Feature Review

CellPress

Special Issue: Cell Biology of Cancer

Microenvironmental regulation of therapeutic response in cancer

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The tumor microenvironment (TME) not only plays a pivotal role during cancer progression and metastasis but also has profound effects on therapeutic efficacy. In the case of microenvironment-mediated resistance this can involve an intrinsic response, including the co-option of pre-existing structural elements and signaling networks, or an acquired response of the tumor stroma following the therapeutic insult. Alternatively, in other contexts, the TME has a multifaceted ability to enhance therapeutic efficacy. This review examines recent advances in our understanding of the contribution of the TME during cancer therapy and discusses key concepts that may be amenable to therapeutic intervention.

The TME orchestrates tumorigenesis and malignant progression

While cancer was long considered a disease defined and driven by genomic instability, chromosomal alterations, and genetic mutations [1], the influence of nonmalignant, stromal cells of the TME is now widely appreciated [2,3]. Tumors are complex tissues comprising not only malignant cells but also genetically stable stromal cells [4], including endothelial cells, fibroblasts, and immune cells among many others (Figure 1), in addition to the extracellular matrix (ECM) they produce. As in healthy organs, the various cellular compartments of the microenvironment are not mere bystanders, but instead critically regulate tumorigenesis [5]. This extends not only to tumor initiation, malignant progression, and metastasis but importantly also to response to therapy. Moreover, the realization that distinct stromal cell types in different contexts can exhibit tumor-promoting or opposing tumoricidal capacities has further complicated our understanding of cancer biology. While the role of the TME during tumorigenesis has recently been reviewed in detail elsewhere [2,3], this review focuses on how the TME regulates therapeutic response, a field that has been rapidly expanding in recent years. As in the context of malignant progression, the TME exhibits a multifaceted ability to influence therapeutic outcome in either a positive or a negative manner. Harnessing this expanding knowledge to improve therapeutic response or even to develop new treatment options through normalization and re-education of the TME is increasingly within reach. The recent clinical success of immune checkpoint inhibitors serves as an illustrative example of this goal. A brief overview of the major components of the TME highlighted in Box 1 provides the necessary background to introduce the reader to the different concepts contributing to both TME-intrinsic and -acquired/-adaptive resistance with regard to traditional anticancer therapies, molecularly targeted therapies, and agents targeted against the TME itself, which are summarized in Box 2.

Therapeutic response is significantly influenced by the TME

Although an increasing number of cancers can be treated successfully if detected at an early stage, the presence of disseminated disease or recurrence of the primary tumor still confer a poor patient prognosis [6,7]. This is due in part to the current paucity of effective therapeutic options in this setting [8]. An initial response to treatment is often followed by disease progression, which, accompanied by a diminution of therapeutic options, ultimately leads to treatment failure and death from recurrent or metastatic disease [9]. Intriguingly, at least some of the traits that promote metastasis appear to be intertwined with resistance to chemotherapy [10–12]. In line with a tumor cell-centric view, this lack of a sustained treatment response has been largely attributed to either intrinsic or acquired therapeutic resistance of the malignant cells via a plethora of mechanisms including increased drug efflux, drug inactivation, altered DNA repair machinery, dysregulation or alteration of the drug targets, upregulation of growth factor and survival signaling, and evasion of apoptosis [8,13]. These mechanisms appear to be partially fueled by a pre-existing intratumoral heterogeneity that supports the outgrowth of resistant clones [14].

In addition to tumor cell-intrinsic mechanisms, an increasing number of examples of TME-mediated resistance have been reported, representing an alternative means to interfere with therapeutic efficacy. Early seminal work by Teicher *et al.* elegantly linked the development of resistance to chemotherapy *in vivo* to interactions with the host's normal tissues [15]. The discrepancy they observed between the *in vitro* efficacy of, and *in vivo* resistance to, a panel of various chemotherapies has subsequently been confirmed and extended by numerous studies [16,17], providing many examples of where TME-mediated resistance may be at play. However, there are also instances where

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Keywords: tumor microenvironment; stroma; therapeutic resistance; chemotherapy; radiotherapy; immunotherapy.

