cancer and breast cancers treatment [80]. They can specifically target to tumor sites. This ability is attributed to α4 and β1 integrins on the macrophage plasma membranes, which bind to the vascular cell adhesion molecule-1 of many tumor cells. And it is noteworthy that proteins on macrophage plasma membranes, like integrin α4 and macrophage-1 antigen (Mac-1), help macrophages to penetrate BBB, then specifically target to brain tumors. Gao *et al* designed a macrophage membrane-coated laser-responsive shape changeable nanomedicine, named I-P@NPs@M. After accumulated in tumor site, the chlorin e6 (Ce6) in I-P@NPs@M could convert 650 nm laser into reactive oxygen species (ROS). Next, ROS could change the spherical micelles into nanofibers for strong retention in tumor region, as well as performing pharmaceutical functions both directly killing tumor cells by PDT and stimulating the dimeric PTX generate monomeric PTX. Such a combinational chemo/PDT successfully suppressed tumor growth and inducing ICD [81]. Cai *et al* designed a macrophages membrane-camouflaged 1,2-Distearoyl-sn-Glycero-3-Phospho-ethanolamine-N- [Amino (Polyethylene Glycol) 2000] (DSPE-PEG(2000)-NH_2) loaded NIR Ib fluorescence dye IR-792 NP (MDINPs). This NP had excellent photostability irradiated by 808 nm, long circulation and the ability to penetrate BBB. As mentioned above, integrin α4 and Mac-1 on membrane-coated particles help to adhere to epithelial cells and activate signal pathway to the breakdown of tight junction, which also attribute to increase the permeability of BBB due to the expression of corresponding receptors to both integrin α4 and Mac-1 on brain endothelial cells. NIR-Ib fluorescence imaging *in vivo* showed that MDINPs selectively accumulated at tumor site. To treat glioblastoma model, the mice were injected with NPs, and irradiated with 808 nm laser 24 h post injection for 5 min. The whole orientation of MDINPs was guided by NIR-Ib fluorescence imaging. As a result, PTT successfully suppressed the growth of glioblastoma, and prolonged survival times of mice [82]. Liu *et al* even utilized cancer cell-macrophage hybrid membrane to coat NPs, in order to take advantage of characteristic membrane proteins of both cancer cells and macrophages [83].

4.3. Macrophage-derived exosomes coating strategy

Exosomes derived from M1 and M2 phenotypes inherit proteins from parental macrophages, so M2 phenotype-derived exosomes promote tumor invasion and metastasis, while counterpart of M1 phenotypes can inhibit tumor invasion and metastasis. The latter create a pro-inflammatory environment, then enhance antitumor activity via the caspase-3 pathway [84]. Combined with affinity between cancer cells and macrophages, M1 macrophages-derived exosomes are now widely utilized to deliver drugs to improve drug delivery efficiency and efficacy. By now, exosome derived from macrophage, especially M1 phenotype macrophage, has been regarded as a promising particle carrier and platform in targeting

and treating tumor cells, and there has emerged lots of studies on it [85, 86].

