Cancer chemoresistance and therapeutic strategies targeting tumor microenvironment

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ABSTRACT: Anticancer drug resistance, also known as a chemoresistance, continues to be the greatest challenge in cancer therapies. Chemoresistance is acquired during cancer treatment due to several mechanisms such as genetic alterations in the drug target, drug inactivation, and increased drug efflux from cancer cells. Therefore, a cure for cancer is challenging for most patients. The current focus on cancer research needs to be re-evaluated to resolve this issue. In recent years, many efforts have been devoted to understanding the interactions between malignant and non-malignant cells in the tumor microenvironment (TME) during cancer progression and treatment. Several TME-targeted therapeutic strategies have been developed and utilized in clinical applications due to an increased recognition of TME as a key driver of tumor progression and chemoresistance. An additional challenge to effective cancer therapy is targeted delivery of therapeutic agents to the tumor site without harming surrounding healthy tissues and organs. The therapeutic efficacy of anticancer drugs is significantly hindered by non-specific biodistribution and low bioavailability of the drugs at tumor sites. In this regard, nanomedicines, including nanoparticle-based drug delivery systems, have been employed to improve the safety and efficacy of cancer therapeutics via TME targeting. This review provides a summary of the cellular components of TME, their roles in cancer progression and chemoresistance, and various TME-targeted therapeutic strategies and clinical trials.

KEYWORDS: cancer, chemoresistance, tumor microenvironment, nanomedicine

INTRODUCTION

Cancer is a leading cause of death worldwide. In 2020, an estimated 1.8 million new cancer cases and 606,520 cancer deaths are expected in the United States. The cancer incidence is projected to increase by 45% from 2010 to 2030 [1]. To cope with this surge, a wide variety of cancer treatment strategies, including surgery, radiation therapy, chemotherapy, and more recently targeted therapy and immunotherapy, have been used to keep cancer under control. However, chemotherapy remains the most common type of cancer treatments. And while cancer chemotherapy is expected to have high efficacy, approximately 97% of new anticancer drugs fail in clinical trials due to lack of efficacy and/or safety issues [2].

Chemoresistance is one of the leading causes of cancer treatment failure and is responsible for most of cancer-related deaths. Chemoresistance is a complex phenomenon caused by several host and tumor-related factors such as drug target mutations, genetic and epigenetic alterations, DNA damage repair, cell death inhibition, and drug inactivation [3]. There remains an urgent need to focus on developing new therapies to reduce the failure in clinical trials. Tumors have been traditionally believed to be clonal. Intratumoral genetic heterogeneity is observed across many cancers and is recognized as a key contributor to chemotherapy resistance and poor prognosis in cancer patients [4]. Therefore, a deeper understanding of the mechanisms underlying tumor heterogeneity and chemoresistance is critically needed.

Recent studies have begun to focus on targeting the tumor microenvironment (TME) as a novel treatment approach since the traditional approach of targeting tumor cells alone has not achieved a successful outcome. The physiochemical characteristics (e.g., hypoxia and low pH) of TME and its heterogeneous cell population, including stromal cells, blood vessel cells, and immune cells are potential targets for novel anticancer therapies. The intent of this review is to summarize existing knowledge...
on the TME, its composition and key cellular constituents, as well as their roles and potential interventions in tumor progression and chemoresistance. Additionally, we will discuss different mechanisms of chemoresistance in order to provide some understanding of the possible causes of chemotherapy failure and tumor relapse. We will also review current therapeutic strategies and nanoparticle-based drug targeting strategies to overcome these limitations.

COMPOSITION OF THE TUMOR MICROENVIRONMENT

Tumor is a highly complex tissue encircled by extracellular matrix (ECM) and stromal cells [5]. The complex functional ecosystem in which cellular and non-cellular components reside and interact is defined as the TME. It comprises of proliferating tumor cells, stromal cells (e.g., cancer-associated fibroblasts, blood vessel cells, lymphatic endothelial cells), infiltrating inflammatory cells, and a variety of myeloid cells such as dendritic cells and macrophages. The major cellular components of TME are summarized in Fig. 1. Increasing evidence indicates the essential roles of these TME cells in tumor initiation, progression and metastasis [5]. The following sections will summarize key cellular players of the TME, including cancer-associated fibroblasts, inflammatory cells, blood and ECM that are involved in tumor progression and chemoresistance.