0962-8924/
 http://dx.doi.org/10.1016/j.tcb.2014.11.006

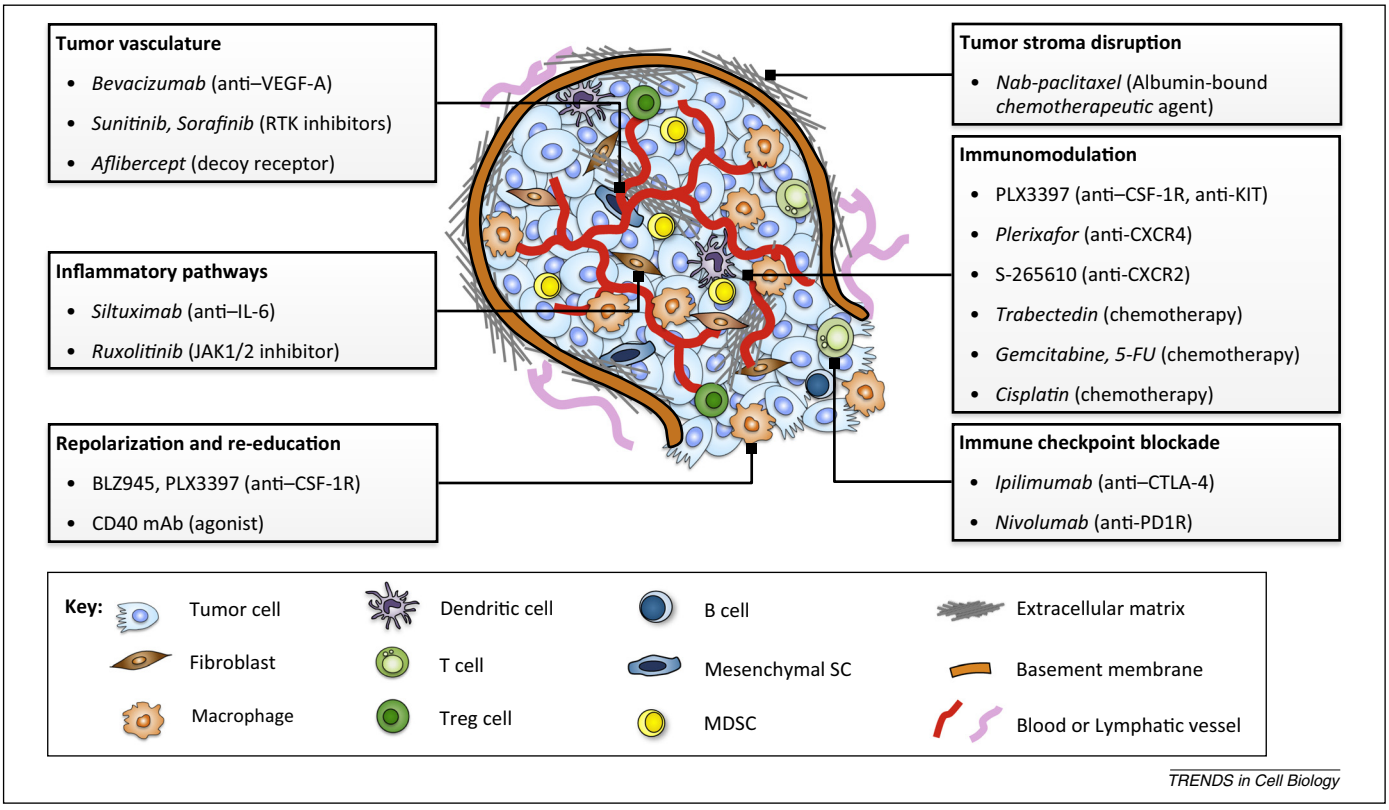

Figure 1. Major constituents of the tumor microenvironment (TME) and TME-targeted therapies. The TME comprises various cell types that modulate treatment response and are putative candidates for therapeutic intervention. The tumor vasculature can be targeted with various drugs, such as the vascular endothelial growth factor (VEGF)-A antibody bevacizumab, the multitarget receptor tyrosine kinase (RTK) inhibitors sunitinib and sorafenib, and the decoy VEGF receptor aflibercept [260]. Inflammatory pathway activation can be inhibited by the interleukin-6 (IL-6) antibody siltuximab [79] or the pan-JAK inhibitor ruxolitinib [166]. Cancer-associated fibroblasts are activated by multiple growth factors and cytokines within the TME and in turn acquire a proinflammatory phenotype and become a major source of soluble mediators that drive angiogenesis and enhance tumor cell survival. The immune cell compartment within the TME exhibits extraordinary plasticity: tumor-associated macrophages (TAMs) and myeloid derived suppressor cells (MDSCs) orchestrate an immunosuppressive and protective phenotype that extends to T cells, T regulatory (T_{reg}) cells and B cells. Repolarization or re-education of macrophages or other myeloid cells can be achieved by colony-stimulating factor 1 receptor (CSF-1R) inhibition (for example, BLZ945) [162] or agonistic CD40 antibodies that activate antigen-presenting cells (e.g., dendritic cells) to process and present tumor-associated antigens to local cytotoxic T lymphocytes [158,167]. This immune landscape within the tumor can be sculpted by inhibition of critical cytokine axes such as CSF-1R and/or KIT (PLX3397) [168], chemokine (C-X-C motif) receptor (CXCR) 4 (plerixafor), and CXCR2 (S-265610) [169]. The chemotherapeutic agent trabectedin has been proposed to selectively deplete monocytes and/or macrophages [170]. Both gemcitabine and 5-fluorourocil (5-FU) have been shown to deplete MDSCs [171,172]. Platinum-based cytostatic drugs can not only alter macrophage polarization but also induce increased antigen-presenting ability of dendritic cells. The blockade of immune checkpoints is another promising avenue of therapeutic intervention. This can be achieved through blockade of cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) (ipilimumab) or the programmed death 1 (PD1) receptor (nivolumab). Finally, several extracellular properties also shape the therapeutic response, such as high interstitial fluid pressure and changes in the composition of the extracellular matrix (ECM). Albumin-bound nab-paclitaxel has been postulated to disrupt the stromal composition [173]. FDA-approved drugs are indicated in italics while agents in preclinical or clinical trials are non-italicized.

radiotherapy (RT) and certain chemotherapies require an active immune cell response for optimal efficacy, as in the case of immunogenic cell death [18]. Interestingly, a simple quantification of the tumor-to-stroma ratio in breast and colon cancers predicts worse clinical outcome in patients undergoing adjuvant chemotherapy as an independent variable [19,20]. Furthermore, analysis of stromal gene expression in various cancers not only yielded tumor type-specific prognostic benefit [21,22] but also exhibited predictive value regarding response to neoadjuvant chemotherapy [23]. Thus, analysis of the TME could convey significant clinical information to aid in the evaluation of treatment options.

TME-mediated therapeutic resistance can be broadly separated into two types. Inherent or intrinsic resistance is present before drug or RT exposure and is therefore evident without any selective pressure. This type of resistance is based on the multitude of pre-existing reciprocal interactions between tumor cells and the surrounding TME. This is in contrast to tumor cell-intrinsic resistance, which is due to existing genetic alterations within the biochemical or molecular target [8]. Acquired TME resistance, by contrast, evolves in response to the effects of therapy and is defined by an adaptive host response to therapeutic perturbation. This can result in pronounced changes in the microenvironment and the emergence of new tumor–TME dialogs operating at the local and/or systemic level.

Ultimately, the protective effect of the TME on tumor cells can lead to persistent residual disease that further increases the risk of recurrence [17]. Therefore, deciphering this complex network and introducing targeted perturbations will be critical for improving therapeutic efficacy and ultimately patient survival. However, it is essential to emphasize that these effects are organ, context, and stage dependent, as the TME can also confer a beneficial effect on treatment response. This concept has been demonstrated both in drug screens that incorporate the tumor stroma [16] and in many studies revealing the importance of various immune cell types in modulating therapeutic efficacy (reviewed in [18]).

In the following sections we discuss intrinsic and acquired responses of the TME to traditional, cancer cell-targeted, and microenvironment-targeted therapies.

Box 1. Major components of the TME

Vasculature

The formation of blood vessels during malignant progression is a critical tumor property acquired at an early stage of tumorigenesis [174] and is therefore considered to be a hallmark of cancer [1]. The activation of this 'angiogenic switch' is not only essential for adequate supply of the tumor with nutrients and oxygen to allow growth beyond a certain size, but also facilitates subsequent metastatic spread. Compared with normal blood vessels, which display a highly organized architecture, the vasculature within a tumor typically exhibits altered anatomical, structural, and functional properties such as aberrant recruitment of pericytes, increased vascular permeability, and turbulent blood flow [175]. Although angiogenesis can be driven by various molecules, VEGF-A is often considered to be the prototypical angiogenic factor in malignant disease. Accordingly, its expression is inversely correlated with overall survival in various cancers [176–178]. While tumor cells are major contributors of VEGF-A, TME cells represent additional sources [179].

Cancer-associated fibroblasts

CAFs constitute a large proportion of the non-epithelial or stromal cells within the tumor. CAFs are considered to be functionally distinct from normal fibroblasts, which can inhibit malignant transformation [180,181], as they can support progression of premalignant lesions in prostate cancer [182] and enhance metastasis in breast cancer [180]. However, CAFs can have tumor-suppressive functions in pancreatic ductal adenocarcinoma [34,35]. CAFs are stimulated to proliferate by growth factors and cytokines such as TGF-β, FGF-2, and PDGF that are abundant in the TME [183,184]. Following activation, CAFs become a major source of secreted growth factors, including VEGF-A, which promotes angiogenesis and vascular permeability [179]. Additionally, CAFs produce proinflammatory factors that drive leukocyte infiltration [185].

Inflammatory cells

Cells of the innate immune system not only provide an essential nonspecific defense against pathogens but also regulate tissue homeostasis and wound healing [2]. Their physiological functions therefore predispose them to play a major role in the inflammatory reactions that occur in cancers [5]. Accordingly, many of the immune cells within the TME are of myeloid origin and are either tissue resident or recruited by cancer cells from the BM and the spleen to enhance their survival, growth, invasion, and dissemination [186]. Tumor cells can be nurtured by TAMs through a plethora of signals [187]. Notable examples include a paracrine loop involving EGF/CSF-1 signaling between TAMs and tumor cells [188–190] and the activation of WNT signaling in TAMs [191,192]. Furthermore, TAMs constitute a major source of proteases, such as cysteine cathepsins, that drive cancer progression [193] via regulation of angiogenesis and tumor growth [194]. Similarly, TAM-secreted MMPs not only degrade the ECM [195,196], but also increase the availability of ECM-bound factors such as VEGF-A [197].

Extracellular matrix

ECM is produced by all of the cell types within the TME, resulting in an intricate fiber network that not only provides structural support but also integrates local signals and regulates cellular movement, proliferation, and differentiation [84]. While collagens and fibronectin provide mechanical strength, proteoglycans contribute growth factor and cytokine-binding properties [198]. TME-associated ECM is fundamentally different from that of the normal tissue stroma [199] and serves as a guiding scaffold for chemotaxis and tumor cell invasion [200]. Paradoxically, increased ECM synthesis [201] and pronounced crosslinking of collagen fibers in the malignant stroma further enable tumor cell invasion [202]. Deregulation of collagen crosslinking also results in alterations of the biomechanical properties of the ECM, significantly increasing tissue rigidity [199,203]. Finally, desmoplastic tumor stroma constitutes a physical barrier for drug delivery and influences the architecture of the tumor vasculature [30].

Effects of pre-existing TME properties on therapeutic efficacy

The intrinsic mechanisms through which the TME modulates drug response involve pre-existing properties of the tumor including a chaotic, frequently inefficient vascular supply, elevated interstitial fluid pressure (IFP), a pronounced desmoplastic stroma, increased tissue rigidity, and the presence of niches within the tumor that protect cancer cells from therapeutic insults. As several of these parameters have been previously reviewed [24–28], we only briefly summarize these topics in the context of drug delivery in the TME and focus in more depth on newly emerging areas of TME-mediated intrinsic resistance including the role of protective niches (Figure 2).

