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Immunotherapy and tumor microenvironment

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Abstract

Recent exciting progress in cancer immunotherapy has ushered in a new era of cancer treatment. Immunotherapy can elicit unprecedented durable responses in advanced cancer patients that are much greater than conventional chemotherapy. However, such responses only occur in a relatively small fraction of patients. A positive response to immunotherapy usually relies on dynamic interactions between tumor cells and immunomodulators inside the tumor microenvironment (TME). Depending on the context of these interactions, the TME may play important roles to either dampen or enhance immune responses. Understanding the interactions between immunotherapy and the TME is not only critical to dissect the mechanisms of action but also important to provide new approaches in improving the efficiency of current immunotherapies. In this review, we will highlight recent work on how the TME can influence the efficacy of immunotherapy as well as how manipulating the TME can improve current immunotherapy regimens in some cases.

Keywords

Tumor microenvironment; Tumor-infiltrating immune cells; Checkpoint blockade; CAR T-cell therapy; chemokine; cytokine

1. Introduction

Led by immune checkpoint inhibitor and chimeric antigen receptor (CAR) T cell therapies, cancer immunotherapy has shown remarkable long-term efficacy in patients with a variety of cancers [1–4]. Conventional therapies for cancers, such as radiation and chemotherapy, usually target the tumor cells themselves and can induce positive responses in the majority of patients. Despite initial responses to these conventional therapies, relapse and resistance often occur in patients with advanced cancer after prolonged treatment [5]. In significant contrast to conventional therapies, immunotherapy targets the immune system to provoke a systemic response against tumors. Clinical trials with immune checkpoint inhibitors have

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none

shown unprecedented durable responses [6], although these positive responses are limited to a small fraction of patients. The top priorities in the immunotherapy field therefore include understanding the mechanisms of action in detail and how we can extend the positive responses to a broader range of patients.

The immune system can recognize tumor antigens and kill tumor cells *in vitro* [7, 8]. However, recognition of the tumor antigen alone is not sufficient for the host to eradicate established tumors *in vivo* [9–11]. An established tumor is a complex tissue composed not only of tumor cells but also of stromal cells, inflammatory cells, vasculature, and extracellular matrices (ECM), all of which are defined together as the tumor microenvironment (TME) [12, 13]. Successful tumor control by immunotherapy requires the activation of the immune system, expansion of the effector cells, infiltration of activated effector cells to the tumor tissue, and destruction of the tumor cells (Figure 1). However, the TME usually prevents effective lymphocyte priming, reduces its infiltration, and suppresses infiltrating effector cells, which leads to a failure of the host to reject tumors. The mechanisms accounting for the resistance to immunotherapy include the following: 1) an inhibitory microenvironment or lack of antigen stimulation/co-stimulation for immune cells, especially T cells, within the TME that may promote tumor growth and immune escape; 2) biological barriers around tumor tissues that can lead to inadequate numbers of immune cells migrating into tumor sites; 3) exhausted or short-lived activation of antigen-specific T cells with limited repertoires that fail to suppress tumor growth; and 4) poor direct or indirect antigen presentation in lymphoid tissues that lead to a lack of T-cell priming due to insufficient release of tumor antigens to the draining lymph node by the TME. Thus, a better understanding of the interactions between immunotherapy and the TME may provide new approaches to improve the response rates of current immunotherapies. As the contributions of the TME in conventional therapies have recently been reviewed [12], we will focus on the developments in understanding the interactions between immunotherapy and the TME.

2. Interactions between immunotherapy and the TME

2.1 Immunomodulatory antibodies

2.1.1 Checkpoint blockade antibodies—Immune checkpoints refer to a series of pathways that can regulate T cell activity as either co-inhibitory or co-stimulatory signals [14], and they function to protect the host against autoimmunity under normal conditions [15, 16]. Increasing evidence suggests that tumors use many of these pathways as important mechanisms to escape antitumor immune responses [6, 17, 18]. Among them, inhibitors targeting programmed cell death protein 1 (PD-1) and its ligand, PD-1 ligand (PD-L1 or B7H1), have shown the most impressive efficacy in clinical trials [3, 4]. PD-1 is mainly expressed on activated T cells [19]. Although PD-L1 expression is limited in normal tissues, it is greatly increased on some tumor cells [20]. Interestingly, PD-L1 expression can be upregulated on many cells if they are stimulated by inflammatory cytokines, especially interferons (IFNs) [20]. PD-L1 engagement of PD-1 on T cells inhibits their activation and induces exhaustion [21]. A paradigm has been proposed suggesting that tumor-expressed PD-L1 inhibits T cells located within the tumor, which leads to a failure of the host rejecting the tumor. This idea is supported by the initial observation that patients with PD-L1–positive