Cancer-associated fibroblasts

Cancer-associated fibroblasts (CAFs), the most dominant component of TME, are a subpopulation of fibroblasts having a similar phenotype as myofibroblasts. The spindle-shaped CAFs play a vital role in remodeling ECM of TME. However, the origin, subtypes and functions of CAFs are not fully understood due to their heterogeneity and absence of specific markers [6]. The potential origins of CAFs are normal fibroblasts, mesenchymal cells, endothelial cells, and epithelial cells. Several studies have been carried out to identify CAF markers, including α-smooth muscle actin (α-SMA), fibroblast-activated protein-a (FAP-a), podoplanin (PDPN), desmin, integrin β, and neuron glial antigen (NG2) [7, 8]. CAFs produce more collagen and other ECM proteins as well as pro-tumor factors than normal fibroblasts. In wound repair, myofibroblasts are transiently activated; they either undergo apoptosis or revert to the original fibroblast phenotype. Unlike the normal process of wound healing, CAFs present at the tumor site are perpetually activated, resulting in growth and invasion of the tumor cells. A number of studies have revealed that tumor initiation and growth depend on the activation of CAFs [8, 9]. For example, activation of CAFs by carbon nanomaterials was shown by our group to promote tumor growth caused by existing lung tumor cells and nanomaterial-induced neoplastic cells [8]. Inhibition of CAFs by genetic ablation of PDPN, a CAF biomarker, was also shown to abrogate CAF-induced tumor promotion and reduce cancer stem cell (CSC) population in the tumor mass [8]. Since CSCs are known to be a key driver of tumor metastasis and chemoresistance [10], these results suggest that CAFs may have a broader role in tumor progression and chemotherapy resistance than originally thought. The results also suggest that CAFs and specifically PDPN could be a potential therapeutic target for cancer treatment.

Immune cells

The immune system plays a critical role in defending the body against invading or infectious agents. However, the composition of immune cells and their abundance vary significantly with disease progression and prognostic factors [11]. Immune cells from both innate and adaptive immune systems are recruited to the TME. Helper T cells (CD4+) and cytotoxic T cells (CD8+) are two major types of T cells [12]. Antigen-experienced (CD8+) T cells and memory (CD45RO+) T cells are excellent prognostic indicators due to their ability to kill tumor cells selectively. Interleukin-2 (IL-2), interferon-γ (IFN-γ), and tumor necrosis factor-β (TNF-β) are pro-inflammatory cytokines that are produced by CD4+ T helper 1 (Th1) cells. The high density of these cells in the TME correlates with a good prognosis [13].

B cells infiltrating the tumor tissues, also known as tumor-infiltrating B (TIB) cells, are mostly found in tertiary lymphoid tissues adjacent to the TME. However, the importance of TIB cells in regulating anti-tumor immunity and tumor progression is still a matter of debate. TIB cells are generally thought to promote anti-tumor immune responses. It has now been suggested that TIB cells are associated with improved clinical outcomes and survival in HER2+ and triple-negative breast cancer patients [14].

Vascular cells

Tumors are surrounded by a complex vascular network to provide nutrients and oxygen, and to evacuate carbon dioxide and metabolic waste from tumor cells to maintain sustainable growth and sur-
Tumor vascular network is irregularly distributed in the TME and inefficient in many ways, i.e., it is fragile, tortuous, and extremely leaky/permeable. Also, the angiogenic switch remains almost always turned on during tumor progression, resulting in tumor promoting vasculature. A variety of pro- and anti-angiogenic growth factors secreted by stromal cells and myeloid cells initiate tumor vascularization by accessing the blood vessels from surrounding stroma.

Vascular endothelial growth factor (VEGF), also known as vascular permeability factor (VPF), is a potent angiogenic factor that promotes neovascularization. An overexpression of VEGF is observed in a variety of cancers, including breast, lung, kidney, bladder and ovarian cancer. In addition to VEGF, platelet-derived growth factor (PDGF), transforming growth factor (TGF), fibroblast growth factor (FGF), and tumor necrosis factor (TNF) are some of the pro-angiogenic factors associated with tumor angiogenesis. In particular, FGF signaling which is mediated by two major oncogenic pathways, phosphatidylinositol 3-kinase/Akt (PI3K/Akt) and mitogen-activated protein kinases (MAPK), promotes angiogenesis.

**Tumor-associated macrophages**

Tumor-associated macrophages (TAMs) are often the principal constituent of TME in solid tumors and account for up to 50% of tumor mass. TAMs perform diverse functions in tumor growth, immune regulation, tumor angiogenesis, metastasis and chemoresistance. Macrophages are broadly categorized as M1 (classical-activated macrophages) and M2 (alternative-activated macrophages) depending on their phenotype. Both phenotypes play contrasting roles in tumor pathogenesis and evolution. M1 macrophages play an anti-tumorigenic role, while M2 macrophages are pro-tumorigenic. An increasing number of studies have revealed that TAMs exhibit the M2 phenotype. M2 macrophages are characterized by secretion of anti-inflammatory cytokines such as IL-4, IL-10 and IL-13, which play a critical role in tissue repair and tumor progression.

