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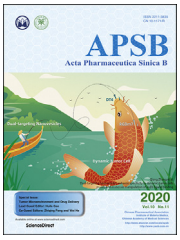

REVIEW

Nanomedicine-based drug delivery towards tumor biological and immunological microenvironment

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Combinational therapy

Abstract The complex tumor microenvironment is a most important factor in cancer development. The biological microenvironment is composed of a variety of barriers including the extracellular matrix and associated cells such as endothelia cells, pericytes, and cancer-associated fibroblasts. Different strategies can be utilized to enhance nanoparticle-based drug delivery and distribution into tumor tissues addressing the extracellular matrix or cellular components. In addition to the biological microenvironment, the immunological conditions around the tumor tissue can be very complicated and cancer cells have various ways of evading immune surveillance. Nanoparticle drug delivery systems can enhance cancer immunotherapy by tuning the immunological response and memory of various immune cells such as T cells, B cells, macrophages, and dendritic cells. In this review, the main components in the tumor biological and immunological environment are discussed. The focus is on recent advances in nanoparticle-based drug delivery systems towards targets within the tumor microenvironment to improve cancer chemotherapy and immunotherapy.

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1. Introduction

Cancer is a leading cause of death globally. Due to the complexity of tumors, the development of effective anti-cancer therapies remains challenging. Tumor tissue includes not only malignant cells but also other cells that are recruited to the tumor area. Together these cells create the tumor microenvironment[1]. In the tumor microenvironment, cancer cells are usually surrounded by cellular and non-cellular components, which consist of extracellular matrix, immune cells and stromal cells[2]. The tumor microenvironment supports tumor progression, drives therapeutic response or resistance, and creates immunological tolerance. It is heterogeneous and complex, in terms of composition, spatial arrangement, and other features such as receptor expression levels[3−5]. For example, in glioblastoma, epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptor (PDGFR) are over expressed in cancer cells located in different proportions of the tumor. Cancer cells with EGFR are present in poorly vascularized areas, while cancer cells with PDGFR are present near the endothelial cells[6]. Another example of the heterogeneity of the microenvironment is the cancer-associated fibroblasts, which have different markers-associated with various functions in different tissues[7].

Although the tumor microenvironment can be highly heterogeneous at the advanced stages, many common features are shared among various types of tumors. Therefore, targeting malignant and metastatic components in tumor microenvironment are potential strategies for tumor treatment[2,8,9]. Conventional drug delivery systems may fail to deliver adequate quantity of therapeutics to kill cancer cells effectively without undesirable side effects due to the complexity of the tumor microenvironment. Different drug targeting strategies can be used to increase the therapeutic efficiency, achieve tumor specificity, reduce the side effect and multidrug resistance, as well as improve the pharmacokinetic profile[10,11]. An example of targeted therapy that has been intensively studied is nanoparticle-based drug delivery systems. The theory of nanomedicine first came out in the late 1990s, achieved through utilization of the enhanced permeability and retention effect (EPR effect) of the tumors[12]. A variety of materials have been used to make nanocarriers, including: polymers[10,13,14], lipids[15−17], silica[18,19], and metals[20−23]. The nanometer size range of these delivery systems make them ideal for delivery of chemotherapeutic agents to the interstitial space of tumors with enhanced vascular leakiness. In addition to passive targeting, based on their size, nanoparticles can also be modified with targeting ligands that bind to specific receptors overexpressed on tumor cells to achieve active targeting. Currently, there are several anticancer nanomedicines approved by the U.S. Food and Drug Administration (FDA), such as Doxil (doxorubicin liposomes), DaunoXome (daunorubicin liposomes) and Abraxane (paclitaxel albumin-bound nanoparticles)[24−27]. A list of nanomedicines currently under clinical trial can be found in a recent published review[28]. However, the heterogeneity of tumors can lead to low therapeutic efficacy, acquired resistance and side effects[29]. Nevertheless, targeting and modulating tumor microenvironment using nanomedicines are important strategies to improve cancer treatment outcomes.

The complex physiological features of the tumor microenvironment include hypoxia, acidosis and high tumor interstitial fluid pressure, etc.[4]. Tumor hypoxia is a result of overconsumption of oxygen due to rapid tumor development. About 50%−60% of solid tumors exhibit decreased oxygen levels[30]. Hypoxia is associated with tumor growth, metastasis and resistance to tumor therapies. Therefore, down-regulating hypoxia could potentially improve treatment outcomes[31,32]. Acidosis is another hallmark of the tumor microenvironment. Rapid glycolysis in tumor cells leads to acidic extracellular pH in the tumor microenvironment. The acidic pH may affect the conformation, solubility, binding affinity, as well as the delivery of many antitumor drugs[33,34]. pH-sensitive nanoparticle drug delivery systems can potentially enhance the antitumor outcome[35,36]. Another feature of the tumor microenvironment is the dysregulated enzyme expression[34]. Enzymes play a critical role in the metabolic processes in tumor progression. Nanomedicines have been designed to be responsive to enzymes that overexpress in the tumor microenvironment, such as matrix metalloproteinases and hyaluronidase[37−39].

Nanomedicine-based drug delivery strategies also provide complementary opportunities for cancer immunotherapy. Cancer immunotherapy uses or involves components of the immune system to treat cancer. With the recent approval of chimeric antigen receptor (CAR) T cell therapy[40,41] and immune checkpoint inhibitors[42−44], cancer immunotherapy has become a most attractive anticancer strategy. Although this approach has strong potential, clinical researches have shown that many cancer patients do not show a therapeutic response to immunotherapy as a result of tumor heterogeneity. In this review, the current therapies targeting the tumor biological microenvironment, as well as the tumor microenvironment targeted therapies involved in cancer immunotherapy are discussed.

2. Targeting the tumor biological microenvironment

Apart from the malignant cells, the tumor microenvironment includes immune cells, stromal cells and extracellular matrix. As shown in Fig. 1, immune cells in the tumor microenvironment include: T lymphocytes, B lymphocytes, NK cells, dendritic cells, tumor-associated macrophages, and tumor-associated neutrophils.