tumor cells are more likely to respond to anti-PD-1 therapy [3]. With the growing number of patient samples, however, some patients with PD-L1-negative tumor cells have also been observed to respond to PD-1/PD-L1-blockade therapies [22]. Additionally, recent retrospective clinical studies show a high correlation between responses to PD-L1 blockade and PD-L1 expression on tumor-infiltrating immune cells [23]. These studies raised the possibility that PD-L1 expression on cells in the TME besides the tumor cells may also play important roles for immune evasion.

In order to increase the response rate to checkpoint blockade therapy, several combination therapies have been developed [24]. Among them, the combination of anti-PD-1 with anti-cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) has shown the best improvement in clinical trials [25–27]. CTLA-4 is another checkpoint molecule mainly expressed on regulatory T cells (Tregs) [28]. Blocking this pathway by anti-CTLA-4 depletes Tregs in tumor tissues, results in the expansion of antigen-specific cytotoxic T lymphocytes. The synergistic effects are mainly restricted to the local TME, as blocking lymphocyte trafficking after tumor is established has no effect on the synergy [29].

2.1.2 Immunostimulatory mAbs—In contrast to PD-1 and CTLA-4 that inhibit immune responses upon activation, the CD40/CD40 ligand (CD40L) pathway represents a different group of immune checkpoints that promote immune responses [30]. CD40 is expressed on B cells, monocytes, and dendritic cells (DCs). CD40L is primarily expressed on activated T cells and platelets [31]. Signaling through CD40 activates antigen-presenting cells and induces co-stimulatory and MHC molecule expression, resulting in increased antigen presentation and T-cell priming [32]. Agonist antibodies to CD40 have been developed to mimic CD40L engagement and can activate immune response both *in vitro* [33–35] and *in vivo* [36–38]; furthermore, several of these antibodies have entered clinical trials and show promising results [39, 40]. Interestingly, although the original paradigm suggests that T-cell priming is increased downstream of CD40 activation, later studies indicate that T cells may be dispensable, at least in some tumor models [39]. Specifically, tumor regression induced by anti-CD40 depends on macrophages, but not T cells, in a mouse model of pancreatic ductal adenocarcinoma. Anti-CD40 activates macrophages, which then translocate to tumor tissues and induce the depletion of tumor stroma, leading to tumor regression [39]. The idea that stromal cells may play a more important role for the efficacy of CD40 agonist antibody treatment is not only supported in tumor models but also in other disease models. Indeed, Bouchlaka et al. has found in a recent study that systemic injection of CD40 agonist antibody together with IL-2 induces a cytokine storm and lethality in aged, but not young, mice [41]. A follow-up study shows that CD40 signaling induces macrophage activation within the visceral adipose tissues [42]. These activated macrophages produce very high TNF levels, leading to a cytokine storm. Interestingly, one possible reason to explain why lethality is found only in aged mice is that they tend to accumulate more fat in their organs than young mice. These possible adverse effects should be taken into account when applying the various CD40 therapies to cancer patients, as the incidence of many cancers tend to be higher in aged people [43]. The CD40/CD40L story is an interesting case demonstrating that the TME can sometimes be the major target of immunotherapy rather than just the tumor cells.

2.2 CAR T cells

The overall CAR T-cell concept is that T cells can be engineered to express tumor-antigen-specific CARs, which serve to activate T cells upon recognizing tumor antigens [44, 45]. A typical CAR consists of a single-chain variable fragment (scFv) linked to an intracellular signaling module. After binding to tumor antigen through the scFv, the CAR activates T cells in an antigen-specific and MHC-independent manner [44]. Clinical trials with CAR T therapy have shown incredible efficacy against acute lymphoblastic leukemia, with a complete response rate of nearly 90% [46, 47]. However, attempts to apply CAR T therapy to solid tumors have been less successful [48, 49], and extensive efforts have been devoted to increasing CAR T cell activity inside solid tumors as well as their targeting specificity.