Drug delivery, vascular remodeling, and drug escape are modulated by the TME

An abnormal tumor vasculature, increased IFP, and altered ECM constitute major obstacles that prevent chemotherapeutic drugs from effectively penetrating tumor tissue (Figure 2A). One tumor type in which these properties have been investigated in depth is pancreatic ductal adenocarcinoma (PDA), in which a desmoplastic tumor stroma driven by hedgehog (Hh) signaling is a hallmark feature [29]. In a genetically engineered PDA mouse model, various strategies to improve chemotherapy delivery have been explored, including inhibition of Hh signaling [30] and the exogenous introduction of hyaluronidase to enzymatically degrade hyaluronic acid in the ECM [31]. However, recent Phase II clinical trials combining gemcitabine with the Hh inhibitor saridegib failed to demonstrate a clinical benefit in PDA [32]. It therefore remains to be seen whether a recently described tumor-suppressive effect of activated Hh signaling in PDA, via downregulation of integrin expression and transforming growth factor beta (TGF-β) activity that in turn diminishes stromal myofibroblast activation, might account for this observation [33]. Two recent studies reported the tumor-suppressive effects of cancer-associated fibroblasts (CAFs) and a Hh-driven stroma in PDA [34,35], again demonstrating the often dichotomous roles of cells in the TME as discussed above.

Drug delivery into the tumor can also be influenced by vessel functionality and vascular leakage [36]. This was elegantly demonstrated by live imaging in a mouse mammary cancer model where vascular permeability, which was highest in intermediate tumor stages, facilitated doxorubicin penetration [37]. When matrix metalloproteinase (MMP) 9 was deleted in the host stroma, vascular leakage was dramatically increased, resulting in improved doxorubicin delivery into the tumor. A counterpoint to these observations is the phenomenon of vessel normalization, in which vessel density and morphology along with pericyte and basement membrane coverage are normalized, resulting in decreased vascular leakiness and enhanced drug delivery [28,38,39]. These findings highlight the complex interplay between intra- and extravascular factors during drug delivery. Angiogenesis inhibitors, such as the anti-vascular endothelial growth factor (VEGF)-A antibody bevacizumab, are thought to act in part through

Box 2. Therapeutic approaches to treating malignancies

Chemotherapy

Conventional chemotherapy, first introduced into the clinic during the 1940s in the form of DNA-alkylating agents and antimetabolites [204], has since been expanded to encompass a wide variety of drug classes and combinations thereof and still constitutes the mainstay of many systemic drug regimens in solid and hematological malignancies. Chemotherapy is often combined with local therapeutic interventions such as surgery and RT in either the adjuvant or neoadjuvant setting. The mechanism of action of many cytotoxic drugs results from their ability to perturb biological pathways that are required for a cell to progress through the cell cycle and maintain its genomic integrity. The major downside of this mode of action is its inability to distinguish between malignant and normal cells, leading to severe systemic short- and long-term side effects. This extends not only to healthy, nonaffected tissues but also to the TME within the tumor. Furthermore, exposure to chemotherapy leads to a general tissue-damage response that triggers an influx of inflammatory cells into the TME, which can alter therapeutic efficacy [18,106,107].

Surgery and radiation therapy

Both surgery and ionizing radiation are major cornerstones of most cancer treatment approaches. While these modalities are inherently aimed at local control, they result in local and systemic effects that encompass the TME [205,206]. Especially in surgery, the physiological wound-healing response mechanisms exhibit a considerable degree of similarity to the TME [207,208]. A plethora of proliferative and angiogenic factors, many of which originate in the stromal compartment, are found to be altered in the serum of patients after surgery [209]. Consequently, it has been postulated that the wounding response contributes to the progression of minimal residual disease and distant, dormant micrometastases after surgery [210–212].

RT has profound consequences on the TME, beyond its direct cytotoxic effects on the tumor cells [206], that can ultimately alter therapeutic responses. Experimental studies show a significant difference between the radiosensitivity of GBM cells *in vitro* versus *in vivo* that can be attributed to the TME [213]. Due to the fact that sufficient oxygenation is a major determinant of radiosensitivity, this observation is inherently connected to the physical nature of the tumor vasculature *in vivo*.

Oncogene-targeted therapy

An increasing understanding of the genetic alterations that drive cancer has led to a new era of 'targeted' therapeutic agents. The prototype for this concept is imatinib mesylate, an inhibitor of the fusion tyrosine kinase protein BCR–ABL [214]. For the first time, an oncogenic driver could be targeted with specificity and striking efficacy. This proof of concept spurred the development of a broad range of TKIs. However, the notion of targeted therapy includes not only inhibitors in the form of small molecules but also therapeutic antibodies.

One paradigmatic example of these two strategies is the family of di-/oligomeric human EGFRs (HERs). EGFR (HER1/ErbB1) is constitutively activated due to a mutation in a small subset of non-small-cell lung cancers (NSCLCs), which forms the rationale for treatment with EGFR inhibitors such as erlotinib [215] in these patients. Furthermore, a large proportion of NSCLC and CRC cases exhibit increased EGFR expression. In CRC, the addition of the EGFR-specific antibodies cetuximab or panitumumab confers a clinical benefit [216]. However, reliable predictive biomarkers that identify patients most likely to benefit from this therapy are not equally well established across different tumor types [217]. Modulation of EGFR activity by and within the TME through the provision of alternative EGFR ligands might account for the therapeutic effect observed in EGFR-negative cases [218,219].

Recently, new TKIs have entered the spotlight. One notable example is vemurafinib, which inhibits BRAF, a member of the ERK protein kinase pathway that is frequently mutated in malignant melanoma [220]. While vemurafinib can have an impressive initial response rate [221], this is overshadowed by the emergence of secondary resistance in most cases. There is increasing evidence that the TME contributes in part to this phenomenon [102,104].

TME-targeted therapy

VEGF-A is a major driver of tumor angiogenesis and therefore the VEGF-A-neutralizing antibody bevacizumab can be considered as the first approved therapeutic agent that perturbs a key axis of tumor cell–TME crosstalk (see Figure 1 in main text). Bevacizumab is effective in metastatic CRC and NSCLC when combined with conventional chemotherapy [222,223]. A similar efficacy was observed with several multi-kinase TKIs (e.g., sorafenib, sunitinib), which target VEGFR2 and other RTKs and are approved for multiple cancer types including advanced renal carcinoma.

Currently the most advanced approach to therapeutically utilize the antitumor activity within the TME is the blockade of immune checkpoints. This concept has recently had considerable clinical success in patients with advanced malignant melanoma. Both the inhibition of cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) with the antibody ipilimumab [224] and the blockade of the programmed death 1 (PD1) receptor via the antibody nivolumab resulted in increased overall survival [225] (see Figure 1 in main text). This effect appears to be even more pronounced when the two therapeutic agents are combined [226]. Phase III trials investigating whether this can be translated to other malignancies such as lung and renal cancer are currently under way. Another promising approach is the reprogramming rather than depletion of TAMs via CSF-1R inhibition (see Figure 1 in main text). The feasibility of this concept was recently demonstrated in mouse models of GBM [162] and PDA [163] and it will now be critical to investigate whether this reprogramming can be similarly achieved in patients.

normalization of the tumor vasculature [28,40] (Figure 1). Recent preclinical studies have also shown that immunotherapy efficacy can be enhanced in combination with vessel-normalization strategies [41]. The clinical experience that angiogenesis inhibitors exert their greatest benefit in combination with conventional cytotoxic drugs in certain cancers supports their use within multimodal treatment regimens [42,43]. However, this is often accompanied by the rapid emergence of resistance [43], which is discussed in the following section on acquired resistance in the TME.

Equally as important as drug delivery into the tumor is the rate of clearance of chemotherapeutic agents by the malignant cells. For instance, integrin 'outside-in' signaling via the extracellular signal-related kinase (ERK)/mitogen-activated protein kinase (MAPK) signaling pathway upregulates the multidrug resistance protein ATP-binding cassette, subfamily C (CFTR/MRP), member 1 (ABCC1) in T cell leukemias, which then actively expels doxorubicin [44]. Tumor-associated macrophages (TAMs) have been shown to mediate gemcitabine resistance in PDA by upregulating cytidine deaminase, the enzyme that metabolizes gemcitabine to its inactive form, in the tumor cells [45].