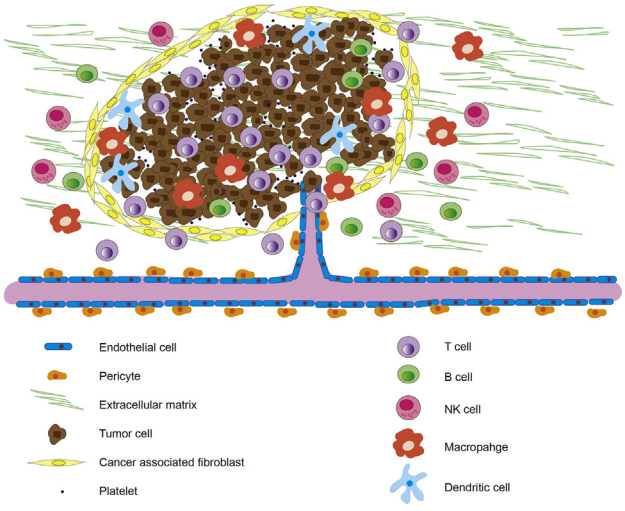

Figure 1 Tumor biological and immunological microenvironment.

Stromal cells are connective tissue cells, such as fibroblasts, pericytes, vascular and endothelial cells.

2.1. Extracellular matrix

The extracellular matrix (ECM) is the non-cellular component of cells in all tissues. It is a complex network of extracellular macromolecules, including collagen, enzymes, and glycoproteins. ECM provides physical scaffolding and biochemical support to the surrounding cells[45], which is necessary for cell growth and development, as well as other key biological processes, such as inflammation, differentiation and migration[46].

In general, the extracellular matrix in the tumor tissue is often dense and stiff compared with the normal cell ECM, and this may contribute to tumor growth and metastasis[47]. ECM is part of all the biological barriers, the dense and stiff ECM in heterogeneous tumor tissues can prevent nanoparticle penetration and delivery, which leads to low accumulation and hinders the therapeutic efficacy[46]. Details on interactions between nanoparticles and the extracellular matrix have been reviewed by Engin et al.[46] and Bandara et al.[48]. Multiple strategies have been investigated to overcome the dense and stiff biological barrier of the ECM to improve the anti-tumor efficiency of nanomedicines, such as using collagenase and hyaluronidase to "loosen" the ECM. Collagen is the major ECM structural component-associated with fibrosis[49]. Common methods to target collagen includes approaches involving the promotion of collagen degradation[50], collagen synthesis inhibition[51,52] and collagen cross-linking remediation[53].

Collagenase[54,55] is the enzyme that breaks down collagen and increases the penetration of the nanoparticles. It has been shown that collagenase can be used to improve drug delivery in cancer treatment. For example, Kato et al.[56] injected collagenase intravenously in a lung tumor xenograft model followed with liposome/plasmid DNA complex (lipoplex) treatment to improve gene expression and lipoplex accumulation. Abdolahinia et al.[57] synthesized collagenase and metformin-conjugated gold-nanoparticles and were able to show increased cytotoxicity in a breast cancer cell 3D spheroid culture model using these nanoparticles. Xu et al.[58] developed a collagenase-modified polymeric micelle formulation, where the collagenase was utilized to digest the collagen fibers thus enhancing the permeation of the nanoparticles into tumor tissue. In addition, this drug delivery system was designed using a pH-sensitive ligand to allow nanoparticle expansion in size after reaching the tumor microenvironment and thus prolonging retention. Utilizing this strategy, cisplatin nanoparticles achieved excellent anticancer efficacy in a mouse model, releasing drug into the mitochondria of the cancer cells.

Hyaluronic acid is a glycolsaminoglycan that contributes to low elasticity and high gelation pressure in tumor tissues, and promotes tumor growth, metastasis and angiogenesis[59]. Hyaluronidase[60,61] is an enzyme that has been used to degrade hyaluronic acid in the tumor ECM and has been shown to alleviate progression of many cancers, such as bladder cancer and brain cancer[62,63]. PEGPH20, a PEGylated recombinant human hyaluronidase, has been studied in multiple clinical trials in combination with chemotherapeutics, such as gemcitabine and nab-paclitaxel[64–67].

Hyaluronidase conjugated nanoparticles have also been investigated in efforts to improve their therapeutic efficiency[68]. Zhou et al.[69] reported that PLGA-PEG nanoparticles conjugated with hyaluronidase demonstrated enhanced tumor penetration and anti-tumor efficacy in a 4T1 syngeneic mouse breast tumor model. These methods often require complicated chemical conjugation reactions. Lee et al.[70] utilized pulsed high intensity focused ultrasound to break down the dense ECM structure of an A549 tumor mouse model, leading to enhanced tumor infiltration of drug-loaded nanoparticles. Using this strategy, the tumor targeting efficiency was shown to be increased by 2.5 times compared to the control group, which was not treated with high intensity focused ultrasound.

Many other ECM components, such as tenascin-C[71], fibronectin/fibrin[72,73], and galectin-1[74], have been targeted to improve nanomedicine delivery[75]. For example, tenascin-C is a glycoprotein overexpressed in the ECM of many tumors, such as breast cancer[76], lung cancer[77] and ovarian cancer[78]. It plays a critical role in supporting tumor proliferation and metastasis[79]. Tenascin-C is almost absent in the ECM of normal cells and can be a good ECM target[79]. Kang et al.[80] synthesized Ft peptide to target both tenascin-C in the ECM and neuropilin-1 in neovasculature and glioma cells. Paclitaxel-loaded Ft nanoparticles (Ft-NP-PTX) enhanced the antitumor efficacy with prolonged survival rate in a glioblastoma mouse model. Lingasamy et al.[81] developed a bi-specific peptide PL1 (PPRRGLIKLKTS) that can target both tenascin-C and fibronectin. PL1 nanoscale model payloads (iron oxide nanoworms and metallic silver nanoparticles) were shown to accumulate in glioblastoma (GBM) and prostate carcinoma models. Reduced tumor growth and increased survival were observed in a glioblastoma mouse model after PL-1 nanoworm treatment.

Targeting ECM for anti-cancer therapy is a promising strategy. Despite all the efforts and progress, there remain challenges and problems. For example, tumor collagen depletion can lead to cytokine release and potential inflammatory responses that promotes tumorigenesis[82]. Evidence has shown enhanced tumor progression after collagen depletion due to the elevated access of tumor cells to the blood stream[83,84]. Excessive breakdown of ECM components could cause ECM to lose its function and collapse its structure and worsen the therapeutic outcomes[85,86]. Further studies of the diverse properties and functions of the ECM are necessary to utilize ECM-targeted therapy.