Since CAR T cells can efficiently lyse tumor cells *in vitro*, one possible reason why CAR T cells do not work as well in solid tumors *in vivo* may be due to the inability of the T cells to penetrate tumor barriers. In a pleural malignancy model, Adusumilli et al. evaluated the efficacy of CAR T therapy after different routes of administration and found that local delivery of CAR T cells into the pleura more efficiently induced complete remission than systemic delivery, requiring 30-fold fewer CAR T cells [50]. Interestingly, although systemic delivery of large CAR T cell numbers can accumulate a similar number of cells within the tumor tissue as the locally delivered T cells, the functional antitumor effects of the systemically delivered CAR T cells are still weaker [50]. These data suggest that the TME can not only hinder T cells from entering the tumor tissues but also rapidly reduce their activity as they penetrate through the tumor.

The TME can form several biological barriers around the tumor tissue to hamper lymphocyte penetration. One of these barriers is the ECM [12]. In order for activated T cells to reach tumor tissues and destroy their targets, they usually produce enzymes that actively degrade the ECM [51]. In an elegant study, Caruana et al. show that one of those enzymes, heparanase, is downregulated in CAR T cells [52]. Heparanase is an endoglycosidase that cleaves the heparan sulfate chains of the heparan sulfate proteoglycans, an important ECM component [53]. Thus, the heparanase deficiency in CAR T cells greatly weakens their ability to penetrate the tumor barrier. Strikingly, CAR T cells engineered to overexpress heparanase show a significantly enhanced capacity to degrade the ECM, resulting in enhanced tumor infiltration and antitumor activity [52].

2.3 Manipulating the chemokine/cytokine profile of the TME

2.3.1 Targeting chemokines—The fact that leukocyte trafficking and migration are tightly regulated by chemokines raises the interesting idea that manipulating the TME chemokine profile may be able to recruit sufficient numbers of effector cells into tumor tissues for tumor destruction [54–56]. Among all chemokines, those that mediate T cell chemoattraction, such as Chemokine (C-C motif) ligand 21 (CCL21) and C-X-C motif chemokine 10 (CXCL10), are of special interest [57].

CCL21, a CCR7 ligand, is an important chemokine that mediates DC and T cell homing to both lymphoid and non-lymphoid tissues [58]. In an early study, intratumoral injection of CCL21 completely eradicates tumors in lung cancer models [59]. Moreover, tumor

regression mediated by CCL21-expressing DCs is accompanied by a significant increase in tumor-infiltrating CD8⁺ T cells, leading to tumor eradication [60]. In another study, Shields et al. knocked down CCL21 in a melanoma model [61] and surprisingly found that tumor cells expressing high CCL21 levels established an immune tolerant TME, which then facilitated tumor progression. In contrast, CCL21-deficient tumors grow much slower and induce tumor-specific T cell responses [61]. The conflicting conclusions from these studies may be due to different TMEs, such as the differing types of tumor models or CCL21-expressing cells. Collectively, these studies suggest that the effector and target cells should be chosen with care when using chemokines to modify the TME.

Another chemokine that recruits T cells is CXCL10, a CXCR3 ligand [62]. In a recent study, da Silva et al. demonstrate that dipeptidylpeptidase 4 (DPP4) is the enzyme responsible for inactivating CXCL10 in tumor tissues [63]. Inhibition of DPP4 enzymatic activity preserves active CXCL10, which increases CXCR3⁺ lymphocyte trafficking to tumor tissues. The number of tumor-infiltrating T cells, which express high CXCR3 levels, is significantly increased after DPP4 inhibition. This study further shows that the DPP4 inhibitor can be used in combination with other immunotherapies, including adjuvant-based immunotherapy and checkpoint blockade molecules [63]. The idea that increasing tumor-infiltrating lymphocytes (TILs) can improve current immunotherapies is attractive, even though the improvements shown in this study are not dramatic. It remains possible that more than one chemokine may be involved.

2.3.2 Targeting cytokines—The overall efficacy of chemokines in tumor immunotherapy are less impressive than other immunotherapies, implying that targeting a single chemokine may not be sufficient to recruit enough lymphocytes to completely control the tumor. In fact, when comparing chemokine profiles in tumors with different sensitivities to immunotherapy, our lab found that several chemokines related to T-cell trafficking were significantly different (unpublished data). In contrast to chemokines, cytokines are a broad group of proteins that can modulate immune responses [64]. Cytokines can directly activate immune effector cells or stimulate tumor stromal cells, leading to the production of chemokines and adhesion molecules for lymphocyte recruitment [65]. These functions suggest that targeting cytokines may also be efficient approaches for tumor immunotherapy depending on various tumor microenvironments.

