Nanomedicine targeting the tumor microenvironment: Therapeutic strategies to inhibit angiogenesis, remodel matrix, and modulate immune responses

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Abstract

Increasing attention has been given to the tumor microenvironment (TME), which includes cellular and structural components such as fibroblasts, immune cells, vasculature, and extracellular matrix (ECM) that surround tumor sites. These components contribute to tumor growth and metastasis and are one reason why traditional chemotherapy often is insufficient to eradicate the tumor completely. Newer treatments that target aspects of the TME, such as antiangiogenic and immunostimulatory therapies, have seen limited clinical success despite promising preclinical results. This can be attributed to a number of reasons, including a lack of drug penetration deeper into the necrotic tumor core, nonspecific delivery, rapid clearance from serum, or toxic side effects at high doses. Nanoparticles offer a potential solution to all of these obstacles, and many recent studies have shown encouraging results using nanomedicine to target TME vasculature, ECM, and immune response. While few of these platforms have made it to clinical trials to date, these strategies are relatively new and may offer a way to improve the effects of anticancer therapies.

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Keywords: Tumor microenvironment; Cancer immunotherapy; Nanomedicine; Angiogenesis

1. Introduction

Classically, cancer has been described as a disease involving uncontrolled cell growth. Now, however, it is apparent that cancer growth and metastasis are not solely dependent on the tumor cells themselves, but involve pathologies in the surrounding tumor microenvironment (TME) as well. In the 19th century, Stephen Paget’s “seed and soil” hypothesis postulated that cancer “seeds” or metastases preferentially established secondary tumors at specific sites (the “soil”) [1,2]. Only within the past few decades have researchers focused on anticancer treatments which target the TME rather than the actual cancer cells.

The TME contains various cell types, such as fibroblasts, myofibroblasts, adipocytes, and immune cells, as well as extracellular matrix (ECM), and blood and lymphatic vasculature [3]. Increasing evidence suggests that the TME is a crucial part of cancer development, proliferation, and metastasis [4]. The TME also contributes to the failure of many conventional cancer therapies to completely eradicate the tumor. Nanomedicine offers a way to circumvent or take advantage of the properties of the TME. As illustrated in Fig. 1, this review will discuss nanoparticle-mediated targeting of TME aspects including vasculature, ECM, and immune cells, with a focus on modulating the immune response. Select studies are summarized in Table 1.

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1.1. Abnormal tumor vasculature and interference with drug delivery

Blood vessel development is critical for the continued growth and progression of solid tumors. Unlike normal blood vessels, structurally and functionally abnormal tumor vasculature impedes the efficient delivery of both oxygen and therapeutics at effective concentrations to all cancer cells [5–10]. This phenomenon is mainly caused by an imbalance of pro- and anti-angiogenic factors, which leads to endothelial cell proliferation, migration, and new vessel formation. This contributes to the formation of poorly organized blood vessels, impaired blood flow, increased hypoxic regions within the tumor, and higher interstitial fluid pressure (IFP) [10,11].

Table 1

A summary of nanoparticle-mediated therapies targeting properties of the tumor microenvironment.