2.2. Endothelial cells and angiogenesis

The vascular niche is a key component in tumor microenvironment[87]. The development of new blood vessels from pre-existing vessels is called angiogenesis. Angiogenesis is a major contributor to tumor growth and metastasis, and this makes it a promising target for cancer treatment[88].

Clinically approved anti-angiogenic agents, such as thalidomide, bevacizumab, sunitinib, sorafenib and pazopanib, have led to significant therapeutic improvement in cancer treatment[87]. Angiogenesis-targeted delivery systems have been extensively studied to effectively deliver anti-angiogenic agents to tumor microenvironment with minimized systemic toxicity[89]. For example, receptors that overexpressed during angiogenesis, such as the epidermal growth factor receptor (EGFR), can be used as targets to prevent angiogenesis[90,91].

The major mechanism of angiogenesis depends on interaction between the components in ECM including endothelial cells, pericytes and stoma cells[87,92]. Endothelial cells are frequently targeted in antiangiogenic therapies and a variety of ligands can be used for this purpose, such as peptides, antibodies, and cationic components[93]. The over expression of αv integrins on the endothelial cell surface is usually a marker of angiogenesis. The

integrins facilitate the growth of newly formed vessels by attaching to tumor ECM proteins and/or neighboring cells[94]. Therefore, many drug delivery systems modified with ligands which can bind to integrins have been developed to target angiogenesis for cancer therapy. For example, the cyclic RGD (Arginine-Glycine-Aspartic acid) peptide is an essential motif that specifically binds to the integrin in tumor endothelial cells. Zhan et al.[95] synthesized paclitaxel-loaded cyclic RGD modified PEG-PLA micelles and demonstrated that the micelles enhanced the anti-glioblastoma effect in U87MG glioblastoma xenografts with a significantly longer median survival time, when compared to the PEG-PLA paclitaxel micelles or paclitaxel controls. Sakurai et al.[96] encapsulated an anti-angiogenic siRNA in an RGD peptide modified lipid nanoparticle (RGD-LNP). The continuous treatment of RGD-LNP encapsulating siRNA effectively improved the overall survival of metastasized lung model mice. A variety of other peptides can also be used to target endothelial cells, such as the NGR (asparagine-glycine-arginine) motif[97], and the CGKRK peptide[98].

Cancer nanomedicines must penetrate and accumulate in tumors through the tumor microenvironment vascular network in order to take effect. EPR effect-based delivery systems have yet to led to success in the clinical setting due to the heterogeneous properties of tumors. Therefore, it is essential to facilitate the delivery of the nanomedicine. Antiangiogenic therapy has drawn a lot of attention and has shown good results in many preclinical studies. However, this type of therapy could activate multiple signaling pathways, such as the vascular endothelial growth factor (VEGF) pathway, which may lead to drug resistance, tumor invasion and metastasis[99,100]. Gyanchandani et al.[100] studied molecular changes in a bevacizumab resistant xenograft model using human-specific microarray analysis. Increased levels of fibroblast growth factor-2 (FGF2) were observed. FGF-2 is a pro-angiogenic factor regulated by the overexpression of upstream genes in extracellular signal-regulated kinase (ERK) pathways, including phospholipase C (PLCg2), frizzled receptor-4 (FZD4), chemokine [C-X3-C motif] (CX3CL1), and chemokine [C-C motif] ligand 5 (CCL5). Upregulation of FGF-2 induces tumor resistance against cancer therapy.

2.3. *Pericytes*

Pericytes are another promising target for anti-cancer treatment[101]. Pericytes are perivascular cells that surround the endothelium of capillaries and vessels. Pericytes interact with endothelial cells and are important cellular components of tumor microenvironment[102]. The function of pericytes in tumor progression is complicated and not fully understood as yet[103,104]. For example, pericytes are found to regulate angiogenesis and control endothelial cell proliferation through the vascular endothelial growth factor (VEGF) signaling pathway[105,106]. For drug delivery for the treatment of brain cancers, overcoming the blood–brain barrier (BBB) is a major challenge. Pericytes are embedded in the basement membrane and ensure the integrity of the BBB. Increased brain permeability of both low and high molecular tracers was observed in pericyte-deficient mouse models[107].

Several researches have shown improved anti-cancer efficiency using drug delivery systems modified with peptide moieties that bind to specific proteins on pericytes. For example, nanoparticles were developed to target the NG2 receptor, a proteoglycan highly expressed in tumor pericytes. The TH10 peptide (TAASGVRSMH) has been conjugated to docetaxel-loaded nanoparticles (TH10-DTX-NP)[108]. The TH10 peptide was screened and isolated through phage display and has a strong affinity for the NG2 receptors on pericytes. Antitumor effects have been observed using TH10-DTX-NP in a melanoma experimental lung metastasis mouse model with prolonged survival. The TH10 peptide enhanced the nanoparticle internalization through interaction between TH10 and NG2.

Another strategy to enhance nanoparticle permeation into tumor tissue is to reduce pericyte coverage. Chaudhuri et al.[109] demonstrated that the administration of a smoothened inhibitor, erismodegib, could significantly enhance doxorubicin-loaded nanoparticle accumulation into the adenocarcinoma cell-enriched tumor region. Smoothened inhibitors may disrupt the hedgehog-signaling pathway (Hh) leading to the deactivation of tumor fibroblasts. In this study, the decrease in the pericyte coverage of the vascular endothelium structure was observed to be-associated with enhanced nanoparticle distribution, contributing to the improved therapeutic effect in a mouse model of pancreatic cancer.

There are still challenges to target pericytes for cancer therapy, including a limited knowledge of the identification, ontogeny, and progeny of pericytes[110]. Pericytes depletion may also fail to improve the anti-tumor effect and lead to unexpected tumor growth[111]. Moreover, low pericyte coverage has been reported to be-associated with tumor metastasis and poor prognosis[110,112]. A deeper understanding of the interaction between tumor pericytes and tumor progression is needed.