For example, Xie *et al* engineered a self-activatable photo-EV for synergistic trimodal antitumor therapy (figure 3(B)). They loaded bis[2,4,5-trichloro-6-(pentyloxycarbonyl) phenyl] oxalate (CPPO), Ce6 and prodrug aldoxorubicin (Dox-EMCH) in M1 macrophages exosomes (M1CCD). After actively targeting to tumor cells, M1 exosomes repolarized M2 to M1 macrophages and produced H_2O_2. Ce6 would be activated by chemical energy that generated by the reaction between H_2O_2 and CPPO, enabling both chemiluminescence and singlet oxygen ($^1O_2$) mediated PDT. $^1O_2$ release rendered membrane rupture, leading to the leak of Dox-EMCH, which would rapidly be activated into Dox in acid environments and migrate into tumoral hypoxic areas to fight against tumor. As the result of verification to the synergism feasibility of immunotherapy, PDT, and chemotherapy in tumors, obvious immune modulation effects and PDT effects had been observed. Besides, fluorescence imaging of tumor slices demonstrated the distribution of Dox intratumor. The final treatment effect also confirmed perfect synergism of the three treatment modalities in the specific TME [72]. Wu *et al* used macrophages exocytosis to get membrane characterized exogenous cargos containing Fe_3O_4 (Fe_3O_4-magnetic vesicles). They fed mouse macrophages with superparamagnetic Fe_3O_4 NPs, and the cells would release them by top-down procedure. The released vesicles membrane were inserted with lipid of 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[dibenzocyclooctyl(polyethylene glycol)] (DSPE-PEG-DBCO) into the phospholipid bilayers to be functioned with DBCO [87]. Huang *et al* focused on exosomes derived from M1 macrophage proinflammatory utility in cancer vaccine as an immunopotentiator [88]. And Xie and Pu also developed exosome nanobioconjugates (M1 Exo-Ab), which was composed by M1 macrophage exosomes with immune-stimulatory anti-CD47 antibodies and anti-signal regulatory protein alpha antibodies linked with pH-sensitive benzoic-imine bonds [89].

5. Other immune cells in tumor treatment

Apart from those well-known immune cells like T cells, NK cells and macrophages mentioned above, there still lots of immune cell play roles in TME and have the potential in therapeutic utilities. Although there might be nonnegligible obstacles, researchers still make great effort in developing those reserved cells into tumoral fighters [17].

DCs possess elaborate membrane seems like the dendrites of nerve cells. They constitute a rare immune cell population within tumor and lymphoid organs, but serve as a major class of sensor cells whose main job is to encounter pathogens, then trigger themselves to produce mediators that activate other immune cells, like T cells. DCs functions vary with their environmental signals, which are detected by surface-expressed and intracellular receptors. They can promote immunity but also can drive tolerance in the TME [90]. Zhang and Feng used cytomembranes of cancer and DCs to offer a biologically derived platform for the combination of immunotherapy and traditional tumor therapy. The fused cell membrane can effectively express whole cancer antigens and immunological co-stimulatory molecules, so this platform shared the same tumor targeting character with parent cancer cells [91]. Zhang and Pu also fused immunologically engineered cancer cell with DCs membrane as the cancer vaccine shell, to camouflage a NIR-II absorbing polymer core. Pre-engineered progress made tumor cells (4T1 tumor cells) and DCs have enhanced level of damage-associated molecules patterns (DAMP, a receptor that can sensed by immune cells) and T cell-stimulating factors [92].

Neutrophils are one of the phagocytic cells, which are the most numerous and important cells in innate immune responses. They take up various microorganisms by phagocytosis, then efficiently destroy them in intracellular vesicles under the help of degradative enzymes and other antimicrobial substances, which are stored in their cytoplasmic granules. Basically, neutrophils charge for targeting inflammation instead of tumors via surface adhesion molecules like lymphocyte function-associated antigen-1 (LFA-1), the IL-6 receptor, and the TNF-α receptor. Their migration is specific and highly regulated by several membrane proteins and cytokines/-chemokine gradients [93]. It had been revealed that PMA-activated neutrophils show morphological changes with the release of nuclear chromatin, as well as things contained inside cells [94]. This process is called neutrophil extracellular traps (NETs) [95]. Besides, neutrophils have a native ability to traverse BBB and blood brain tumor barrier (BBTB). Combined with specific chemotaxis to inflammatory sites, remarkable phagocytosis, and special release mechanism via NETs, Zhang *et al* strikingly proposed a neutrophils-mediated drug-delivery system against resected glioma (figure 4(B)). After surgical treatment, there would occur abundant local brain inflammation with a burst of inflammatory factors, such as IL-8 and TNF-α, to activate neutrophils migrate to the post-surgical site in brain. To construct this engineered neutrophil (PTX-cationic liposomes/neutrophils, PTX-CL/NEs), PTX were loaded in liposomes, then internalized by neutrophils (figure 4(A)). The PTX-loaded liposomes would be released by a conformational change, which was contributed to NETs. After being intravenously administrated, PTX-CL/NEs tracked along the chemotactic gradient towards the inflamed site [96]. This ground-breaking drug delivery system provided a perspective to utility a less high-profile immune cell in neoplastic targeting