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
<th>Target and effects</th>
<th>Status</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vasculature</td>
<td>PLGA nanoparticles with combrestatin</td>
<td>Inhibits angiogenesis, can be combined with antitumor agents such as doxorubicin</td>
<td>Preclinical</td>
<td>[21]</td>
</tr>
<tr>
<td>ECM</td>
<td>Polymer nanoparticles with lodamin</td>
<td>Inhibits angiogenesis and tumor growth</td>
<td>Preclinical</td>
<td>[22]</td>
</tr>
<tr>
<td>ECM</td>
<td>PEG nanoparticles with hyaluronidase</td>
<td>Degrades ECM, improves drug penetration into tumor core</td>
<td>Preclinical</td>
<td>[24]</td>
</tr>
<tr>
<td>ECM</td>
<td>Polymer nanoparticles with anti-LOX antibodies</td>
<td>Inhibits LOX, suppresses tumor growth</td>
<td>Preclinical</td>
<td>[14]</td>
</tr>
<tr>
<td>Immune</td>
<td>Gold nanoparticles with TNFα</td>
<td>Immune stimulant, administers TNFα systemically without side effects</td>
<td>Phase I (US)</td>
<td>[61]</td>
</tr>
<tr>
<td>Immune</td>
<td>Liposomal mifamurtide</td>
<td>Activates monocytes and macrophages</td>
<td>Approved (Europe)</td>
<td>[62]</td>
</tr>
<tr>
<td>Immune</td>
<td>Adenovirus nanoparticles</td>
<td>General immune stimulant</td>
<td>Phase I (US)</td>
<td>[63]</td>
</tr>
<tr>
<td>Immune</td>
<td>Liposomal TNFα or IL-12</td>
<td>General immune stimulant</td>
<td>Preclinical</td>
<td>[28–30]</td>
</tr>
<tr>
<td>Immune</td>
<td>Polymer or glucan nanoparticles with siRNA</td>
<td>Delivers siRNA to TAMs to polarize to M1 phenotype</td>
<td>Preclinical</td>
<td>[46,47]</td>
</tr>
<tr>
<td>Combination</td>
<td>CRLX101 (polymeric nanoparticle with camptothecin) with bevacizumab</td>
<td>Anti-angiogenic inhibitor of HIF-1 combined with conventional chemotherapy</td>
<td>Phase II (US)</td>
<td>[64]</td>
</tr>
<tr>
<td>Combination</td>
<td>Liposomal sorafenib and CXCR4 antagonist</td>
<td>Anti-angiogenic combined with immune stimulant</td>
<td>Preclinical</td>
<td>[48]</td>
</tr>
<tr>
<td>Combination</td>
<td>HA-based nanoparticle with TRAIL and cilenitide</td>
<td>Anti-angiogenic drug combined with antitumor drug</td>
<td>Preclinical</td>
<td>[49]</td>
</tr>
<tr>
<td>Cell-Based</td>
<td>Iron nanoparticles conjugated to NK cells</td>
<td>NK cells accumulate in tumor with external magnet</td>
<td>Preclinical</td>
<td>[53]</td>
</tr>
<tr>
<td>Cell-Based</td>
<td>Gold nanoparticles conjugated to T cells or internalized by monocytes and macrophages</td>
<td>Immune cells traffic to tumor, cause damage via photoablation</td>
<td>Preclinical</td>
<td>[54,55]</td>
</tr>
<tr>
<td>Cell-Based</td>
<td>Liposomal IL-15/IL-21 conjugated to T cells</td>
<td>T cells stimulated by own payload, enhances expansion and antitumor effects</td>
<td>Preclinical</td>
<td>[56]</td>
</tr>
</tbody>
</table>
Due to vascular hyperpermeability, the gradients between vascular and interstitial pressure are not maintained, resulting in elevated interstitial pressure within the tumor. Reduced lymphatic drainage from the tumor further exacerbates elevated IFP within the tumor. High IFP hinders effective penetration of anticancer agents into the deeper core of solid tumors [11].

Due to the leaky tumor vasculature, nanoparticles can accumulate within the tumor and its surrounding environment more effectively than free drugs. This phenomenon, referred to as the enhanced permeability and retention (EPR) effect, is further enhanced by poor lymphatic drainage around the tumor, effectively trapping the nanoparticles at the site [12]. Recent studies also demonstrate that anti-angiogenic or anti-vasculature therapies have shown promising results in enhancing drug delivery and boosting anticancer efficacy [5,9–11,13]. The combination of passive targeting via the EPR effect and active targeting with an anti-angiogenic conjugate confers an advantage of nanoparticle-mediated therapies over soluble drugs.

1.2. Abnormalities of tumor ECM and effects on tumor progression

Major ECM components include collagen, glycoproteins, proteoglycans, elastin, and hyaluronan as well as ECM-associated enzymes and growth factors which direct cell proliferation and differentiation [14]. However, in a hypoxic TME, biophysical and biological characteristics of the ECM are altered, contributing to tumor progression, invasion, and metastasis. Stromal stiffness is significantly increased in breast tumor tissue stroma as compared to normal tissue stroma [7,8,15]. Lysyl oxidase (LOX) is a major player that upregulates crosslinking of collagen fibers with other ECM components [15]. Furthermore, the interstitial space is mostly composed of collagen. In the TME, the collagen content is higher than that of normal tissue and is a major barrier of interstitial drug penetration [14]. Interactions between cancer cells and the various components of the ECM serve as one of the main obstacles that prevents effective penetration of chemotherapeutic drugs into tumor tissue.

1.3. Immune dysfunction and opportunities for nanomedicine in the TME

The TME contributes to chronic inflammation and dysregulation of immune cells. In a healthy patient, both innate and adaptive immune cells partake in cancer immunosurveillance. In the TME, chronic exposure to inflammatory cytokines often results in immune cell anergy and a lack of antitumor response. Over time, these immune cells themselves start to secrete pro-tumorigenic mediators and contribute to maintaining the TME. Immune suppressor cells also accumulate within the TME.