Another mechanism by which drug availability can be altered is through the expulsion of cytostatic drugs from tumor cells via extracellular vesicles (EVs) [46,47] and the horizontal transfer of multidrug efflux transporters by EVs [48]. Tumor cell-derived EVs can also act as decoys for therapeutic antibodies such as rituximab [49] and trastuzumab [50]. Recent data additionally implicates stromal–tumor EV transfer in promoting chemoresistance. For example, endothelial cell (EC)-derived EVs conferred increased resistance of breast and ovarian cancer cells to doxorubicin and paclitaxel [51]. It has recently been

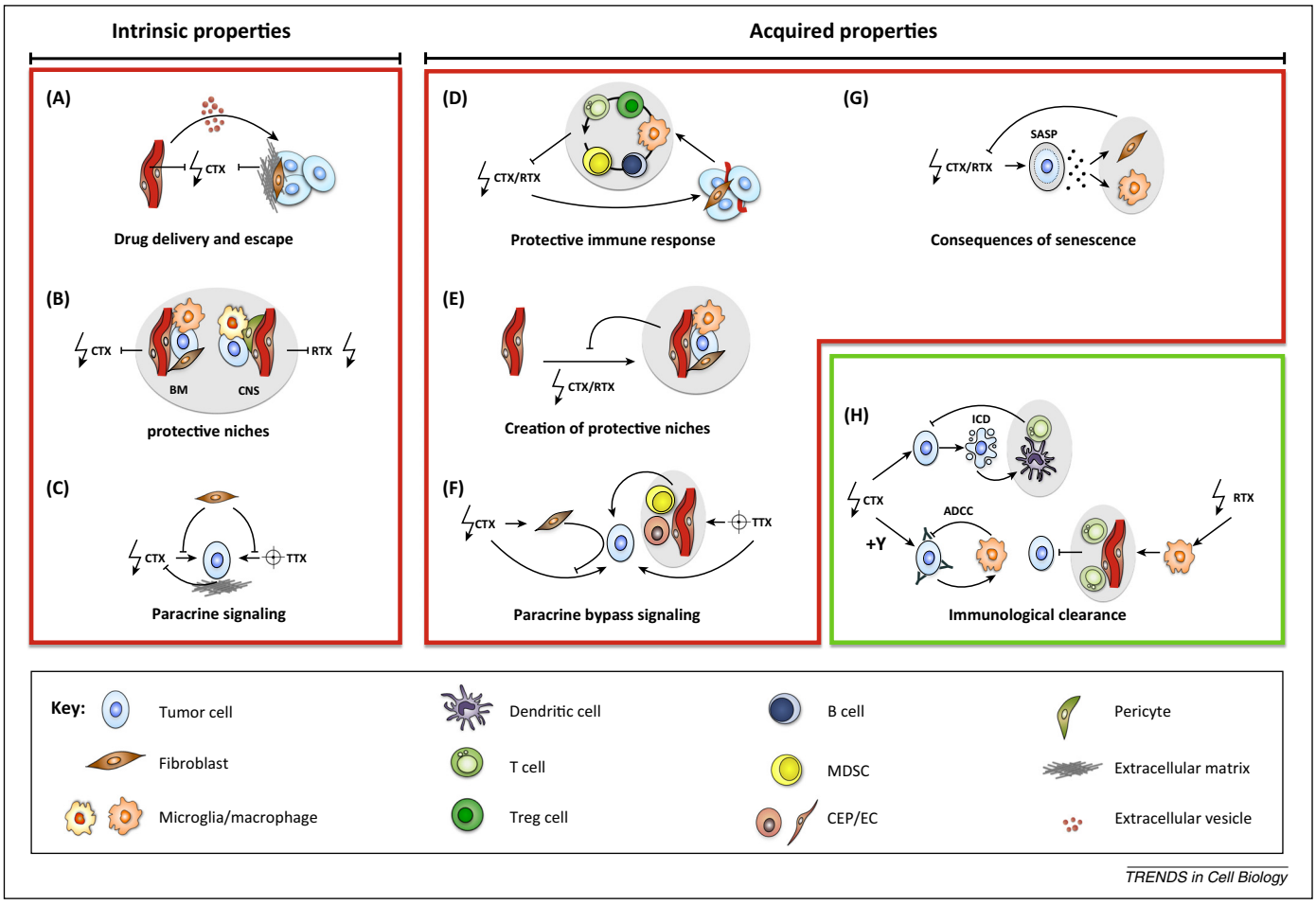

Figure 2. Intrinsic and acquired contributions of the tumor microenvironment (TME) to therapeutic response. The TME alters therapeutic efficacy through both intrinsic traits and properties acquired after exposure to therapy. This applies to chemotherapy (CTX), radiotherapy (RTX), and targeted therapies (TTX). The intrinsic properties of the TME that modulate therapeutic response include: **(A)** the alteration of drug delivery and clearance; **(B)** the utilization of pre-existing protective niches within the bone marrow (BM) and central nervous system (CNS) to shield malignant cells from therapeutic insult; and **(C)** the co-option of prewired paracrine signaling loops within the stroma to counteract therapeutic interventions. **(D)** In response to therapy the TME can orchestrate a protective immune response that is defined by a plethora of multidirectional interactions between different immune cell populations. **(E)** Furthermore, therapeutic interventions can lead to the emergence of newly created protective niches within the TME that function as safe havens. **(F)** In addition, paracrine bypass signaling pathways can override the effects of both conventional and targeted therapies, while **(G)** the senescence-associated secretory phenotype (SASP) can dramatically change the signaling equilibrium within the TME toward a therapy-attenuating state. **(H)** However, the TME can also substantially augment therapeutic efficacy by several mechanisms that ultimately result in an increased immunological response. This can result from immunogenic cell death (ICD) of tumor cells that activates antigen-presenting dendritic cells and cytotoxic T cells, promotion of increased antibody-dependent cell-mediated cytotoxicity (ADCC) by macrophages through cyclophosphamide, and the reprogramming of macrophages by low-dose radiation to facilitate normalization of the tumor vasculature and recruitment of cytotoxic T cells. Mechanisms that attenuate the therapeutic response are highlighted in red; therapy-ameliorating effects are marked in green. Arrow-headed lines indicate a positive or activating connection and bar-headed lines illustrate an antagonizing function.

demonstrated that stromal exosomes, which are rich in noncoding RNA, elicit a signal transducer and activator of transcription (STAT) 1 response mediated by the pattern recognition receptor retinoic acid-inducible gene 1 (RIG-1) in basal-like breast cancer cells. This augments juxtracrine Notch 3 signaling, which ultimately expands tumor-initiating cells (TICs) that are known to exhibit increased resistance to chemotherapy [52]. Interestingly, EVs also exert a protective effect on nonmalignant populations within the TME: prostate cancer cell-derived EVs blunted apoptosis of fibroblasts following treatment with actinomycin D in an ERK-dependent manner [53]. In a similar fashion, EVs carrying TGF-β from injured epithelial kidney cells led to fibroblast activation that ultimately resulted in fibrosis, a process that closely mirrors the tumor stroma [54]. Exchange of cellular contents can also occur via tunneling nanotubes, as recently demonstrated for the transfer of mitochondria between ECs and cancer cells, which led to doxorubicin resistance [55].

Taken together, these representative examples not only provide substantial evidence for a protective effect of the tumor stroma via direct regulation of drug accessibility and turnover, but also highlight the intricate interactions within the TME that go beyond simple bystander effects.

Protective niches in the TME confer survival signals

Several cellular niches exist within tumors, including the perivascular niche and the bone marrow (BM) niche, which are critical for supporting cancer stem cells (CSCs)/TICs [56]. While CSCs/TICs have been postulated to be inherently therapy resistant [57], it is now becoming apparent that pre-existing niches can also confer protective signals non-cell autonomously in the face of therapeutic intervention, enabling a subset of cells to survive and thus re-establish a malignant tumor (Figure 2B). Here, we discuss examples of both soluble factor- and ECM-mediated intrinsic resistance to various therapies in different TME niches

and how these niches can be altered to support adaptive resistance as a result of therapeutic intervention.