2.4. *Cancer-associated fibroblasts*

Cancer-associated fibroblasts (CAFs) are a type of mesenchymal stromal cells abundant in tumor microenvironment that play important roles in cancer progression[113,114]. CAFs provide physical support for tumor cells and are important in stroma remodeling. They are the major cell type involved in collagen production and crosslinking which contribute to increased ECM stiffness[115]. CAFs are also associated with tumorigenesis and are involved in immune evasion[116].

CAFs could cause lower tumor penetration of nanomedicines and lead to poor therapeutic outcomes. Anti-cancer-associated fibroblast therapy has become a promising strategy to improve drug sensitivity. For example, Zhang et al.[117] used the clinically-relevant cyclooxygenase-2 (COX-2) inhibitor, celecoxib, to improve the delivery of paclitaxel-loaded micelles. Celecoxib leads to reduced CAFs, disruption of the fibronectin bundle and improved tumor perfusion. As a result, the penetration and accumulation of paclitaxel-loaded micelles were enhanced and their therapeutic benefits improved in human lung A549 tumor xenografts. Chen et al.[118] modified navitoclax-loaded nanoliposomes with peptide FH (FH-SSL-Nav). Small molecule navitoclax can induce the apoptosis in CAFs and peptide FH can specifically bind to tenascin-C. The peptide liposome FH-SSL-Nav exhibited increased cellular uptake and cytotoxicity *in vitro*, as well as improved antitumor efficacy in a Hep G2 tumor mouse model. A recent report[119] has shown that the tumor cell-promoting behavior of CAFs can be attenuated by gold-core silver-shell hybrid nanomaterials. In the *in vivo* study, the nanomaterial showed promising results in inhibiting tumor metastasis.

A key challenge in CAF research is the lack of simple nomenclature of CAFs and the fibroblast subtypes for broader use in cancer and stromal biology. There is also a lack of robust biomarkers for CAFs detections in the clinical setting[120]. Deeper understanding of the CAFs origin, diverse function, heterogeneity

Table 1 Quick guide for phenotype identification of the main immune cells within the tumor microenvironment.

Lineage	Cell		Function	Positive	Ref.
Lymphoid	T cells	Cytotoxic T cells	Immunostimulatory	CD3, CD8	133,134
		Helper T cells	Immunostimulatory	CD3, CD4	135
		Gamma delta T cells	Immunostimulatory	CD3, $\gamma\delta$TCR	136,136
		NK T cells	Immunostimulatory	CD3, CD161, CD94, CD1d-α-GalCer	137,138
		Memory T cells	Immunostimulatory	CD3, CD27, CD45RO	139,140
		Regulatory T cells	Immunosuppressive	CD4, CD25, FoxP3	141,142,143
	B cells	Plasma cells	Immunostimulatory	CD38, CD138	144
		Memory B cells	Immunostimulatory	CD19, CD20, CD27, CD38	145
		Regulatory B cells	Immunosuppressive	CD19, IL10, CD1d	144,146
	NK cells		Immunostimulatory	CD16, CD56	147
Myeloid	Tumor-associated macrophages (TAMs)	M1 phenotype	Immunostimulatory	CD68, iNOS, HLA-DR, CD80	148,149
		M2 phenotype	Immunosuppressive	CD68, CD163, VEGF, CD206	148,149,150
	Myeloid derived suppressor cells (MDSC)	Polymorphonuclear (PMN-MDSC)	Immunosuppressive	CD11b, CD15 (or CD66b), CD33	151
		Monocytic (M-MDSC)			
	Tumor-associated neutrophils (TANs)		Can be pro- or anti-tumor activity	CD66b	152
	Dendritic cells (DC)		May be defective in TME as terminally differentiated myeloid DC	MHCII, CD103 or CD11b	153,154,155

and plasticity will be beneficial in modulating CAFs for anti-cancer therapy[120]. The design of sophisticated nanoparticulate drug delivery systems targeting CAFs relies on a more fundamental understanding of CAFs.

2.5. *Platelets*

Platelets are anucleate blood cells that are present in the tumor microenvironment. Besides their role in blood coagulation, platelets have been recognized for supporting tumor growth and metastasis[121,122]. Platelets interact with tumor cells through different ways. In brief, tumors relying on the vascular network for growth can induce aggregation, activation, and secretion of the platelets flowing through the tumor vessels[122]. Platelets not only protect tumor cells from blood sheer stress and immune cell-mediated elimination[123], but also interact with other components in the tumor microenvironment, such as endothelial cells, pericytes, fibroblasts and immune cells thereby contributing to tumor progression and inflammation[124].

Targeting platelets using nanoparticle-based drug delivery systems can potentially inhibit tumor metastasis. For example Zhang et al.[125] designed nanoparticles modified with the tumor-homing pentapeptide CREKA (Cys-Arg-Glu-Lys-Ala) to deliver platelet inhibitor (ticagrelor). These nanoparticles were determined to efficiently inhibit platelet-tumor cell interaction and block tumor cell transition into mesenchymal-like invasive cells in a mammary tumor xenograft mouse model. Interestingly, platelet drug-loading and platelet membrane biomimetic systems are also very popular for tumor therapy[126–128]. Xu et al.[129] conjugated doxorubicin-loaded platelets with anti-CD22 monoclonal antibodies for tumor targeting. The platelet drug carriers prolonged the circulation time of doxorubicin. Enhanced antitumor activity was observed both *in vitro* and *in vivo*. Wang et al.[130] prepared platelet membrane (PLTM)-coated nanoparticles loaded with the anti-cancer drug bufalin (PLTM-CS-pPLGA/Bu NPs). Increased accumulation of the nanoparticles and more effective tumor growth inhibition were observed in H22 hepatocellular carcinoma tumor-bearing mice treated with platelet membrane-coated nanoparticles (PLTM-CS-pPLGA/Bu NPs) when compared to uncoated groups (CS-pPLGA/Bu NPs).

The platelet-like nanoparticles have demonstrated promising preclinical results. However, scale-up manufacturing and a thorough investigation of safety have yet to be established, which are necessary to translate the technology. Targeting platelets for cancer therapy may interfere with the hemostasis of cancer patients[131]. The risk-benefit of targeting platelets should be balanced as some of the platelet targeting approaches may impair their critical physiological function in coagulation. A better understanding of the interaction between platelets and other components in the tumor microenvironment is very important to improve therapeutic outcomes.