T cells are part of the adaptive immune system that have antitumor effects, but are suppressed in the TME. Foxp3+CD25+CD4+ regulatory T cells (Treg) are recruited to the tumor site and secrete inhibitory cytokines such as IL-10 and transforming growth factor-β (TGF-β) [16]. Myeloid-derived suppressor cells dampen immune responses by hindering antigen presentation by dendritic cells (DCs) and impairing CD8+ T cell cytotoxicity [17].

The TME also impacts the function of innate immune cells, including DCs and natural killer (NK) cells. DCs are antigen-presenting cells which stimulate T cell response to tumor antigens. DCs pulsed with tumor peptides are being researched as a cancer vaccine, but have seen limited clinical efficacy due to the inhibitory effects of the TME [18]. Rather than being activated by an antigen-specific receptor, NK cells are stimulated by a complex balance of inhibitory and stimulatory signals. Under normal conditions, their cytotoxic abilities are inhibited by engaging with HLA class I present on healthy autologous cells. Tumors tend to downregulate HLA class I, leaving NK cells uninhibited and able to target the cancer cells. In the TME, cancer cells are often able to escape NK cytotoxicity by suppressing NK cells with IL-10 and TGF-β [19].

Macrophages play roles in both the innate and adaptive immune systems. Macrophages with the M1 phenotype have enhanced cytotoxicity and contribute to an inflammatory environment by upregulating the release of pro-inflammatory cytokines and reactive oxygen species. Within the TME, macrophages can be polarized from the M1 to the immunosuppressive M2 phenotype [18]. Tumor-associated macrophages (TAMs) can also modulate the ECM by signaling to increase collagen secretion or by releasing matrix metalloproteases (MMPs), which break down ECM and can lead to metastasis. Additionally, TAMs can secrete proangiogenic factors and contribute to tumor vascularization [20]. All of these functions make TAMs an important target in cancer immunotherapy.

Numerous studies have used nanoparticles to carry immunomodulatory agents and affect various immune cell populations, including DCs [21] and T cells [22–24]. While many of these have been used in the treatment of autoimmune diseases with the goal of suppressing the immune system, the same nanoparticle platforms can apply to anticancer, immunostimulatory therapies. Depending on their payload, nanoparticles can augment immune responses within the TME as well as inhibit immunosuppressor cells such as Treg. Nano-based delivery systems can reduce undesired toxicities and rapid clearance rates that have hindered the systemic administration of free cytokines in the clinic. Targeted nanoparticles can also deliver their cargo to specific subsets of immune cells for enhanced antitumor effects.

2. Nanomedicine targeting the tumor microenvironment

2.1. Inhibiting angiogenesis for vasculature normalization

The EPR effect has been used as the foundation for passive targeting of nanomedicines to tumor sites. Unique features of vascular pathophysiology and inefficient lymphatic drainage
play a major role in the EPR effect [7,10,13]. Nevertheless, the optimal passive targeting strategy of nanoparticles depends on the degree of tumor vascularization and angiogenesis. As previously mentioned by Jain et al., the irregular and leaky tumor vasculature is also responsible for elevated tumor IFP which could a barrier for efficient transport of drugs. It is now well known that most tumors have increased IFP [25].

Furthermore, in order for most malignant solid tumors to grow and progress, tumors stimulate the formation of new blood vessels through processes driven primarily by proangiogenic proteins such as vascular endothelial growth factor (VEGF) [5,6,20]. Upregulation of these proteins influences endothelial cell migration and proliferation, resulting in excess endothelial cells and abnormal perivascular cells. Due to this poor vascularization, conventional intravenously distributed cytotoxic drugs are limited in their ability to penetrate the tumor core, posing a significant barrier to their anticancer efficacy. This therapeutic obstacle has given rise to a different strategy to cancer treatment: targeting tumor vasculature and angiogenesis using nanoparticle delivery systems to allow greater dissemination of chemotherapeutics. Anti-angiogenic therapies eliminate excess endothelial cells, resulting in normalization of vasculature. This “normalized” vasculature has decreased vessel diameter, density and permeability which lowers interstitial fluid pressure and enhances blood perfusion and oxygen tension [20,25,26].