The perivascular niche (PVN), comprising ECs and pericytes, provides an ideal 'microenvironment within the microenvironment' for cancer cells to resist therapeutic insults. To date, this has been most widely shown in brain tumors, which possess a particularly rich PVN comprising multiple cell types that constitute the blood–brain barrier: astrocytes and often microglia in addition to ECs and pericytes [58–62]. In preclinical models of medulloblastoma, for example, CSCs located within the PVN survive irradiation through several mechanisms including enhanced phosphatidylinositide 3-kinase (PI3K)/v-akt murine thymoma viral oncogene homolog 1 (Akt) signaling [63] or via Yes-associated protein (YAP) expression, which drives insulin-like growth factor 2 (IGF2)/Akt activation [64]. This allows tumor cells to enter mitosis with persistent DNA strand breaks and thus contributes to radioresistance.

It is now becoming evident that PVN-mediated signals can have similarly potent effects in organs other than the brain. PVNs for CSCs have been reported in multiple tumor types including breast, head and neck, and melanoma [65–67] and thus it will be interesting to determine whether PVN factors similarly modulate therapeutic resistance in these organs. In several different mouse models, including B16 melanoma, EC-specific deletion of focal-adhesion kinase (FAK) was recently shown to sensitize tumors to RT and various chemotherapies, including doxorubicin, due to loss of EC-derived protective cytokines. Interestingly, the authors also studied lymphoma patients and found that low FAK expression in blood vessels at the time of diagnosis correlated with a better response to doxorubicin [68]. Recently, EC-secreted paracrine factors, termed angiocrine factors, that have potent roles in liver regeneration and fibrosis have been identified [69]. Related angiocrine factors were shown to promote chemoresistance in B cell lymphoma via a paracrine signaling loop in which lymphoma cells cocultured with ECs induced fibroblast growth factor (FGF)-4 expression, leading to activation of FGF receptor 1 (FGFR1) signaling and induction of Jagged 1 in ECs. Consequently, EC-specific deletion of FGFR1 or Jagged 1 *in vivo* resulted in enhanced sensitivity of lymphoma-initiating cells to doxorubicin [70], supporting consideration of inhibitors targeting these pathways as a means to enhance chemotherapy efficacy. Therefore, the link between CSC maintenance or the induction of 'stemness' by protective niches within the TME and the resulting resistance to therapy, as suggested by several of these studies, warrants further investigation.

Intimate interactions between cancer cells and stromal cells that substantially influence therapeutic outcome have similarly been reported for niches in the BM microenvironment, which can comprise mesenchymal cells, ECs, macrophages, osteoblasts, fibroblasts, and other stromal cell types [71–73]. This is the case for hematological malignancies that develop in the BM [74,75] such as acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), or multiple myeloma (MM), and cancers that metastasize to bone. A paracrine interleukin (IL)-6 axis between the BM stroma and MM cells contributes to chemotherapy resistance via STAT3-mediated protection from apoptosis [76]. Stromal IL-6 expression has also been demonstrated in solid tumors such as gastric, lung, and colon cancer [77–79]. In colorectal cancer (CRC), depleting stromal IL-6 showed greater antitumor effects than targeting tumor cell-derived IL-6. However, recent Phase II trials of the anti-IL-6 antibody siltuximab failed to demonstrate substantial clinical benefits in newly diagnosed or relapsed/refractory MM [80,81]. Likewise, in advanced ovarian cancer or renal cancer the use of siltuximab as a single agent predominantly facilitated disease stabilization [82,83]. It therefore remains to be seen whether this therapeutic approach in combination with conventional chemotherapy might provide more beneficial results in solid cancers.

The ECM is an essential component that maintains both primary and metastatic niches in cancer [84,85]. However, interactions between cancer cells and the various components of the ECM go far beyond simple structural support. For example, adhesion of malignant cells to ECM substrates restores cellular polarization of breast cancer cells, which results in resistance to apoptosis on treatment with etoposide [86]. The mechanism of this cell adhesion-mediated drug resistance (CAM-DR) relies on integrin binding to ECM constituents such as collagen, fibronectin, and laminin [11,84,85]. The protective effects of integrin–ECM binding result not only from the upregulation of multidrug resistance transporters [44] but also from the downstream activation of FAK, PI3K/AKT, STAT3, and nuclear factor kappa light chain enhancer of activated B cells (NF-κB) signaling, all of which exhibit antiapoptotic properties [11,87]. While this has been most extensively studied in the context of the BM niche in hematological malignancies, it has also been observed in breast, lung, and pancreatic cancer [24]. Moreover, the protective effect of integrin ligation extends beyond chemotherapeutic agents; it is also involved in mediating resistance to radiotherapy [88–90] and oncogene-targeting therapies such as small-molecule [91–93] and antibody-based receptor tyrosine kinase (RTK) inhibition [94], which can be attributed to bypassing of the targeted pathways.

Paracrine signaling loops in the TME

Classical paracrine signaling loops between different cell types in the TME have been shown to mediate therapeutic resistance (Figure 2C). These studies typically have either investigated well-known factors using candidate-based approaches or, more recently, expanded to large-scale unbiased screens. One prototypic example of the former approach involves chemokine stromal derived factor (SDF)-1, which is produced by BM stromal cells, and its cognate receptor chemokine (C-X-C motif) receptor 4 (CXCR4), which is expressed on cancer cells [95]. SDF-1 protects AML cells from apoptosis induced by the cytarabine [96]. Treatment with the CXCR4 inhibitor AMD3465, an analog of the FDA-approved drug plerixafor (Figure 1), blocked stromal SDF1-induced activation of the prosurvival AKT and ERK signaling pathways in AML [97]. A similar antiapoptotic mechanism has been observed *in vitro* for small-cell lung cancer [98], which has a predisposition for bone metastasis. Outside the BM niche, recruitment of

circulating BM-derived mesenchymal stem cells (BMMSCs) into solid tumors has been described in preclinical models of breast and lung cancer [99]. Therefore, it is tempting to speculate that these BMMSCs could constitute a local source for SDF-1 in solid tumors that similarly modulates therapeutic response [100].

Interestingly, cytokine-mediated protection can circumvent targeting of RTK-mediated signaling pathways, even in kinase-addicted cancers. In AML cells with fetal liver tyrosine kinase-3 (FLT3) gene mutations, coculture with stromal cells significantly diminished the antileukemic effects of the FLT3-targeting inhibitor sorafenib, while CXCR4 inhibition reversed this effect in a preclinical animal model [97]. In another example, AKT inhibitors effectively override stroma-associated cytoprotection of FLT3-mutated AML cells [101]. Soluble RTK ligands can thus rescue kinase-addicted tumor cell lines from targeted tyrosine kinase inhibitor (TKI) therapies due to a convergence at the level of downstream signaling targets [102]. Combined with the observation that stromal CAF-like cells can attenuate endothelial growth factor (EGF) receptor (EGFR) inhibition at the RTK or downstream level in prostate cancer or lung cancer cells, respectively [103], a substantial body of evidence indicates the TME as a source for these alternative activators. Accordingly, hepatocyte growth factor (HGF) was shown to be a stroma-derived factor that mediates resistance to BRAF inhibition in V600E BRAF mutant melanoma [102,104], as well as HER2 inhibition in $HER2^+$ breast cancer [102] and EGFR inhibition in triple-negative breast cancer [105].

Several recent high-throughput screens involving complex matrices of numerous cancer cell lines from various organ sites and molecular subtypes, panels of distinct stromal cell lines, and a broad range of different drugs have demonstrated both the extent and the potency of stromal effects on drug response, which appear to be most pronounced in the context of targeted therapies [75,102,104]. Critically, as shown in these screens, stromal cells are not always mediators of resistance; in several cases they can sensitize to therapy [16]. These types of large-scale approaches have underscored the importance of the TME in modulating therapeutic response to chemotherapeutics and targeted agents, yet remain relatively simple coculture platforms that may not fully recapitulate the *in vivo* TME. In this regard, increasingly sophisticated modeling approaches are becoming more accessible to address these questions, as summarized in Box 3.

Together, the representative examples discussed here indicate the possibility that a cancer cell (sub)population that successfully co-opts cues from the TME will survive and expand under both conventional and targeted therapies. This interplay between the TME and the malignant cells can result from a pre-existing interaction but, equally importantly, can also be introduced by the TME response to a therapeutic intervention.