3. Targeting the immunological microenvironment

The interaction between the tumor microenvironment and the immune cells can influence the clinical outcomes of immunotherapy. Immune cell infiltration and cytokine production leads to heterogeneous inflammatory tumor microenvironment. The main immune cells in tumor microenvironment include: T lymphocytes, B lymphocytes, macrophages, NK and dendritic cells. The functions and components of the immune cells are diverse and complex[1,132] and are listed below in Table 1[133–155].

3.1. *Tumor-associated macrophages*

Tumor-associated macrophages (TAMs) are macrophages that are abundant in the tumor microenvironment. Monocytes generated in the bone marrow and spleen can infiltrate the tumor microenvironment and differentiate into macrophages[156]. Depending on the type of cytokines they are exposed to, the activated macrophages can be classified into two different phenotypes, M1 and M2 phenotype[157]. M1 and M2 macrophages exhibit pro-inflammatory or anti-inflammatory activities, respectively[158]. Likewise, TAMs include the classically activated (immunostimulatory) M1 phenotype as well as the alternatively activated (immunosuppressive) M2 phenotype[159]. The M1 phenotype can switch to the

M2 phenotype and *vice versa* in response to microenvironmental signals, such as cytokines, chemokines, growth factors, as well as signals derived from other cells[160]. This process is called macrophage polarization. TAMs are usually M2 phenotype although they can exhibit either polarization phenotype[161–163]. The TAMs in the tumor microenvironment contribute to tumor progression, survival and metastasis and may result in a poor clinical outcome[164].

Targeting TAMs to prevent tumor progression and metastasis has become a promising anticancer strategy. TAM-targeted therapy is mainly focused on inhibition of macrophage recruitment[165,166], elimination of M2-TAMs[167] or re-polarization of M2-TAMs to M1-TAMs[168,169]. For example, Das et al.[170] reported a pancreatic cancer therapy involving activation of the innate immune receptor retinoic acid-inducible gene 1 (RIG-1) by a short interfering RNA agonist using surface-modified nanoparticles. This resulted in a higher M1:M2 macrophage ratio, increased proportion of cytotoxic T cells over regulatory T cells, as well as a reduction in regulatory B cells and plasma cells. Rong et al.[171] introduced Fe^{3+} into PEGylated polydopamine to form iron chelated nanoparticles (Fe@PDA-PEG). As shown in Fig. 2, Fe@PDA-PEG nanoparticles induced M2-TAMs to M1 repolarization and enhanced anti-tumor efficacy in colon carcinoma and breast carcinoma mouse models. Pang et al.[172] developed PLGA nanoparticles that were coated with M2-macrophages binding peptide (M2pep) to encapsulate PLX3397, a receptor tyrosine kinase inhibitor that was shown to deplete macrophages in tumors[173]. Results showed an increased uptake of M2pep-coated PLGA nanoparticles in M2-TAMs and reduced tumor growth in a mouse melanoma model.

3.2. *Chronic inflammation in tumor development*

Chronic inflammation is critically related to tumor progression[174]. On the one hand, cancers may arise from sites of infection and chronic inflammation, such as colorectal cancer associated with inflammatory bowel disease[175] and esophageal adenocarcinoma associated with reflux esophagitis[176]. On the other hand, tumor progression can often lead to chronic inflammation due to the inflammatory cytokines or other inflammatory stimuli[177]. In the tumor microenvironment, cytokines such as tumor necrosis factor (TNF-α), Interleukins (IL-1, IL6 and IL-10) and transforming growth factor β (TGF-β), play a critical role in cancer-related chronic inflammation[178,179].

For example, IL-1 binding to specific IL-1 receptors (IL-1R) initiates IL-1 signaling[180]. The IL-1R family includes 10 members, which assemble as heterodimers and signal through the MyD88/IRAK/NFκB pathway[177,181]. IL-1R-targeted delivery therapies have been studied to inhibit IL-1 induced inflammation. Shevtsov et al.[182] conjugated recombinant IL-R antagonist (IL-1Ra) to superparamagnetic iron oxide (SPION) nanoparticles to image and target glioblastoma in an experimental rat model. SPION-IL-1Ra nanoparticles significantly improved the lifespan of C6 glioblastoma rats.

Increased levels of immunosuppressive cytokines, such as TGF-β and IL-10, are accumulated in the tumor microenvironment[183]. These cytokines are involved in a variety of activities in tumorigenesis and play important roles in the chronic inflammation processes[184,185]. Combinations of inflammation modulating agents can improve the efficacy of nanomedicines. Zuo et al.[186] demonstrated that the TGF-β signaling pathway inhibition by the TGF-β type I receptor inhibitor LY364947 improves tumor penetration of the nanoparticles carrying siRNA targeting the breast cancer stem cells. This synergistic treatment delayed tumor growth and enhanced cancer stem cell clearance in breast cancer mouse xenografts. Panagi et al.[187] showed that a combination of tranilast (TGF-β inhibitor) with Doxil (doxorubicin liposomes) increased tumor perfusion and oxygenation, reprogramed macrophage polarization towards the M1 phenotype, and caused reduction in tumor size in triple-negative breast cancer (TNBC) mouse models. Moreover, tranilast and Doxil co-treatment also improves the efficacy of immunotherapy of immune checkpoint blocking antibodies (anti-PD-1/anti-CTLA-4).

3.3. *Driving "cold" tumors "hot"*

TME plays essential roles in regulating the interactions between cancer cells and immune cells during tumor progression.