Numerous active vasculature targeting approaches using nanoparticles have emerged to enhance the intracellular concentration of drugs in tumor cells. Sengupta et al. developed a PLGA polymeric nanoscale delivery system targeting tumor cells and the tumor vasculature. Doxorubicin was covalently attached to the inner PLGA core, and the anti-angiogenic agent combretastatin was encapsulated within the outside lipid envelope. Both doxorubicin and combretastatin were successfully encapsulated into their ‘nanocell’ delivery system, enabling the temporal release of two drugs more effectively than with free drugs or simple liposomal formulations. As a result, combretastatin released from the outer envelope was able to induce a rapid vascular shutdown inside the tumor by disrupting the cytoskeletal structures, which first took advantage of the EPR effect and then further trapped the nanoparticles within the tumor after disrupting tumor vasculature. Afterward, doxorubicin from the inner nanoparticle was efficiently taken up by the tumor, improving overall therapeutic index with reduced toxicity [26].

Another study by Benny et al. synthesized nanopolymeric micelles loaded with the angiogenesis inhibitor TNP-470, called Lodamin, and successfully demonstrated improved oral bioavailability, inhibition of tumor growth, angiogenesis and proliferation, without causing any severe side effects in vivo. Tumor growth was significantly decreased upon treatment with Lodamin compared to the free inhibitor, TNP-470 [27]. These studies highlight the uses of nanoparticles by using the EPR effect to prolong the retention of antiangiogenic and chemotherapeutic agents within the tumor and by increasing the bioavailability and vascular uptake of poorly soluble anti-angiogenic small molecule drugs.

2.2. Targeting tumor extracellular matrix

Abnormally dense ECM can cause inefficient penetration of drugs. TME-associated ECM serves as a guiding scaffold for cancer cell proliferation, migration, invasion and angiogenesis [9]. Collagen, the main structural protein of the ECM, can build migration tracks for the tumor cells. Hyaluronic acid (HA) contributes to high IFP, preventing the diffusion and penetration of drugs [8,14,28]. To this end, one of strategies used to improve intratumoral drug delivery with nanoparticles is modification of ECM components. Recent studies, along with clinical trials, have shown that ECM-degrading enzymes, such as collagenase or hyaluronidase, can improve nanoparticle penetration into solid tumors. However these agents have potential risk of increasing tumor metastasis. A PEGylated form of recombinant human hyaluronidase (PEGPH20) has recently been introduced into clinical trials combined with other chemotherapeutics [29]. PEGPH2 was shown to have therapeutic effects for patients with metastatic pancreatic cancer, especially those which expressed high levels of hyaluronidase. Adverse side effects were mild to moderate and none led to discontinuation of the treatment. PEGPH2 warrants additional studies in combination with other chemotherapeutic, in particular for patients with hyaluronidase-overexpressing tumors.

Further studies have been focusing on enzymes such as LOX or proteases like MMPs, which are responsible for remodeling ECM. In the study by Kanapathipillai et al., polymeric nanoparticles coated with a LOX inhibitory antibody was able to selectively bind to ECM and enhanced suppression of mammary tumor growth [15]. Using nanoparticles greatly enhanced the therapeutic effects of anti-LOX antibodies—in vitro, nanoparticle-bound anti-LOX was effective at doses 50 times lower than that of soluble anti-LOX, and had a high therapeutic index in vivo as well.

Another recent study used paclitaxel-loaded PLA nanoparticles which contained a fibronectin-targeting peptide. Fibronectin is one of the main components of ECM, which is abundant in the glioma microenvironment. Mice treated with these targeted nanoparticle-drug conjugates had a survival time nearly 70% greater than other treatment groups [30]. Nanoparticle-assisted targeting of the tumor ECM is a novel approach, and although currently few researchers have studied this potential therapy, it shows significant promise and merits further study.

2.3. Modulating immune response

2.3.1. Administration of general immunostimulatory cytokines

Many of the immunosuppressive effects of the TME stem from an imbalance between suppressive and stimulatory cytokines. While cytokine administration has been largely effective in modulating immune responses in animal models, these successes have not been translated into clinical results due to nonspecific delivery and adverse side effects [31]. For example, IL-2 has been used as a single agent in cancer
immunotherapy and has been approved by the FDA for both metastatic melanoma and renal cell cancer. However, systemic administration carries the risk of serious side effects such as thrombocytopenia and lymphopenia [32]. Additionally, cytokines are often degraded and rapidly cleared from serum [33]. Nanoparticles may offer sustained, specific delivery of cytokines, in part via their passive accumulation within leaky tumor vasculature.