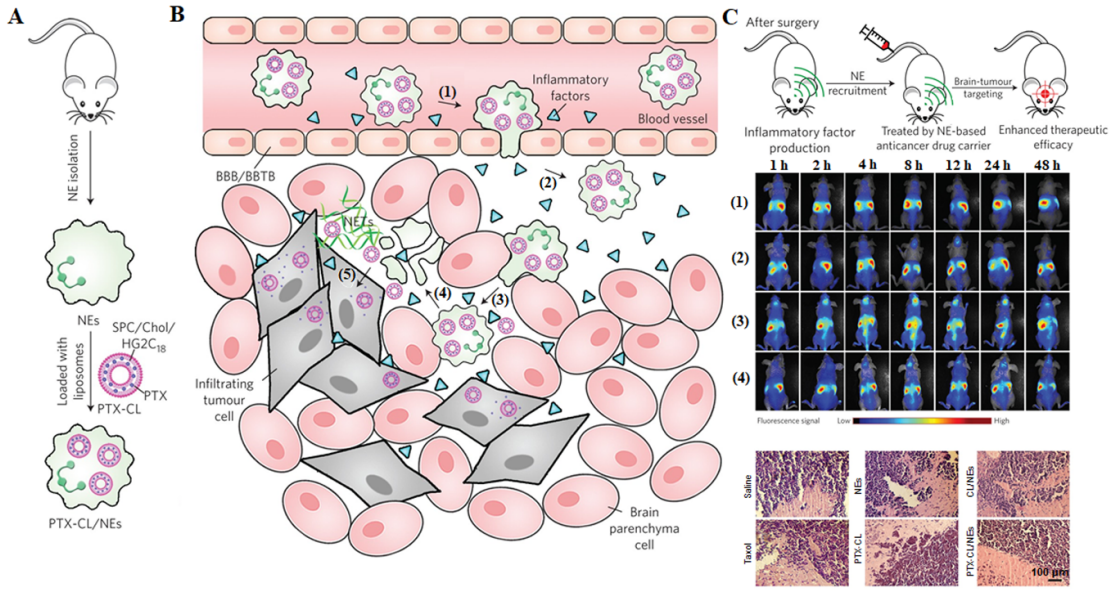


Figure 4. (A) Schematic diagram of the construction of PTX-CL/NEs. (B) Schematic diagram of PTX-CL/NEs targeting process after i.v. injection into mice whose glioma had been resected surgically. (1) Inflammatory factors lead the movement of PTX-CL/NEs followed by the chemotactic gradient. (2) PTX-CL/NEs across BBB/BBTB to transmigrate to the inflamed brain. (3) PTX-CL/NEs penetrate the infiltrating tumor cells. (4) PTX-CL/NEs are sequentially activated by the cytokines which are concentrated, and release the NETs, then result in a concomitant release of PTX-CL. (5) PTX-CL delivers PTX into the infiltrating tumor cells to fight against tumor. (C) Schematic diagram of NE-mediated antitumor drug delivery to inhibit glioma recurrence after surgical tumor removal, and *in vivo* fluorescence images after administrated PTX-CL/NEs of (1) the normal mice, (2) G422-bearing mice, (3) surgically treated G422-bearing mice and (4) the sham-operated mice. And histological observation of the brain from G422-bearing mice after surgical treatment and been administrated with different formulations. Reprinted by permission from [96] Springer Nature.

and treatment, leaving a promising potential in further studies.