Therapy-induced responses and acquired resistance in the TME

In the previous section we discussed intrinsic, pre-existing niches and the physical properties of the TME that contribute to non-cell-autonomous therapeutic resistance. Here we focus on how the TME can also be significantly changed by therapeutic intervention and how this can lead to acquired resistance (Figure 2). One paradigmatic example of TME alterations following therapy involves the response of the innate and adaptive immune system.

The immune response influences therapeutic outcome

The innate immune system is a crucial component of the altered TME after therapeutic intervention (Figure 2D). Macrophage infiltration, in response to paclitaxel-enhanced expression of colony-stimulating factor 1 (CSF-1) and IL-34, appears to be a major contributor to chemoresistance and immunosuppression [106]. Furthermore, in a mouse model of breast cancer, recruitment of chemokine (C-C motif) receptor 2 (CCR2)-expressing monocytic cells occurs following doxorubicin treatment, via stroma-derived CCL2 [37]. In turn, the presence of these CCR2-expressing cells contributes to suboptimal treatment response and tumor re-emergence. Likewise, treatment of murine breast cancers with various chemotherapies leads to increased TAM accumulation, which then enables cathepsin protease B- and S-mediated chemoresistance to paclitaxel, etoposide, and doxorubicin [107].

Macrophages also constitute an important link to the adaptive immune system. Their increased influx into recurrent tumors leads to a surge of regulatory T cells that is accompanied by impaired recruitment of $CD8^+$ cytotoxic T cells [108]. Regulatory T cells also accumulate following RT, as do macrophages, and their transient ablation was shown to enhance RT efficacy in a mouse breast cancer model [109]. A similar observation has been made for myeloid-derived suppressor cells (MDSCs), which drive IL-1β-induced secretion of IL-17 by $CD4^+$ T cells that leads to chemoprotection [110]. Interestingly, there is also evidence for a chemoprotective role of B cells involving the education of TAMs toward a tumor-supporting phenotype by the activation of the Fc receptor [111]. Accordingly, B cell depletion resulted in increased recruitment of $CD8^+$ cells and an enhanced therapeutic response.

The complexity of the immune system's response to therapeutic intervention is underscored by contradicting observations that support a positive contribution of the immune system to improved chemotherapeutic efficacy (Figure 2H). Interestingly, apoptosis, long thought to be immunologically silent, has been shown to result in the production of proinflammatory cytokines such as IL-8 and CCL2 [112]. Furthermore, several chemotherapeutic agents, such as anthracyclines and platinum derivatives, are able to induce immunogenic cell death that culminates in the release of damage-associated molecular patterns (DAMPs) [113,114]. It has been proposed that these mechanisms ultimately improve the anticancer effects of chemotherapy through increased antigen-presenting ability of dendritic cells and a subsequent T cell response [115]. The latter has been linked to infiltration of innate IL-17-producing γδ T cells that precedes the invasion of $CD8^+$ T cells [116]. The importance of a robust $CD8^+$ response is evident not only in the setting of conventional chemotherapy but also when mouse mammary tumor virus (MMTV)-neu transgenic mice were treated with the HER2 inhibitor

Box 3. Model systems for investigating TME-mediated resistance

While cancer drug screening assays were historically performed using transplantable tumor models, this approach has been superseded by tumor cell culture systems in recent decades [227] (Figure I). This tumor cell-focused approach has considerable advantages such as scalability, reproducibility, and time efficiency; however, one significant downside is the loss of information about the TME. Recently, several studies that incorporate 2D coculture with stromal cells in their screens have uncovered an important contribution of the TME to therapeutic response [75,102,104]. Nonetheless, these systems are still far from fully recapitulating the complex spatial and temporal composition of a tumor and its TME. It has been demonstrated that sophisticated 3D culture systems result in significant cytoskeletal reorganization, appropriate cellular polarity, and the generation of hypoxic gradients [24,228,229], which in turn alter chemoresistance [230]. This can be further expanded to multicellular direct [231] and indirect [232] coculture systems. Another level of complexity can be achieved by using organotypic normal tissue slice-culture systems that allow the investigation of microenvironmental interactions and vessel co-option during tumor colonization [233,234]. In a similar fashion, tumor explants can be studied as slice cultures [235,236]. One main advantage of these *in vitro* and *ex vivo* systems is that they are easily amenable to therapeutic intervention and accurate measurement of therapeutic efficacy. Moreover, specialized imaging techniques such as fluorescence recovery after photobleaching (FRAP) and Förster resonance energy transfer (FRET) [229] can be incorporated to analyze the downstream signaling cascades.

Highly sophisticated imaging techniques in animal models, including confocal [37,237] and intravital multiphoton microscopy [238], allow one to unravel tumor–stroma dynamics that potently impact therapeutic responses. Advanced approaches that combine these imaging modalities with various markers and reporters allow investigators to monitor tumor progression, the TME, and the success or failure of anticancer therapy with remarkable spatial and temporal resolution *in vivo* [239]. However, the tumor model needs to be chosen carefully, as the response to therapy differs greatly not only between orthotopically and subcutaneously transplanted tumors [240] but also between primary tumors and postsurgical metastases [241]. From a translational perspective, it could be that patient-derived xenografts (PDXs) will be developed into valuable models to study tumor–stromal interactions. Currently their usefulness is limited by loss of the human stromal compartment [242] and the necessity for immunodeficient recipient mice. However, the transplantation of PDX tumors into humanized tumor stroma [243] or the utilization of mice with a transplanted, functional human immune system [244,245] is likely to circumvent some of these obstacles.

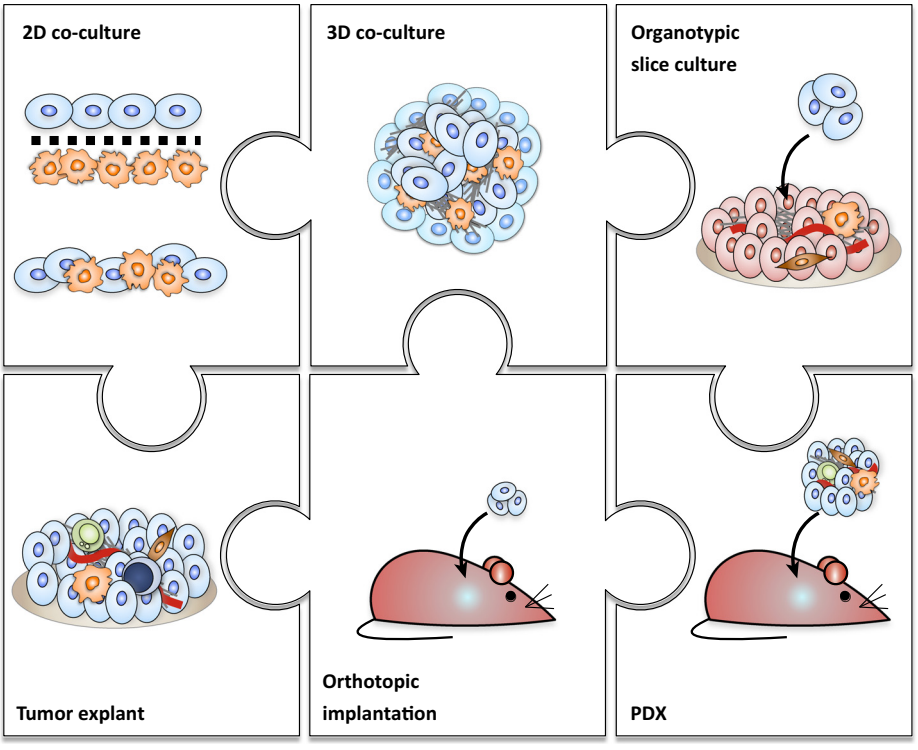

Figure I. Commonly used indirect and direct coculture techniques have yielded valuable information about the role of stromal cells during therapeutic interventions. These cocultures can be further extended to a 3D system to account for spatial relations within the tumor microenvironment (TME). Organotypic slice cultures allow recapitulation of the microenvironment of different organs *in vitro*, while *ex vivo* explants of tumors retain the original TME. For preclinical *in vivo* studies, orthotopic implantation of tumor cells permits the investigation of tumor cells within the appropriate organ-specific TME. The correlation between therapeutic response in the mouse model and clinical efficacy can potentially be increased when patient-derived xenografts (PDXs) are used. It is important to note that the experimental strategies presented here are neither exhaustive nor in a hierarchical order. They rather represent complementary approaches to investigate the role of the stroma in therapeutic response.

lapatinib [117]. In the context of antibody-dependent cell-mediated cytotoxicity (ADCC), low-dose cyclophosphamide has been shown to promote macrophage-mediated killing of antibody-targeted cells in the bone marrow. This is due to an acute secretory phenotype of the tumor cells after cyclophosphamide exposure that increases the phagocytic capacity of macrophages through tumor necrosis factor (TNF)-α, VEGF, and CCL4 [118].