One early study used liposomes to encapsulate TNFα and, after confirming that the liposomal TNFα had comparable in vitro cytotoxicity to free TNFα, suggested using this strategy to improve systemic cytokine anticancer therapy [34]. Since then, many researchers have reported improved circulation times and antitumor effects of cytokines using liposome carriers. Incorporating IL-2 into multilamellar liposome vesicles prolonged circulation times and decreased hematologic toxicities when administered intravenously to rats [35]. Cytokines can have a synergistic effect when administered with conventional chemotherapeutic drugs; dramatic tumor shrinkage was observed in rats injected with Doxil and liposomes containing TNFα, even though either treatment alone only resulted in slowed tumor growth [36].

Directly injecting nanoparticle-cytokine formulations into the tumor have also yielded encouraging results. Polyactic acid (PLA) microspheres encapsulating IL-12 or GMCSF were intratumorally injected into mice bearing subcutaneous tumors, and a combination of the two cytokines yielded the best results, including fewer metastases and longer survival times following a single injection [37]. Intratumoral injections of PLA microspheres carrying either IL-12 or TNFα in B16 melanoma-bearing mice resulted in tumor eradication, induction of a tumor-specific memory T cell response, and tumor rejection upon rechallenge [38]. These studies indicate that nanoparticle-mediated cytokine release can overcome the immunosuppressive effects of the TME.

2.3.2. Enhancement of T, natural killer, and dendritic cell function

Instead of stimulating general immune responses via cytokine delivery, many studies have used nanoparticles to target specific subsets of immune cells within the TME. T cells are one of the most common types of immune cell found within the TME, and higher cytotoxic T cell infiltration is often correlated with better survival [39], while higher numbers of Tregs may be associated with worse prognoses [40]. Given these observations, T cells are a natural target when remodeling the TME. One group used polyethylene glycol (PEG) micelles to carry the natural polyphenol, curcumin, to tumor sites in a murine melanoma model. The curcumin-PEG micelles increased CD8⁺ T cell cytotoxicity and decreased numbers of Tregs in vivo. These results were further augmented when combined with a lipid-based vaccine containing tumor associated antigens (TAA)s and Toll-like receptor (TLR) agonist 9. Curcumin is poorly soluble, but its bioavailability has been improved by nanoparticle platforms, including liposomes [41], poly(lactic-co-glycolic acid) nanoparticles [42], and NIPAAM-based nanoparticles [43]. Other groups have focused on targeting Tregs with anti-CD4 antibodies. Anti-CD4 therapy is in clinical trials and inhibits Treg’s immunosuppression, but the high doses required often lead to unwanted side effects [44]. By utilizing a nanoporous silica delivery system, one group hypothesized that therapeutic antibodies such as anti-CD4 can be slowly and locally released into the TME [45].

NK cells have the ability to eradicate tumors, but often are not abundant in the TME or are suppressed. Nanoparticle-mediated enhancement of NK function and infiltration into the tumor is a less common but promising antitumor strategy. Mica has been shown to possess inherent immunostimulatory properties [46]. Inspired by these findings, one group used mica nanoparticles to both increase the susceptibility of cancer cells to NK cells and to increase the numbers and cytotoxicity of NK cells in a murine breast cancer model [47]. A common strategy to induce TAA-dependent cytotoxicity is to pulse DCs with epitopes from the desired antigens and to inject the DCs as a cancer vaccine, inducing strong immune responses against the TAA-bearing cells. Some groups have targeted DCs with nanoparticles in order to improve their maturation and antigen presenting abilities. TLRs can stimulate DCs, but are hydrophobic and have short half-lives [48]. Dominguez et al., optimized their cancer vaccine by covalently conjugating antibodies against RNUA, a TAA, and against CD40, a surface marker expressed by DCs, to PLA nanoparticles. The particles trafficked to RNEUT tumors and then drew DCs into proximity of the tumor with the anti-CD40 antibodies. The nanoparticles induced formation of DC-tumor cell conjugates and rejected 100% of the tumor challenges [49].

Some of these nanoparticles have immunostimulatory properties just based on their materials, while others triggered an immune response due to their cargo. These studies highlight just a few of the variety of nanoparticle-based therapies aimed at inhibiting suppressor immune cells or augmenting the responses of antitumor immune cells.