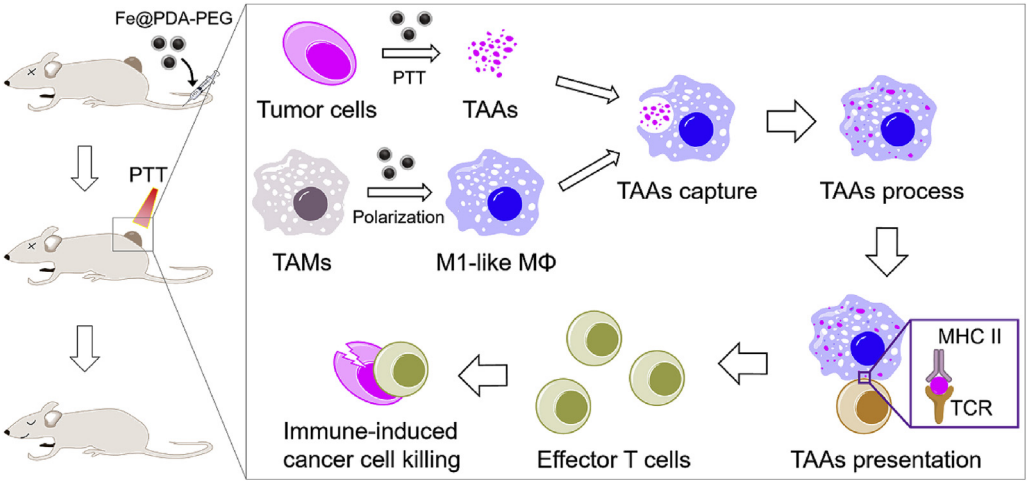

Figure 2 Iron chelated melanin like nanoparticles (Fe@PDA-PEG) induced M2-TAMs to M1 repolarization. Combining with photothermal therapy (PTT)-induced tumor-associated antigens (TAAs) release altered the tumor microenvironment to immune-induced cancer cell killing mode. (MΦ, macrophages; MHC II, major histocompatibility complex class II; TCR, T cell receptor). Reprinted with the permission from Ref. 171. Copyright © 2019 Elsevier.

Immunologically, the TME can be broadly classified as 'hot' or "cold" based on the tumor antigenicity[188]. "Hot" tumors are T cell-inflamed and highly immunogenic. The infiltrated tumor-specific T cells lose their tumor-killing capacity due to immune evasion. One of the immune evasion mechanisms is *via* the expression of the programmed death-ligand 1 (PD-L1), which can bind to the programmed death protein 1 (PD-1) on T cells and deactivate the T cells, which are the major players in the adaptive immune system. Therefore, the "hot" tumors are usually good responders to immune checkpoint inhibitors. In contrast, "cold" tumors are poorly immunogenic, there are less lymphocyte infiltrations and more immune suppressing cells[189]. Tumor immune evasion as a result of the immune responses likely happens at an early stage and affects the immune cell trafficking. Cold tumors, like pancreatic cancer, typically respond poorly to the checkpoint inhibitor therapy[163].

To improve immunotherapy outcomes of "cold" tumors, activating the innate immune system has become an attractive strategy to increase tumor immunogenicity and turn "cold" tumors to "hot" tumors.

The innate immune system plays an integral role in the activation of adaptive immunity. Innate immune cells (*i.e.*, macrophages and dendritic cells) activate inflammatory signaling through pattern recognition receptors (PRRs) in response to the binding of pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs)[190].

3.3.1. *Activate stimulator of interferon genes (STING) pathway*

One strategy to activate the innate immune system to increase tumor immunogenicity is through activation of the STING pathway. STING is an endoplasmic reticulum (ER) transmembrane protein and mediates the innate immune responses induced by cytosolic DNA[191]. Upon sensing the DNA, enzyme cyclic GMP-AMP synthase (cGAS), a cytoplasmic PRR, cyclizes GTP and ATP and produces cyclic GMP-AMP (cGAMP). cGAMP binds to STING and activates the transcriptional gene signaling and host immune responses[192]. Activating STING is a promising way to stimulate the immune suppression reversion and induce tumor regression in experimental cancer models[163,193–196]. Consistently, multiple STING agonists are in clinical trials for immunotherapy and combinational therapy with immune checkpoint inhibitors[192]. Targeted delivery of STING agonists could potentially activate immune suppressed tumors[197–199]. Cyclic dinucleotide (CDN) agonists of STING are widely studied immunotherapeutics to activate innate immunity.

The use of nanoparticulate drug delivery systems can facilitate the intracellular delivery of STING therefore inhibiting tumor growth. In a recent publication, Chattopadhyay et al.[200] described the development of hollow polymeric nanoshells using PLGA to encapsulate STING for intracellular delivery. This resulted in synthetic immunogenic cell death (sICD) of cancer cells in three mouse tumor models. The sICD was in synergy with chemotherapeutic administration into the mouse models and resulted in enhanced therapeutic effect and restrained tumor progression. Shae et al.[199] utilized STING-activating nanoparticles (STING-NPs) as a drug carrier with high bioavailability, to enhance the cytosolic delivery of the STING CDN agonist cGAMP. STING-NPs activate the STING signaling pathway in the tumor microenvironment, increase the immunostimulatory potency of immunotherapeutic cGAMP and induce the immunosuppressive "cold" tumors to immunogenic "hot" tumors. STING-NPs exhibited improved therapeutic efficacy in a melanoma mouse model, with decreased tumor growth rate and increased survival time in comparison to the immunotherapeutic cGAMP.

3.3.2. *Activate Toll-like receptors (TLRs)*

Another promising way to activate the innate system to increase tumor immunogenicity is through TLRs activation. Among the PRRs, TLRs have been most extensively investigated. TLRs are expressed across many immune cells, including T cells, B cells, dendritic cells, macrophages and NK cells. TLR-mediated signaling pathways play a critical role in the initiation of adaptive immune responses[201]. TLR signaling pathways include various signaling components, such as MyD88, Toll-interacting protein (TOLLIP), IL-1R-associated kinase (IRAK) and TNF receptor-associated factor 6 (TRAF6)[201]. Lately, TLR agonists have drawn extensive attention in cancer immunotherapy[202]. Several TLR agonists have been approved by FDA, such as Imiquimod (Aldara or R-837) and Bacillus Calmette-Guérin (BCG) vaccine. Moreover, various clinical trials in different cancer patients are ongoing to investigate the safety and efficacy of TLR agonists for monotherapy or combinational therapy[203].

Targeted delivery of TLR agonists has also been extensively studied to improve the TLR-mediated immune-stimulatory effects for cancer immunotherapy[204–209].

For example, multiple studies have been conducted to stimulate TLR 7 and TLR 8, which are structurally conserved receptors. Therefore, in certain circumstances they recognize the same ligand[210]. One of the best-characterized TLR7/8 agonists is the imidazoquinoline, such as imiquimod. Imiquimod has been approved by the FDA for topical use in basal cell carcinoma and has also been investigated under clinical trials in metastatic melanoma and localized bladder cancer patients[211].