The most extensively studied cytokines in terms of tumors are the type I IFNs, which are expressed by almost all cells and play important roles in antitumor responses [66]. Although systemic delivery of recombinant type I IFN proteins has been used in the clinic to treat some tumors, such treatment has been associated with high toxicity due to the broad distribution of IFN receptors [66]. Several groups have tested a second generation of IFNs that uses an antibody to guide IFNs specifically to tumor tissues. In one study, Xuan et al. generated a fusion protein linking IFN- α to an anti-CD20 antibody [67] and showed that it induced tumor regression in established tumors with higher efficacy and less toxicity than free IFN- α . This antitumor effect is attributed mostly to direct killing and can be independent of adaptive immunity, as this fusion protein can control xenograft tumors grown on nude mice. Knocking down the IFN receptor on tumor cells greatly reduced the antitumor effect, suggesting that the anti-CD20–IFN- α fusion protein mediates its antitumor

effect mainly by directly inducing tumor cell apoptosis [67]. However, T cells might contribute to the overall antitumor activity when T cells are available. In another study, Yang et al. used an anti-EGFR antibody to guide IFN- β (anti-EGFR-IFN- β fusion protein) into tumors for tumor immunotherapy [68]. Similar to the previous study, the anti-EGFR-IFN- β fusion protein also showed potent antitumor activities. Interestingly, in contrast to the anti-CD20-IFN- α fusion protein that directly induces tumor cell apoptosis at a high dose, the data in the Yang et al. study clearly shows that the antitumor effect of the anti-EGFR-IFN- β fusion protein depends on adaptive immunity. Specifically, the anti-EGFR-IFN- β fusion protein targets DCs to increase antigen cross-presentation, leading to increased T-cell priming in the TME; in support of this, a conditional knockout of the IFN receptor on DCs completely abrogates the antitumor effect [68]. The discrepancy between these studies is not surprising. In fact, a similar contradictory phenomenon was observed when free type I IFNs were used for tumor immunotherapy [66], wherein free IFNs directly acted on tumor cells in some cases, while host immunity was likely required to control tumors in others.

Whether host immunity is required for the IFN effect seems to depend on the level of IFNs within the TME. Since a lower dose of IFN- β cannot occupy all the IFN receptors in the tumor tissue, host immunity is thus required for optimized tumor control [68]. In contrast, a high IFN- β dose delivered locally into the tumor tissues can induce destruction of the tumor vasculature, leading to tumor elimination [69]. The latter process is independent of T, B, or NK cells. Taken together, the interesting lesson learned from these studies is that the immunomodulator dose can result in completely different interactions between immunotherapy and the TME.

Sufficient lymphocyte infiltration into tumor tissues is critical for the success of many cancer therapies [70]. In fact, the presence of TILs correlates with a better prognosis to various antitumor therapies in a multitude of cancers [71–73]. An intriguing property of chemokines and cytokines for cancer immunotherapy is their potential to recruit leukocytes, especially T cells, into tumor tissues and change the TME from immunosuppressive to immunosupportive [13]. IFNs have been considered as such candidates [68]. However, preliminary studies in our lab showed that increased TILs were only observed in some tumor models, likely due to multiple downstream effects of IFNs (unpublished observations). Another candidate that can potently increase TILs in the TME is LIGHT (also known as Tumor Necrosis Factor Superfamily member 14 [TNFSF14]), a co-stimulatory molecule that can regulate T-cell activation [74]. Full-length, membrane-bound LIGHT can be secreted as a cytokine through protease digestion. Studies in our lab have shown that forced expression of LIGHT in tumor cells promotes the formation of lymphoid-like structures for direct T-cell sequestration and activation, leading to tumor regression [75, 76]. LIGHT is a ligand protein that binds to two different receptors, Herpesvirus Entry Mediator (HVEM) and Lymphotoxin beta Receptor (LT β R). While LIGHT binding to HVEM delivers a co-stimulatory signal to T cells [74], LIGHT binding to LT β R, which is commonly expressed on non-lymphoid cells, is critical for the formation of secondary and tertiary lymphoid structures [77, 78]. LT β R plays a pivotal role in lymph node formation and in the organization of distinct T-cell and B-cell zones in secondary lymphoid organs. Signaling via LT β R regulates the expression of various chemokines and adhesion molecules that control the migration and positioning of DCs and lymphocytes in the spleen [79]. Thus, in contrast

to targeting just one specific chemokine, LIGHT activation of LT β R signaling in tumor stromal cells induces the expression of multiple chemokines and adhesion molecules for effective T cell recruitment to the TME. This induction of multiple chemokines makes LIGHT more efficient for lymphocyte recruitment and activation than single cytokine or chemokine. Taken together, extensive studies targeting the TME with cytokines that can induce an immunostimulatory environment support the idea that they may be an effective approach for cancer therapy [80].