**MECHANISMS OF CHEMORESISTANCE**

Chemotherapy is a type of cancer treatment that utilizes chemical drugs to kill rapidly growing cancer cells. However, chemoresistance, the insensitivity of cancer cells to the drugs, develops and is the leading...
cause of failure in cancer chemotherapy. The underlying mechanisms of cancer chemoresistance are incompletely understood [21]. Hence, understanding the relationship between cancer progression and chemoresistance in response to chemotherapy is essential to successful development of novel anticancer therapeutics. Drug resistance can be intrinsic (pre-existent) or acquired (drug-induced). Intrinsic resistance indicates the lack of tumor response to initial therapy due to pre-existent resistance in cancer cells. Mutational changes in cancer cells during chemotherapy make them insensitive to the drugs that were once effective. There are many mechanisms that contribute to chemoresistance, including alterations in drug efflux, DNA damage repair, epithelial-mesenchymal transition (EMT), cell death inhibition, drug target alteration, and drug activation (Fig. 2). Table 1 summarizes some known chemoresistance mechanisms of commonly used anticancer drugs.

**Drug efflux mechanisms**

Cancer cells achieve chemoresistance by activating ATP binding cassette (ABC) transporters, also known as drug efflux mechanisms. These are the most abundant transmembrane proteins which can shuttle drugs, metabolic products, and foreign substances through the cell membrane using energy derived from ATP hydrolysis [32]. An overexpression of specific ABC transporter proteins is often associated with chemoresistance in various solid tumors.

Three major types of ABC proteins involved in chemoresistance have been described, including P-glycoprotein (ABCB1)/multidrug resistance protein 1 (MDR1), multidrug resistance-associated protein 1 (MRP1/ABCC1), and breast cancer resistance protein (BCRP/ABCG2) [48]. BCRP is operated as an efflux pump for small molecule drugs. BCRP-associated resistance has been observed in breast cancer and leukemia [49]. MDR1 is associated with drug resistance in the kidney, liver, breast, lung, colon, and blood cancer. MRPI is identified as a biomarker for the prediction of chemoresistance. The common anticancer drugs that are known to increase the expression of efflux pumps in cancer cells are cisplatin, doxorubicin, etoposide, estramustine, and vinblastine [50].

**Epithelial-mesenchymal transition**

Epithelial-mesenchymal transition (EMT) is a process in which cells can transition from an epithelial phenotype to a mesenchymal phenotype. Epithelial cells are characterized by tight cell-cell junctions and cell polarity. The cellular phenotype changes in epithelial cells facilitate the fibroblast-like morphology resulting in increased cell migration and invasion, and resistance to apoptosis [51]. EMT also plays a role in cancer metastasis and is associated with CSC phenotype. Mani and coworkers showed that human mammary epithelial cells that have undergone EMT exhibited similar...
Fig. 2 Mechanisms of chemoresistance in cancer cells.

### Table 1 Mechanisms of chemoresistance in cancer therapy

<table>
<thead>
<tr>
<th>Chemoresistance mechanism</th>
<th>Targeted therapy</th>
<th>Tumor type</th>
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<td>Drug efflux</td>
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<td>ABCC10</td>
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<td>Ovarian cancer</td>
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<td>DNA damage repair</td>
<td>Mismatch repair (MMR), Nucleotide excision repair (NER), Double-strand break repair (DSB)</td>
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<td>Lung cancer</td>
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<td></td>
<td>Double-strand break repair (DSB) by ERCC1</td>
<td>Cisplatin</td>
<td>Non-small cell lung cancer</td>
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<tr>
<td>EMT</td>
<td>Increased vimentin expression, Decreased desmosome and tight junction formation</td>
<td>Adriamycin</td>
<td>Breast cancer</td>
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<td>Expression of a transcriptional repressor of E-cadherin (Zeb-1), Expression of other epithelial markers (EVA1, MAL2)</td>
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<td>Mutation and amplification of BCR-ABL1 gene</td>
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<td>Drug inactivation</td>
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<td></td>
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<td>Epigenetics</td>
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<td>Hypermethylation of MGMT promoter</td>
<td>Fotedustine</td>
<td>Melanoma</td>
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traits to CSCs derived from normal and neoplastic mammary stem-like cells [52]. In lung cancer cells, our group showed that EMT is functionally linked to CSCs as downregulation of E-cadherin, an EMT marker, promoted EMT and increased stemness of the cells [53]. Such downregulation also promoted cell death resistance and invasiveness of the cells [53]. The mechanism by which EMT regulates CSCs in lung cancer cells was shown to involve mesothelin (MSLN) activation [54]. MSLN is a tumor-associated antigen that is overexpressed on various malignant tumor cells. Knockdown of MSLN was found to reverse EMT and attenuate stemness of the cells in addition to inhibiting tumor growth and metastasis [54]. EMT activation is also associated with chemotherapy resistance allowing epithelial cells to escape from drug treatment and various endogeneous and exogenous cellular stresses, including hypoxia, nutrient depletion, and mechanical constraint [55]. Many studies have shown a strong relationship between EMT phenotype and chemotherapy resistance. For example, doxorubicin resistance is developed in breast cancer cells undergoing EMT [56].