In summary, the available evidence indicates that the immune response can both support and obstruct therapeutic efficacy. Therefore, unraveling and exploiting this complexity continues to be a challenging task.

Reactions of the TME beyond the immune response influence therapeutic outcome

An important mechanism that contributes to the altered phenotype of the TME after therapeutic intervention is induction of the senescence-associated secretory phenotype (SASP) (Figure 2G). Originally linked to replicative and genotoxic stress, senescence induction is typically considered a tumor-suppressive mechanism due to the resulting growth arrest. However, in some circumstances the SASP can promote cancer progression [119]. In the context of therapy-induced changes, DNA-damaging agents such as doxorubicin and RT lead to SASP in various tumor types [120]. This is associated with the secretion of various cytokines, proteases, and growth factors including HGF, TGF-β, IL-6, and CCL2 [121–123]. As outlined above, several of these molecules can also dramatically alter therapeutic response. Furthermore, this phenomenon similarly occurs within non-transformed TME cells [124] and may even spread in a paracrine fashion [121]. Thus, SASP induction in the TME can have potentially pleiotropic effects on therapeutic response.

Besides SASP-associated growth factors and cytokines, other signaling molecules have also been implicated in the therapy-altered TME (Figure 2F). For example, both RT and chemotherapy lead to upregulation of wingless-type MMTV integration site family member 16B (WNT16B) in CAFs, which then acts as a paracrine signal that attenuates the effects of anthracycline therapy in prostate cancer cells [125]. The upregulation of WNT16B is mediated via NF-κB, which in turn activates β-catenin-dependent WNT signaling in the cancer cells. The authors demonstrated a similar effect in breast cancer models. CAFs are similarly enriched in CRC during post-therapy tumor recurrence and exhibit upregulation of the cytokine IL-17A, which promotes the maintenance of TICs via NF-κB activation [126].

The BM also constitutes a rich source of cells of varying progeny beyond myeloid cell populations that are mobilized and recruited to the TME in response to therapeutic insult. For instance, BMMSCs have been identified as a source of novel chemoprotective factors: secreted polyunsaturated fatty acids [127]. While apparently only cisplatin-based therapy was capable of inducing their release, these fatty acids were able to confer resistance to various agents and even act on a systemic level.

Marked increases in BM-derived circulating endothelial progenitors (CEPs) in preclinical models and patient samples have been reported following the administration of various chemotherapies including taxanes, doxorubicin, and 5-fluorouracil (5-FU) [128]. In a mouse lung cancer model, the elevated CEPs were subsequently recruited to the tumor and treatment with antibodies targeting VEGF receptor 2 (VEGFR2) or SDF-1 abrogated this accumulation and increased chemotherapeutic efficacy. Interestingly, this synergy did not occur when gemcitabine was used in the combination treatment. Again, this highlights the varying response patterns of the TME between different tumor types to distinct therapeutic interventions.

With regard to TME-mediated resistance to antiangiogenic therapies, the role of non-VEGF angiogenic pathways such as FGF or SDF-1 as possible escape mechanisms is increasingly appreciated [129,130]. Several cell populations within the TME are able to counteract anti-VEGF therapies: $CD11b^{+}Gr1^{+}$ MDSCs and CEPs both contribute to antiangiogenic resistance [128,131,132]. Interestingly, in the case of MDSCs, this did not predict resistance to conventional chemotherapy [132] and was due to the non-conventional mediator of angiogenesis Bv8 [133]. Subsequent studies showed that murine lymphomas refractory to anti-VEGF therapy cause CAFs to secrete platelet-derived growth factor (PDGF)-C, which stimulates EC migration independent of myeloid cell infiltration [134]. Another illustrative example is the paracrine signaling network between T helper type 17 cells (Th17), the TME, and immature myeloid cells or MDSCs. Tumor-infiltrating T_H17 cells induce the expression of granulocyte CSF (G-CSF) in the stromal compartment via IL-17. This in turn attracts MDSCs, which drive anti-VEGF-A resistance [135].

In the case of the commonly used non-hypofractionated RT, hypoxia-inducible factor 1 (HIF-1) induction in the tumor cells leads to increased VEGF-A and SDF-1 expression [136]. While the former directly promotes EC recovery [137], the latter attracts BM-derived myeloid monocytic cells that contribute to vasculogenesis and tumor regrowth [138,139]. Interestingly, upregulation of CSF-1 in irradiated prostate cancers represents an alternative means to increase myeloid cell numbers after RT [140]. Taken together, the angiogenic network exhibits an impressive degree of plasticity involving numerous cell types within the TME that affect the outcome of both antiangiogenic and RT-based therapies. While VEGF-A inhibition in combination with RT and chemotherapy in glioblastoma (GBM) demonstrated increased progression-free survival [141,142], CSF-1 receptor (CSF-1R) inhibition combined with RT is currently being evaluated in Phase II clinical trials.

Finally, various therapeutic interventions can alter the TME at the level of the various niches (Figure 2E) discussed in the section on intrinsic resistance. For example, in a murine model of Burkitt's lymphoma, DNA-damaging agents induced the secretion of IL-6 and tissue inhibitor of metalloproteinases 1 (TIMP-1) from ECs in the thymus [143]. This resulted in specific protection of lymphoma cells in the thymus but not other lymphoid organs and served as a reservoir of cancer cells that subsequently fueled tumor relapse. Consistently, the increased tumor load in the thymus following doxorubicin administration was blunted in IL-6-deficient mice. A recent study in a mouse model of leukemia showed that a BM niche is created by ALL cells following cytarabine and daunorubicin treatment once physiological BM niches have been destroyed due to leukemic dissemination. This leukemia-induced niche evolves in response to therapeutic intervention and withdrawal [144]. Mesenchymal cells, recruited by leukemia cell-secreted CCL3, were found to be the major components that built a therapy-induced niche and evolved from $Nestin^{+}$ cells to α smooth muscle actin $(\alpha\text{-SMA})^{+}$ cells under the influence of TGF-β and ultimately developed into fiber residues. The authors also identified furin-cleaved growth differentiation factor (GDF15) as the effector molecule provided by the niche to activate TGF-βII and confer

chemoprotection and correspondingly found that blocking GDF15 or furin enhanced chemotherapy sensitivity. Other examples of niches that either serve as a 'safe haven' from therapeutic intervention or contribute to metastatic escape include a lymphatic vessel niche in melanoma that promotes survival and enrichment of $CD133^+$ TICs following dacarbazine treatment [145] and a TME stem cell 'unit' that is altered by RT and subsequently confers radioresistance on glioma cells [146].

Therapy-induced changes in differentiation and activation of TME cells

Altered differentiation represents another cell-intrinsic mechanism for cancer cells to develop resistance; for example, via epithelial–mesenchymal transition (EMT) [8,147]. There have been several recent studies revealing how the TME can also influence this process in a paracrine manner, including examples of how alterations to the polarization state of TME cells can influence therapeutic outcome. A fascinating case of cancer cell dedifferentiation was recently uncovered in a melanoma mouse model following adoptive cell transfer (ACT), which resulted in loss of melanocytic antigens and the subsequent development of ACT resistance [148]. Interestingly, this phenomenon was driven by an inflammatory response in the TME: specifically, TNF-α released by tumor-infiltrating T cells and macrophages that mediated the dedifferentiation. The authors therefore propose that ACT for melanoma, which is currently targeted toward melanocytic antigens, be expanded to simultaneously target tumor-specific mutated non-melanocytic antigens, thus ensuring broad recognition of both differentiated and dedifferentiated melanoma cells.