Rodell et al.[207] studied the TLR agonist R848 loaded into β-cyclodextrin nanoparticles (CDNPs) in different mouse tumor models. The CDNP-R848 induced the repolarization of TAMs toward an M1 phenotype in tumor microenvironment. In combinational therapy with a PD-1 inhibitor, improved response rates were observed in a PD-1 resistant tumor model. In Fig. 3, Chen et al.[212] designed a combined therapy involved low-temperature hyperthermia (44 °C) and immunotherapy using NIR (near-infrared) absorbing R848-loaded nanoparticles (R848NPs/+NIR). Results showed elevated immune activation, delayed tumor regression and extended survival in tumor-bearing mouse model.

The activation of anti-tumor activity can also be utilized to develop cancer vaccines based on nanoparticles. Lynn et al.[213] developed personalized cancer vaccines based on self-assembled nanoparticles composed of charge-modified peptide-TLR-7/8a conjugates and peptide neoantigens. The charge-modifying group and hydrophobic block in the platform is tunable for the modification of diverse peptide neoantigens for personalized use. The imidazoquinoline-based TLR-7/8a adjuvant used in the vaccine allows broad activation of human dendritic cell subsets to promote cytokine production, which can activate T-cell immunity. Moreover, the nanoparticles can form in a defined size through a simple self-assembly process.

Some examples of utilization of nano-carriers targeting the TLR for immune cancer therapy are listed in Table 2[212–220] below.

3.3.3. *Activate unconventional T cells*

In addition to the activation of conventional alpha beta T cells, an emerging group of T cells that have been frequently studied are gammadelta T ($\gamma\delta$ T) cells. $\gamma\delta$ T cells link the innate and adaptive immune responses[221]. Multiple studies have shown that $\gamma\delta$ T cells

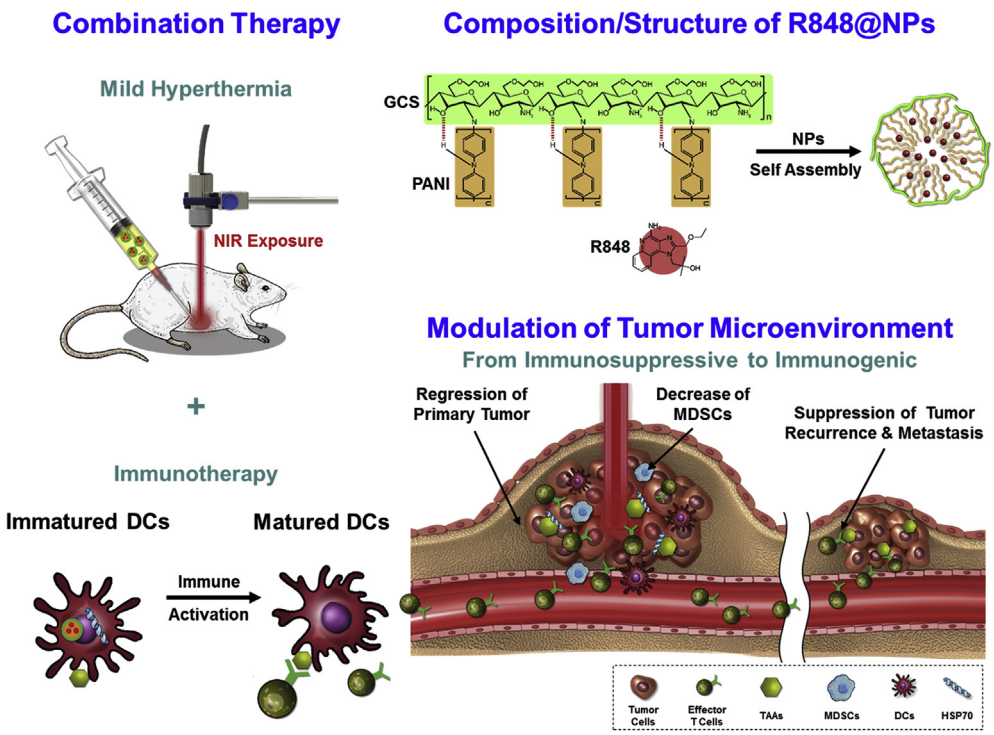

Figure 3 Composition and structure of TLR-7/8 agonist-loaded nanoparticles (R848@NPs). R848@NPs exert low-temperature hyperthermia and activate immune cells in the tumor microenvironment. Reprinted with the permission from Ref. 212. Copyright © 2020 Elsevier.

are abundant components among the tumor-infiltrating lymphocytes[222,223]. $\gamma\delta$ T cells are a less frequent type of T cells (1%−5% of circulating T cells) with a different type of T cell receptor that is composed of a γ chain and a δ chain[224]. Unlike conventional T cells which depend on MHC class I or class II molecules to recognize the antigens (*i.e.*, peptides), $\gamma\delta$ T cells respond to ligands that are fundamentally different. For example, the dominant subset of $\gamma\delta$ T cells in peripheral blood is the Vδ2 T cell, which is sensitive to phosphorus-containing small molecules known as phosphoantigens[225]. Activated $\gamma\delta$ T cells can either directly kill cancer cells or kill them through cytokine production[226,227].

Bisphosphonate drugs, such as zoledronate, risedronate, alendronate and pamidronate, were approved by the FDA to treat osteoporosis. In clinical use, they were found to indirectly activate the gammadelta T cells[224]. Modulating the activation of $\gamma\delta$ T cells using targeted delivery of these small molecules can be a potential way to stimulate anti-tumor immunotherapy. Man et al.[228] utilized alendronate liposomes to activate the [^{89}Zr]Zr (oxinate) labeled $\gamma\delta$ T cells. Positron emission tomography (PET) tracking showed that liposome treatment increased the accumulation of $\gamma\delta$ T cells in tumors in a human breast cancer xenograft mouse model.

The combination of immune-stimulating nanomedicine and immunotherapy has been extensively studied[229]. Engineering of nanoparticles by facilitating the targeted delivery to modulate the immune response in the tumor microenvironment may improve the outcome of anticancer immunotherapy. In addition to the innate immune system, the adaptive immune cells can also be directly targeted by nanoparticles. For example, Yu et al.[230] prepared hyaluronidase-responsive nanoparticles (mCAuNCs@HA) carrying the photosensitizer (pheophorbide A) and the paclitaxel prodrug (PXTK). The immune checkpoint inhibitor anti-PD-L1 peptide (dPPA) is also loaded these nanoparticles and this combination therapy activates CD4$^+$T cells, CD8$^+$ T cells and NK cells *in vivo*. Elevated TNF-α and IL-12 levels were observed. This combination therapy leads to an improved anti-tumor efficacy with the tumor inhibition rate increased to 84.2% in mouse tumor models.