2.3.3. Improvement of immune responses through targeting lymph nodes

While many of the aforementioned nanoparticle systems either circulate in the bloodstream or are targeted to accumulate in the tumor, targeting the lymph nodes is another important area in which nanoparticles can have a great impact. Irvine and colleagues have published several papers on polymer nanoparticles which traffic to lymph nodes. For cancer vaccines to have greater therapeutic effects, vaccine adjuvants must accumulate in lymph nodes, where naïve B and T cells are primed. CpG is a DNA motif which binds TLR9 and has the potential to be a potent immunostimulant; however, free CpG does not accumulate in lymph nodes. Irvine et al., conjugated CpG to a lipophilic albumin-binding domain and demonstrated that these nanoparticle-based subunit vaccines traffic to lymph nodes via albumin hitchhiking. One week after injection, albumin-binding CpG-liposomes accumulation was six times greater than that of soluble CpG in lymph nodes, and this platform also led to sustained tumor regression in murine melanoma models [50].
Direct injection of vaccines into the lymph nodes increases their potency, but rapid vaccine clearance remains problematic. Irvine and coworkers combined nanoparticle-based vaccines with intralymph node injections to circumvent this issue. Combining intralymph node vaccination techniques with a PLGA micro- or nanoparticle-conjugated TLR3 agonist increased accumulation in lymph nodes, boosted T cell cytokine production, and resulted in more persistent DC activation in immunized mice.

In contrast, Swartz and colleagues intradermally injected lymph node-targeting nanoparticle-conjugated TAA and adjuvant in tumor-bearing mice. Despite the different route of delivery, these nanoparticles efficiently accumulated in the lymph nodes. Furthermore, the vaccine had greater therapeutic effects when conjugated to nanoparticles and when injected into the TAA-primed tumor-draining lymph node. The immunosuppressive environment of the tumor-draining lymph nodes was reversed to a more immunogenic environment after vaccine administration [51]. Swartz et al., also delivered hydrophobic DC stimulatory agents including TLR9 agonist and paclitaxel, a TLR4 agonist, using pyridyl disulfide nanoparticles targeted to the tumor-draining lymph nodes. They observed increased DC maturation, greater IL-12 production, and slowed tumor growth using this delivery system [52].

These studies indicate that functionalized nanoparticles have the ability to deliver cancer vaccines adjuvants to lymph nodes and augment immune responses utilizing a variety of routes of delivery. Utilizing nanoparticles can increase circulation time of the adjuvants due to the larger size of the complex, and functionalized particles can actively target crucial areas such as the lymph nodes.

2.3.4. Targeting of tumor associated macrophages

Macrophages are involved in both innate and adaptive immunity and can be pro- or anti-tumorigenic, depending on the signals they receive from the surrounding environment. In the TME, pro-tumorigenic M2 macrophages, or TAMs, suppress cancer immunosurveillance and also promote vascularization. Nanoparticle-based therapies can either neutralize or kill off TAMs entirely or polarize TAMs to a more antitumor M1 phenotype.

One of the challenges with killing TAMs is to reduce the TAM population while leaving the anti-tumorigenic M1 population intact. Zhu and colleagues created a delivery system that was preferentially taken up by TAMs. The desired cargo can be loaded into mannose-modified PLGA nanoparticles and shielded by pH-sensitive PEG, which are shed in the acidic TME to release the drugs to TAMs which overexpress mannose receptors [53]. Another group reported that PEGylated cowpea mosaic virus nanoparticles (composed of viral capsid proteins but no viral nucleic acids) were internalized by TAMs but not M1 macrophages. They suggested that these nanoparticles can be loaded with cytotoxic agents that target the TAM population only, with the M1 population rising as the TAMs decrease [54].

Rather than ablating or inhibiting TAMs, small interfering RNA (siRNA) technology has been used in recent studies to change the TAM phenotype. One group demonstrated the ability of their nanoparticle system to deliver siRNA to TAMs. They developed a triblock polymer nanoparticle composed of a pH responsive block surrounded by a protective shell; this system was functionalized with a TAM-targeting mannose ligand and was able to deliver siRNA effectively in vivo [55]. Zhang et al., used siRNA targeting macrophage migration inhibitory factor (MIF), which is upregulated in TAMs. Glucan-based nanoparticles loaded with anti-MIF siRNA were injected into tumor-bearing mice and significantly reduced tumor growth and metastasis while polarizing TAMs to a more immunogenic phenotype. After treatment, the TAMs expressed fewer M2 markers and more pro-inflammatory cytokines such as TNFα and IL-2, which subsequently enhanced T cell infiltration and function at the tumor site [56]. This may be a more effective method, as TAMs are converted into cells with an antitumor response instead of simply being eliminated.