**DNA repair mechanisms**

Most chemotherapeutic agents kill tumor cells by damaging their DNA, either directly or indirectly. Cells have multiple repair mechanisms to deal with the damaging effect, including ROS scavenging, ataxia telangiectasia mutated (ATM) kinase and checkpoint kinase 1/2 (Chk1/Chk2) activation, and anti-apoptotic signaling via Wnt/β-catenin, Notch, and PI3K/Akt pathways [8]. It is crucial to maintain the steady intracellular levels of ROS under normal physiological conditions in tumor cells in order to promote cell growth and discrete cell death mechanisms. Excessive ROS production can lead to oxidative stress, which is defined as a condition where the rate of ROS production exceeds the capability of antioxidant defense mechanisms [60]. Elevated ROS levels can damage biomolecules, including DNA, proteins and lipids, resulting in cancer cell senescence and apoptosis. The existence of enhanced adaptive stress strategies selectively protects cancer cells from ROS-mediated DNA damage leading to chemoresistance [61].

**Drug target alterations**

The efficacy of an anticancer drug is dependent on its molecular target and an alteration of such target through gene mutation or dysregulated expression, leading to drug resistance. For example, topoisomerase II (Top2) disentangles strands of DNA helix to inhibit DNA entanglement and supercoiling. Top2 mutations lead to cancer cell resistance against Top2 inhibitors such as etoposide and doxorubicin [62]. Certain mutations in protein kinases are also a major cause of drug resistance. Epidermal growth factor receptor (EGFR) inhibitors such as gefitinib and erlotinib are commonly used in targeted therapy of non-small-cell lung cancer (NSCLC) to inhibit EGFR’s kinase activity. EGFR mutation (in-frame deletion in exon 19) leads to acquired resistance to EGFR inhibitors [63]. An overexpression of human epidermal growth factor receptor 2 (HER2) has been reported in 20–30% of breast cancer patients. Trastuzumab, an anti-HER2 monoclonal antibody, is commonly used in breast cancer patients to inhibit cancer cell growth. Several breast cancer patients were reported to have tumor progression despite trastuzumab therapy due to drug resistance caused by HER2 amplification [64].

**Epigenetic alterations**

Epigenetic alterations have been recognized as an important factor in chemoresistance. Epigenetic
mechanisms, mainly DNA methylation and histone modification, play a crucial role in regulating gene expression and cell differentiation [65]. Cytosine in CpG islands of DNA is frequently methylated to 5-methylcytosine (5mC) by DNA methyltransferases. Hypermethylation of CpG islands causes genomic instability leading to tumor suppressor gene silencing. Hypermethylation of secreted frizzled-related protein 5 (SFRP5) gene that acts as a Wnt antagonist in ovarian cancer has been shown to be associated with platinum resistance [66]. Histone modifications, including acetylation and deacetylation, take place on the lysine residues located at the N-terminal of histones and non-histone proteins. Histone acetyltransferases (HATs) and histone deacetylases (HDACs) are the two opposing classes of enzymes that catalyze the aforementioned reactions, respectively [67].

**THERAPEUTIC STRATEGIES TARGETING TUMOR MICROENVIRONMENT**

The efficacy of anticancer drugs is limited by several factors including inefficient distribution of drugs to target tissues or organs, limited drug penetration into tumor mass, and drug degradation. Nanomedicine is emerging as a promising therapeutic platform that utilizes nano-sized drug delivery carriers to increase the therapeutic index of chemotherapeutic agents by improving their delivery to the tumor site [68]. However, TME is structurally complex and highly disorganized with an uneven distribution of blood vessels that limits the distribution of nanoparticles within the TME [69]. There is a need to improve the efficiency of drug delivery and design more effective drug delivery systems to overcome such barriers created by the solid tumors. Therefore, the unique characteristics of TME, such as hypoxia, acidic pH, high enzymatic activity, and specific TME markers should be taken into consideration when designing new drug delivery systems for cancer treatment (Fig. 3).