Although nanomedicine is capable of enhancing the cancer immunotherapy in various aspects, there are still challenges to targeting immune cells using nanoparticle-based immunomodulatory strategies. For instance, T cells are the main players in fighting cancer and are a therapeutically relevant target for *ex vivo* gene delivery. It is challenging to transfect T cells using most commercially-available reagents[231]. Engineering cationic polymers for gene delivery to T cells may be of assistance. Olden et al.[232] designed and evaluated a panel of cationic polymers to deliver genes to T cells. Results show that comb- and sunflower-shaped pHEMA-*g*-pDMAEMA polymers can successfully deliver genes into human T cell line (Jurkat cells), human primary CD4$^+$T cells, and CD8$^+$ T cells with minimal cytotoxicity and the transfection efficiencies were up to 50%, 25% and 18%, respectively.

4. Summary, conclusions and further perspectives

Cancer remains one of the leading causes of death worldwide despite the huge effort and resources that continue to be applied in the area of cancer therapy. Tumors consist of not only the malignant cell masses but also the surrounding tumor microenvironment including cellular and non-cellular components. Dynamic and complex tumor microenvironment promotes tumor progression and is a promising target for anticancer treatment. In addition to targeting the tumor biological and immunological microenvironment as has been discussed here, tumor physical and chemical microenvironments, such as tumor interstitial fluid pressure, flow

Table 2 Immuno-therapeutics targeting TLRs using nanocarriers.

Nano carrier	Agonist and type of TLR	Application	Ref.
Polyaniline conjugated glycol-chitosan nanoparticles	Resiquimod (R848) (TLR7/8)	Synergy between hyperthermia and immune-suppression alleviation	212
Poly (lactic-*co*-glycolic acid) nanoparticle	Racemic mixture '522', stereoisomer '528' (TLR7/8)	Induced high levels of pro-inflammatory cytokines, effective in multiple tumor models	214
Antigen ovalbumin and phospholipid-loaded zinc doped iron oxide nanoparticles	PolyIC (TLR3), imiquimod (TLR7)	Checkpoint inhibition and protective antitumour responses for cancer immunotherapy	215
CpG DNA Nano-cocoon	Synthetic oligonucleotides contain unmethylated cytosine and guanosine (CpG ODN) (TLR9)	Co-delivery of anti-PD-1 with CpG induced immune response and prolonged survival time in mouse model	216
Poly (D,L-lactic-*co*-glycolic acid) (PLGA)/mPEG-PLA nanoparticles	Resiquimod (R848) (TLR7/8)	Nanoparticles detected in dendritic cells and macrophages in the draining lymph nodes with activated immune response	217
Qβ-VLPs as a nano vaccine	B-type CpGs with phosphorothioate backbone (TLR9)	Induced effective $CD8^+$ T-cell responses	218
Magnetite nanoparticles-loaded polyethylene glycol phospholipid micelles	Monophosphoryl lipid A (MPLA) derived from lipooligosaccharide (TLR4)	Long-term protection against repeated tumor challenge in combination with checkpoint inhibition	219
Self-assembled nanoparticles of acetyl modifed glucomannan polysaccharide	Glucomannan polysaccharide (TLR2)	Intratumoral injection of acGM-1.8 suppresses the growth of two tumor models	220

dynamics, pH, enzymes, and hypoxia, have also been widely studied as targeting strategies. With a growing knowledge of tumor microenvironment, various approaches have been applied to enhance the efficiency of nano-therapeutics compared to traditional passive targeting such as EPR effect.

The recent advancement in immunology offers hope for curing cancer. The approval of immune checkpoint inhibitors and CAR-T cell therapy has taken cancer immunotherapy to a new era. However, many cancer patients still do not obtain a therapeutic benefit from immunotherapy due to the heterogeneity of immune responses and various sorts of counter-measures by tumor cells. Unique tumor microenvironment creates immunological tolerance and causes therapeutic resistance. The tumor-infiltrating lymphocytes could become exhausted and express a variety of immune-inhibitory receptors, such as PD-1, LAG-3, CTLA-4 and TIM-3. In this article, several different strategies targeting the tumor microenvironment that can promote immune responses *via* increased drug release, tumor penetration, as well as prolonged immune activation have been reviewed. Nanotechnology-based drug delivery systems have shown promising results in delivering immune-therapeutics to enhance the outcome of cancer treatment in preclinical settings. The synergy between immunotherapy and nanomedicine has been gaining momentum.

Although progress has been made in the development of nanotechnology-based drug delivery systems to target tumor microenvironment, challenges remain, particularly in clinical translation and large-scale manufacturing. Researchers must be cautious about over-engineering the nanoparticulate drug delivery systems (which is a major obstacle hampering commercialization of nanocarriers in general). Stability, manufacturability and bio-performance are three aspects that must be carefully balanced when designing a nanocarrier system involving complex processes, such as chemical conjugation, targeting ligand modification, bioresponsive linker ligation, and particle size control. Academic scientists and pharmaceutical companies are making efforts to improve quality control and manufacturing reproducibility of these systems. For example, our team is leading an effort in continuous manufacturing of nanoparticles to improve quality and throughput while reducing cost. This manufacturing platform consists of a coaxial turbulent jet in co-flow system coupled with process analytical technology originally designed for the production of liposomes[17] and has been extended to other nanoparticles such as polymeric micelles. The nanoparticles (for example, liposomes) can be produced with precise control over particle size in a monodisperse fashion, as well as precise control over other critical quality attributes such as drug loading and coating with targeting ligand. Such manufacturing control can assist in targeting anticancer therapeutics to specific sites, while ensuring quality and safety.

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Author contributions

Jin Li and Diane Burgess conceived and designed this review. Jin Li wrote the manuscript. Jin Li and Diane Burgess revised the manuscript. All of the authors have read and approved the final manuscript.

Conflicts of interest

The authors have no conflicts of interest to declare.

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