2.4. Enhancing antitumor effects with combination therapies

Perhaps a more successful approach to anticancer therapy would be to target a combination of the vascular, ECM, and immune cells within the TME, as well as the actual tumor cells. Within the last year, Liu and coworkers published a study in which liposomes were used to encapsulate anti-VEGF agents and were decorated with a CXCR4 antagonist to target both angiogenic and immune responses in a hepatocellular carcinoma model. CXCR4 is overexpressed in both tumor and immune cells within the TME and served both as a targeting ligand and a method to modulate the immune response. They first tested these CXCR4-targeting liposomes with sorafenib, a clinically approved anti-VEGF small molecule drug, and found that combination therapy was more effective than either treatment alone. They then replaced sorafenib with anti-VEGF siRNA, which when combined with the targeting liposomes, reduced the mean vessel density and inhibited tumor growth as well as prevented TAM infiltration into the tumor site [57].

Combination therapy can also target both the TME and cancer cells themselves. Another group very recently reported a multipart nanocarrier that can deliver multiple anticancer agents and assemble into “drug-delivery depots” within the TME. Their pH-sensitive carrier contained HA, which traffics to tumor sites that overexpress the HA receptor CD44 as well as hyaluronidase (HAase). When near the tumor site, HAase cleaves HA, which triggers crosslinking of other components of the nanocarrier, forming depots which are gradually degraded by the acidity of the TME. When loaded with TNF-related apoptosis-inducing ligand and the anti-angiogenic drug cilenitide, these carriers home to the tumor site, aggregated into depots, provided a sustained release of their cargo, decreased tumor vascularization, and significantly slowed tumor growth with no toxic side effects. In addition, they suggested that this nanoparticle platform also can be used to carry a variety of cargo such as anticancer small molecule drugs and immune modulating agents [58].
Finally, Jiao and coworkers used combination therapy in cancer theranostics, in which a diagnostic agent also has therapeutic benefits. They conjugated gold nanoparticles to a chimeric tumor-targeting antibody, anti-GD2, which had been modified to enhance NK cell function through interaction with the Fc receptor. The nanoparticles successfully trafficked to GD2-expressing cancer cells and enhanced computerized tomography contrast such that even small tumors were visible on diagnostic scans. The Fc regions of the antibodies bound to the Fc receptors of on NK cell surfaces and stimulated an immune response against the tumor cells. Interestingly, the antibodies had a greater effect on NK activation when conjugated to the nanoparticles than when used alone, possibly due to the configuration of multiple antibodies bound to each nanoparticle [59]. Thus, combination therapy can affect multiple TME aspects, target tumor cells and the TME, or provide therapeutic effects as well as diagnostics.

2.5. Transporting antitumor agents using cell-based nanotherapies

Cells can be used as carriers for nanoparticles that are either directly cytotoxic to the tumor and its surrounding environment or nanoparticles which carry chemotherapeutic agents. In most of the current cell-based nanotherapies, the cell serves as a carrier for nanoparticles and their cargo. In 2006, Lee and coworkers coincubated T cells with doxorubicin-loaded magnetic nanoparticles and induced particle uptake via electroporation. Although over half of the T cells died from doxorubicin exposure within 15 h [2], other studies built upon this concept of using cells to deliver nanoparticle-bound cargo [60,61].

Nanoparticles can be conjugated without a payload and still have antitumor potential. In 2007, another group transfected T cells with boron carbone nanoparticles and hypothesized that the loaded cells could traffic to tumor sites, upon which the boron nanoparticles would be irradiated and induce tumor cell death [62]. One group conjugated iron-based nanoparticles to NK cells and then injected them into tumor-bearing mice. By placing an external magnet near the tumor site, they were able to manipulate the movement of the NK cells and increase their infiltration into the tumor 17-fold [63]. Gold nanoparticle-mediated photothermal therapy is being evaluated in clinical trials, but relies on passive accumulation in leaky tumor vessels, which is insufficient in poorly vascularized tumors. One group demonstrated that monocytes and macrophages can endocytose gold nanoparticles and migrate to tumor sites. Once infiltrated into breast cancer spheroids, the nanoparticles were heated with near infrared light and induced photoablation of tumor cells in vitro [64]. A few years later, another group used T cell carriers to enhance gold nanoparticle delivery in vivo, reporting a four-fold increase in accumulation at the tumor site [65]. In these cases, the nanoparticles themselves assist in killing cancerous cells without the use of a chemotherapeutic drug.