TME-produced cytokines such as NF-κB and TNF-α have also been shown to promote mesenchymal differentiation in GBM, resulting in enhanced radioresistance [149]. Macrophages/microglia were enriched in pockets within the tumor that were positive for mesenchymal markers, suggesting a potential link between TAMs and changes in cancer cell differentiation. Macrophages themselves can also be altered by distinct therapies including platinum-based agents, which induce an M2/alternatively activated state *in vitro* [150], and low-dose irradiation that promotes an $iNOS^+$ M1 phenotype [151]. In the case of M1-polarized macrophages, this RT-induced change in polarization toward an immunosuppressive state allows the recruitment of cytotoxic T cells in the tumor and thereby enhances immunotherapy efficacy in animal models [151].

Together, these examples illustrate the diversity of therapies that can induce altered polarization states within the TME and changes in cancer cell differentiation. As this effect is often reversible, this indicates numerous potential targets that could be used in combination with, or following, standard therapeutic interventions.

Concluding remarks

The number of mechanisms by which cancers can develop resistance to various therapeutic interventions inevitably increases as our arsenal of anticancer treatments expands. We can clearly now add TME-mediated resistance to this list and, as indicated by the representative examples we have highlighted here, and by emerging areas of interest (Box 4), these mechanisms of resistance are similarly diverse in their nature. While there are an increasing number of TME-targeted approaches to circumvent resistance (Figure 1), there are also evident challenges in considering how to translate this knowledge to the clinic.

One obstacle is our currently limited understanding of the underlying mechanisms through which some of these therapeutic interventions affect distinct subpopulations within the TME. This is especially evident for the potential immunomodulatory effects of conventional chemotherapeutic agents (Figure 1), which remain poorly understood and thus cannot be utilized to their full potential.

Another challenge is to fully understand and ultimately target microenvironmental contributions to therapeutic response. It is clear that the stromal and immune composition of tumors is complex and highly dynamic and we currently only have a limited perspective on these alterations in certain human tumors, such as through the analysis of stromal gene expression in breast cancer [21] and the recent 'immune landscaping' in CRC [152]. These types of immune cell analysis are being extended to other tumor types through the 'Immunoscore', the prognostic value of which is currently being evaluated [153], but information on stromal cells such as fibroblasts, ECs, and organ-specific TME cell types is not currently being captured in these large-scale approaches. Thus, where possible, comprehensive landscaping of the entire TME before and after therapeutic intervention would significantly enhance these efforts.

Alternative strategies available at present include using computational deconvolution of gene expression data from whole-tumor samples from patients to capture gene signatures of stromal and immune cells [154]. Advanced bioinformatics tools that can discern subpopulations in complex tissues [155,156] and identify factors that could be targeted concomitantly in tumor cells and the TME [157] may provide new insights into potential 'double-hit' therapeutic strategies. Similarly, profiling the RTK ligands present in the TME (for example, via ELISA of tumor biopsies), represents another attractive approach, given their potent roles in mediating resistance [102,104]. These examples serve as a reminder that complementary technological approaches will be required to dissect the TME response to therapy that relies in part on physiological tissue-specific survival pathways.

It is also critical to remember that the TME plays a multifaceted role and can exhibit tumoricidal capacities, often through interplay with the immune system. A recent study revealed a surprising, tumor-promoting effect of CAF depletion in PDA [34]. Taken together with the observation that the tumor stroma can also enhance therapeutic efficacy [16,104] and the examples discussed here where immune cells are required for optimal therapeutic response, it is reasonable to propose that re-education of the TME rather than elimination of an entire cellular compartment will be substantially more beneficial [3,158–163] (Figure 1).

The idea of re-education raises a currently unmet need: how to translate the 'activation state' of the TME, which encompasses the cellular and molecular composition of the TME and can be envisioned as an intricate and dynamic

Box 4. Emerging areas in TME-mediated resistance: the microbiome and the metabolome

In this review, we focus on how different cellular and extracellular components of the TME can profoundly impact therapeutic response and outcome. However, it is becoming increasingly evident that bacteria in the host can modulate the TME to influence the initiation and progression of cancers in the gastrointestinal (GI) tract [246]. Moreover, there are examples in which the gut microbiota can promote disease progression in other organ sites, including the liver [247] and breast [248,249]. Thus, the microbiome is emerging as a major player in many diseases, including cancer, obesity, and metabolic syndromes, and is likely to influence both local and systemic therapeutic response. Two recent studies showed that the response to chemotherapy or immunotherapy could be negatively impacted by the use of broad-spectrum antibiotics that disrupted the intestinal microbiota [250,251]. Cyclophosphamide chemotherapy, which has immune-modulating properties, was found to increase intestinal permeability resulting in an altered bacterial composition in the gut and the entry of certain Gram-positive bacteria into the spleen and lymph nodes [251]. Interestingly, cyclophosphamide converted naïve $CD4^+$ T cells in these organs to Th17 cells, which contributed to the immune response against the tumor. This beneficial effect could be blocked by antibiotic treatment or by using germ-free mice, demonstrating the critical role of these bacterial species in promoting the cyclophosphamide response. Iida *et al.* also examined immunotherapies in their study and found an impaired antitumor response in animals pretreated with antibiotics, which they attributed to decreased TNF production by tumor-infiltrating myeloid cells. Together, these studies demonstrated that the microbiome can have a potent effect on the efficacy of distinct therapies through interactions with different immune cell types in the TME.

Given that gut microbiota can be profoundly disrupted by chemotherapy and radiotherapy [252], often resulting in a substantial decrease in microbial diversity, strategies to restore microbial balance could have a profound impact not only on the health of the patient but also on therapeutic response. 'Beneficial' bacteria have been shown to stimulate host immune cells (regulatory $CD4^+CD25^+$ lymphocytes) to suppress mammary cancer in mice [253], raising the intriguing possibility that altering the microbiome could similarly be considered as a novel strategy to circumvent TME-mediated therapeutic resistance in GI malignancies and possibly other organs.

Another previously underappreciated layer of complexity that needs to be incorporated into the multifaceted network of tumor–TME interactions is the role of the metabolome [254,255]. There are emerging data demonstrating a 'reverse' Warburg effect beyond the malignant cells; that is, anabolic, aerobic glycolysis occurring in the tumor stroma that provides the metabolite lactate to tumor cells [256]. Interestingly, the metabolic reprogramming of CAFs within the TME appears to be linked to their production of tumor-promoting IL-6 and TGF-β [257]. Initial preclinical studies aimed at disrupting the synergistic metabolism between tumor compartments proved to be feasible and thus might constitute a promising avenue to pursue [258,259].

As indicated by these examples, and the emergence of additional important stromal cell types in the TME including adipocytes and nerves [3], the complexity of the TME extends far beyond the local environment to involve a multitude of systemic interactions within the host. Thus, targeting the TME in the context of evading or overcoming therapy resistance will increasingly require a sophisticated understanding of the physiology of the patient.

network, into predictive biomarkers. This applies to both conventional cytotoxic and targeted therapeutic interventions. Initial studies that have highlighted the ability of tumor stroma-related gene signatures to predict therapeutic response to chemotherapy demonstrate that this is a feasible concept [23,164]. However, the fact that putative markers are very likely not dichotomous variables, but rather gradual and multivariable, makes this a challenging task. This difficulty is illustrated by the current lack of a definite predictive biomarker for anti-VEGF therapies, although this treatment modality has been in use for many years [165]. Nonetheless, there is a critical need to integrate these questions into future trial designs to ultimately confer clinical benefit and shield patients from unnecessary harm. Achieving this objective will thus be a major goal in the coming years and will necessitate a close dialog between investigators working on the TME and clinical oncologists.

Acknowledgments

The authors apologize to researchers whose work is not cited due to space limitations. They thank Oakley Olson and Daniela Quail for critical reviews of the manuscript. Research in the Joyce laboratory is supported by the National Institutes of Health (CA181355, CA148967), the American Cancer Society (RSG-12-076-01-LIB), and the Breast Cancer Research Foundation. F.K. is supported by a postdoctoral fellowship from the Deutsche Forschungsgemeinschaft (KL 2491/1-1).

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