**Acidic tumor microenvironment**

The acidic environment of TME (pH 6–7 range) has been widely utilized to enhance drug release from nanoparticles by triggering pH-dependent structural transformation of the nanoparticles. Nanoparticles are generally stable at the extracellular pH (∼7.4) of normal tissues and blood, whereas they are gradually dissociated at the extracellular pH (6.0–7.0) of solid tumors due to protonation of pH-sensitive moieties present on them. Acid-sensitive linkers or ionizable groups are commonly used pH-sensitive moieties for TME drug delivery and targeting [70]. Acid-soluble inorganic nanoparticles have increasingly been used in many cancer chemotherapy applications because of their tunable physicochemical properties. Dong and co-workers developed mono-dispersed calcium carbonate nanoparticles modified with polyethylene glycol (PEG) to incorporate Mn²⁺-chelated chlorin e6 (Ce6(Mn)) and doxorubicin (DOX) for cancer therapy [71]. This delivery system is rapidly dissociated at a mild acidic environment (pH 6.5–5.5) and effectively releases both DOX and Ce6(Mn) at the tumor site.

**Hypoxia**

It has been shown that tumor hypoxia plays a significant role in tumorigenesis and drug resistance. Tumor hypoxia, also known as a deprivation of oxygen in tumor cells, is proven to be a pivotal driver of neovascularization and EMT, which facilitate rapid tumor growth [72]. Hypoxic tumor cells are located at a distance from blood vessels, making them less responsive to chemotherapeutic agents [73]. As a result, hypoxic cells are substantially more resistant to chemotherapy or radiotherapy. Increasing research efforts have been devoted to improving tumor oxygenation. Yang’s group reported pH-responsive catalase (CAT) and Ce6 hollow silica nanoparticles capable of light-triggered ROS generation [74]. CAT-loaded nanoparticles were rapidly degraded in the acidic environment of TME and induced tissue oxygenation by converting H₂O₂ into H₂O and O₂ via CAT-mediated enzymatic reaction.

**Targeting neoplastic vs. TME cells**

Over the past decades, cancers have been mistakenly identified as a mono-cellular disorder, and most approved anticancer drugs target neoplastic cells [75]. Limitation of such approach and an increasing recognition of the role of TME in cancer progression and chemoresistance have led to a new focus on TME cells, such as CAFs and TAMs. Nanoparticles have been used to overcome the penetration limitation by selectively targeting CAF-associated proteins, such as α-SMA and FAP-α [76]. Cleavable amphiphilic peptide nanoparticles (CAP-NPs) were designed to be specifically responsive to FAP-α. Drug-loaded CAP-NPs rapidly disassembled upon cleavage of FAP-α that resulted in efficient drug release and deep tumor penetration [77].

Most macrophage-targeted therapies are mainly focused on limiting macrophage recruitment, depleting TAMs, initiating immune responses, and blocking TAM-mediated tumor promotion [78].
Receptor-mediated endocytosis is often used as a means to target TAMs for drug delivery. TAM surface markers, such as mannose receptor, folate receptor, galactose receptor, legumain, and CD163 receptor have been explored for macrophage-targeted therapies [65]. Huang and co-workers developed acid-sensitive, galactosylated cationic nanoparticles to deliver oligonucleotides (CpG, anti-IL-10, and anti-IL-10R) to suppress pro-tumor functions and initiate anti-tumor activities of TAMs. The results showed that the galactosylated nanoparticles were able to target TAMs by binding to galactosylated lectin moieties on the cell surface [79].

FINAL NOTE
Chemoresistance continues to be the leading cause of failure in cancer chemotherapy. Therefore, new knowledge is critically needed to improve our understanding of the drug resistance problem and identify new drug targets for more effective treatment of cancers. This review provides an overview of the composition of TME, its roles in tumor progression and chemoresistance, as well as its exploitation as therapeutic and drug delivery targets. In recent years, several therapeutic strategies including small molecule and antibody-based therapeutics have been developed to target the TME. It is likely that other therapeutic strategies such as siRNA, miRNA and other gene-based therapeutics will be developed for advanced cancer treatments. Such development will require effective drug delivery systems that target the TME. By exploiting the unique features of TME, i.e., hypoxia, acidic pH, and redox status, as well as specific surface markers of TME cells, such targeting is feasible and several nanoparticle-based drug delivery systems have been developed for such purpose. Thus, nanomedicine is promised to revolutionize the field of cancer therapy by improving drug targeting ability and minimizing chemoresistance and relapse.

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