2.6. Utilizing nanoparticles to augment immune therapies

Adaptive T cell transfer has been explored as a means to eradicate solid tumors, but clinical results have been underwhelming due to limited T cell expansion within the immunosuppressive TME. Stephan et al., used a bioengineered alginate matrix to harbor T cells and embedded silica particles containing IL-15 superagonist and anti-CD3 to stimulate T cell expansion within the matrix. The T cells migrated along collagen fibers out of the matrix, and the stimulatory cytokines and antibodies increased T cell proliferation 22-fold. When implanted near the tumor site in a murine breast cancer model, this system delivered localized immunotherapy and resulted in tumor regression and increased survival due to greater expansion and infiltration of T cells. This platform can be modified to deliver other immune cells with antitumor properties or adjuvants to stimulate an immune response [66], and demonstrates how bio-active materials can be used to deliver cells which can modulate the TME.

Another group applied cell-based drug delivery to immune adjuvants. Irvine et al., described a method of conjugating PEGylated, maleimide-functionalized liposomes to free thiols found on T cell surfaces. They were able to load T cells with liposomes containing IL-15 and IL-21 and demonstrated that these cytokines mimicked autocrine release kinetics and resulted in a 10-fold increase in T cell expansion when compared to the same nanoparticles systemically infused instead of directly conjugated to T cells [60]. In this way, T cells were sustained by cytokines from nanoparticles attached to their cell surfaces instead of relying on systemic cytokine doses.

3. Conclusion

Solid tumors are highly heterogeneous and grow in a complex microenvironment consisting of the extracellular matrix components, fibroblasts, vasculature and immune cells. It is crucial to develop nanoparticles that can adapt the TME and improve the selective targeting of anti-cancer drugs to tumors [5,9,25,26]. The sensitivity of tumors to chemotherapy is highly influenced by the surrounding tumor vasculature. Vascular development is critical for tumor and growth progression and furthermore, anticancer drugs gain access to tumors via blood vessels, making vasculature an attractive target for improving cancer therapy. Recent advances have been made to employ nanotechnology in tumor vasculature-targeted drug delivery, enhancing the therapeutic efficacy of several anticancer drugs. In addition, several studies have shown a tumor vasculature targeted delivery system for integrated combination therapy of vascular disrupting or anti-angiogenic agents and chemotherapeutic agents, resulting in more effective and less toxic anticancer therapies.

One of major structural components of the TME is the ECM, with the most common constituents being collagen and hyaluronic acid. Altered ECM processing and accumulation of ECM with an abnormal composition play important roles in...
tumor progression, invasion and metastasis [8,14,67]. To suppress cancer growth and increase therapeutic efficacy, cancer nanotherapeutics have been developed to deliver drugs that target the altered physical properties of the ECM.

Nanoparticles can affect immune cells and their responses within the TME. Cytokines encapsulated within various nanoscale carriers provide more sustained release and reduced toxicity while being systemically delivered to induce general immune stimulation. Alternatively, nanomedicine can be functionalized to enhance specific subpopulations of immune cells, such as T cells, NK cells, and DCs. Nanoparticles can carry cytotoxic agents or siRNA to either kill or modify TAMs, respectively.

Combination therapy may be more effective than single agents. Multiple TME components, such as immune cells and vasculature, can be targeted using a nanoparticle carrier, or both TME and cancer cells can be targeted at once. These strategies often have been shown to work synergistically.

Combination therapy can also involve combining therapeutics and diagnostics into one nanoparticle system. Combination therapy is in its early stages and seems promising, but even with highly functionalized nanoparticles, delivery is still often reliant on the EPR effect, which is not always sufficient for delivery into poorly perfused tumors.

Using immune cells as chaperones for drugs that target tumor cells and the TME may increase accumulation in otherwise inaccessible tumor sites. Most often, T cells are used to carry either cytotoxic or immune-boosting agents to tumors, but other immune cells can potentially be used as “Trojan horses” as well. In other disease models, such as Alzheimer's disease [68] and HIV [69], macrophages were loaded with nanoparticles containing therapeutic agents and were able to cross the blood brain barrier to deliver the cargo. Macrophages may also prove to be useful in delivering loaded nanoparticles to typically inaccessible tumor sites, including those within the brain. Many different cell types can be used as “pharmacists” to deliver nanoparticle-conjugated small molecule drugs, cytokines, siRNA, and myriad other agents in order to overcome effects of the TME. Cell-based therapies may prove to be the most effective anti-cancer treatment and have shown promise in overcoming many hurdles presented by the TME.

Q2 Conflict of interest statement

None.

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References


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