Basophils are the least abundant leucocytes in the circulation, but rapidly expand in the bone marrow when inflame occurs [97]. Their functions have been further regarded by recent reports that they act important role in acquired immunity regulation, protective immunity to pathogens, and immunological disorders, for instance allergy and autoimmunity [98]. They have even been associated with myeloid neoplasm, most remarkably chronic myelogenous leukemia in recent studies. Go *et al* reviewed medical reports of patients who tested BCR-ABL, and found that myeloid neoplasm-bearing patients had higher absolute basophil counts compared to the others [99]. Basophils also manifest potential in regulating immune responses because of their ability of producing IL-4 and affecting Th2 cell responses after adoptive transfer [100].

Same as basophils, eosinophils are chief characters in defensing against parasites which are too large to be engulfed by macrophage or neutrophils, and recruitment of them into tissues can be mediated by multiple chemokine receptors in response to inflammation [17]. Lee *et al* proposed a hypothesis called LIAR hypothesis suggested that accumulating tissue eosinophils are actual regulators of local immunity and/or remodeling/repair in both health and disease. They believed that the correlation of tumor infiltrating eosinophils and tumor growth make LIAR hypothesis more reliable. Based on their theory and observation, eosinophils may show diverse effects relying on the particular tumor immune microenvironment [101]. Albert *et al* revealed that eosinophil could mediate tumor growth inhibition while CD26 (DPP4) was inhibited [102]. And it had also been shown that activated eosinophils were recruited into developing colorectal cancer and exerted antitumor activities [103].

However, both eosinophils and basophils have not been employed as drug delivery vehicles most likely because of their short portion in the blood, which leads to great problems in obtaining enough cells, hence leaving huge blank to investigate [17].

6. Perspectives and challenges

Immune cell-based drug delivery strategy demonstrated a very promising direction for cancer treatment in the future. In this review, we discussed recent advances on oncologic drug delivery strategy based on T cells, NK cells, macrophages and some other immune cells like neutrophils and DCs, exhibiting delicate and inspiring designs. However, there still remain some obstacles to be further solved in order to promote the real applications in clinical. Those challenges should be overcome in following aspects.

(a) Safe obtainment and standardized preparation of immune cells. The number one issue

that hinders clinical applications of immune cell-based drug delivery is cell source. Although these cells can be obtained from both healthy population and patients by sedimentation methods, NH_4Cl separation method or ficoll/percoll separation method, nondestructive separation and extraction of these cells with high efficiency still faces challenge. Therefore, great efforts should be paid to develop novel technologies to solve this problem. Besides that, cellular expansion *in vitro* is another concern. Development of novel immune cell bio-reactor combining optimized cellular culture conditions to achieve continuous, stable cell production may be a promising direction, thereby proving abundant high-quality immune cells.

(b) Development of more engineering technologies. Exploration of more functionalized methods to engineer cells or bio-engineer particles with biocharacters is also essential. Methods employed now include adoptive cellular immunotherapy (like CAR cell immunotherapy), click reaction on the surface, cells loading, membrane mimicking, exosome coating, etc. The proposed methods must be highly controllable, cost-effective and community-friendly, having the potential in quantity production with excellent qualities, which could contribute to further utilities. Or the current methods applied on rare-matched cells, like utilities of membrane derived from NK cells and CAR Macrophages. The shift of technologies sometimes means unexpected success, and mixture of high-profile cells and mature technologies makes approaches more reliable and implementable.

(c) Exploitation of more immune cell types. Moreover, the application of those minor-used immune cells in tumor therapy remains to be further explored, for lots of them actually act important roles in tumor invasion, elimination or growth, which lead to great research values. The extend of cells probably results in extension of variety of diseases, providing potential possibilities in serving as a more universality or more precise delivery systems in future diagnose and treatments.

Data availability statement

No new data were created or analyzed in this study.

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