2.4 PRR agonists

Pattern recognition receptors (PRRs) are a class of conserved proteins expressed by innate cells that can sense danger signals from pathogens or cellular stress [81]. Signaling through PRRs can induce the expression of immunostimulatory cytokines, leading to the activation of innate and adaptive immunity [82]. Several PRR agonists have been developed to mimic danger signals and have been applied in both preclinical and clinical studies [83]. Among them, CpG has the most potent antitumor activity and has been extensively used in mouse studies, either alone or in combination with other therapies [84]. When targeted to the tumor tissues, an antibody-guided CpG can induce complete tumor regression [85, 86]. DC activation and a systemic antitumor immune response accompany this process. However, it should be noted that the antitumor effects of CpG are more impressive in mouse models than in humans, likely due to differences in the TLR9 expression pattern [87].

Most synthetic PRR agonists are derived from microbe-associated molecular patterns. However, PRR signaling in the TME under physiological conditions were largely unknown until recently when two studies revealed a central role for the stimulator of IFN genes (STING) pathway in recognizing danger signals inside the TME [88, 89]. STING is an adaptor protein that links PPRs to IRF3 activation [81]. Woo et al. show that the STING pathway plays an important role in recognizing tumor-derived DNA, leading to IFN- β production [88]. Deficiency in the STING pathway dampens IFN- β production, resulting in defective tumor control in mice. Interestingly, a complementary study from Deng et al. showed that STING was also critical for recognizing tumor DNA released after radiation therapy [89]; furthermore, they demonstrated that cGAMP, a STING agonist, could amplify the antitumor effects of radiation therapy.

3. Conclusions

The immune response is a dynamic and complex process wherein different mechanisms balance each other in order to protect the host, local tissues and cells, including tumor cells from immune destruction. The TME is one location in which both immunosuppressive and immunosupportive mechanisms converge, but suppressive mechanisms usually take over. The recent success of checkpoint blockades is a proof-of-concept showing that manipulating different signaling pathways can create therapeutics that are more efficient than ever before. On the other hand, relatively low response rates in patients indicate that current immunotherapies are still impeded and not able to release their full potential in many situations. Thus, there is still much room left to improve our understanding of these immunotherapeutic mechanisms. As our knowledge about the TME and immunotherapy

expands, more approaches will undoubtedly emerge to harness the TME and improve our current immunotherapies.

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Abbreviations

CAR	chimeric antigen receptor
TME	tumor microenvironment
PD-1	programmed cell death protein 1
PD-L1	PD-1 ligand
DC	dendritic cell
IFN	interferon
CTLA-4	cytotoxic T-lymphocyte-associated antigen 4
CD40L	CD40 ligand
ECM	extracellular matrix
CCL21	Chemokine (C-C motif) ligand 21
CXCL10	C-X-C motif chemokine 10
DPP4	dipeptidylpeptidase 4
TIL	tumor-infiltrating lymphocyte
HVEM	herpesvirus entry mediator
LTβR	lymphotoxin beta receptor
PRR	pattern recognition receptor
STING	stimulator of IFN genes

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Highlights

- The TME can dampen or enhance the efficacy of immunotherapy.
- Immunotherapy targets both the TME and tumor cells.
- Manipulating the TME can improve current immunotherapies.

Figure 1. Immunotherapy and the tumor microenvironment (TME)

A successful tumor control induced by immunotherapy requires the activation of the immune system, expansion of the effector cells, infiltration of activated effector cells to the tumor tissue, and destruction of the tumor cells. Tumor barriers can greatly dampen those processes, while immunotherapy aims to enhance them. Effector T cells can be inhibited by checkpoint molecules, such as PDL1, expressed in the TME. The inhibition by PDL1 can be overcome by anti-PD1/PDL1. Stimulatory checkpoint antibodies are used to activate immune cells. But some antibody, eg anti-CD40, can also work on stroma cells for optimized tumor control. The ECM forms a barrier preventing T cells reach to the TME for tumor destruction. However, the infiltration can be enhanced by inducing/delivering cytokines/chemokines to the TME.