

Present and future of cancer immunotherapy: A tumor microenvironmental perspective (Review)

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Abstract. Modulation of the tumor microenvironment is becoming an increasingly popular research topic in the field of immunotherapy, and studies regarding immune checkpoint blockades and cancer immunotherapy have pushed cancer immunotherapy to a climax. Simultaneously, the manipulation of the immune regulatory pathway can create an effective immunotherapy strategy; however, the tumor microenvironment serves an important role in suppressing the antitumor immunity by its significant heterogeneity. A number of patients with cancer do not have a good response to monotherapy approaches; therefore, combination strategies are required to achieve optimal therapeutic benefits. Targeting the tumor microenvironment may provide a novel strategy for immunotherapy, break down the resistance of conventional cancer therapy and produce the foundation for personalized precision medicine. The present review summarized the research regarding cancer immunotherapy from the perspective of how the tumor microenvironment affects the immune response, with the aim of proposing a novel strategy for cancer immunotherapy and combination therapy.

Contents

1. Introduction
2. Formation and development of the tumor immunosuppressive microenvironment
3. Heterogeneity of the tumor microenvironment
4. Immunotherapies targeted to the tumor microenvironment
5. Conclusions and perspectives

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1. Introduction

Cancer immunotherapy is emerging as a beneficial tool for cancer treatment by activating the immune system to produce antitumor effects (1). Recently, cancer immunotherapy, particularly immune checkpoint therapy, has progressed and provided novel strategies for the treatment of cancer. The most advanced approach to therapeutically utilize the antitumor activity is via immune checkpoint inhibitors. This strategy has recently achieved notable clinical success in patients with numerous malignant cancer types; for example, in patients with advanced melanoma, the blockade of cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) via the antibody ipilimumab and the inhibition of the programmed death 1 (PD-1) receptor via the antibody nivolumab resulted in improved overall survival time (2,3). In comparison to conventional therapies for cancer, including radiation and chemotherapy, cancer immunotherapy primarily targets the immune system or tumor microenvironment rather than tumor cells themselves, and can induce a synergistic effect in combination therapies (4). However, the efficacy of cancer immunotherapy is limited to only certain patients, due to not all patients responding to these immunomodulatory maneuvers, and there are notable differences between individuals (5). The manner in which to improve the efficacy of patients with cancer is fast becoming the focus of cancer immunotherapy.

There is increasing evidence demonstrating that the differences in the outcome of cancer immunotherapy are attributed to the heterogeneity of the tumor microenvironment (6). The tumor microenvironment consists of tumor cells, tumor-infiltrating immune cells, cancer-associated fibroblasts (CAFs), the tumor vasculature and the extracellular matrix (ECM), which collectively can promote tumor transformation, protect the tumor from host immunity, support tumor growth and invasion, foster therapeutic resistance and provide niches for dormant metastases to thrive (7). The presence of malignant tumor cells initiates a series of changes that can transform the tumor environment into one that can promote cancer progression. The orchestration of these changes involves recruitment and activation of CAFs, migration of immune cells, stroma remodeling, development of tumor vascular networks, upregulation of the suppressive receptors on tumor cells and reprogramming of cell metabolism (8). The complexity of these changes results in the heterogeneity of the tumor microenvironment. Furthermore, the tumor immunosuppressive microenvironment is formed

with the development of tumor proliferation and the increasing heterogeneity of the tumor microenvironment, which may influence the cancer immunotherapy.

In the present review, the progression of tumor microenvironment heterogeneity, its development and the effect on immunotherapy, and the present and future of cancer immunotherapy from the perspective of the tumor microenvironment are summarized.

2. Formation and development of the tumor immunosuppressive microenvironment

Although tumor cells initially instigate the formation of the tumor microenvironment, the mutual influence and co-evolution among tumor cells, stroma components and immune cells continuously promote the development of immunosuppressive progress (7). Tumor cells utilize the negative regulatory mechanism of the immune system, in order to establish a full range of immunosuppressive states in the tumor microenvironment, which will create the conditions for their survival and development (9).

Effects of tumor cells on the tumor microenvironment. Tumor cells promote immune escape by forming an immunosuppressive microenvironment. Antigens expressed on the surface of tumor cells are usually in a defective state. The decreased or absent expression of major histocompatibility complex class I restricts the activation of the tumor-infiltrating lymphocytes (TILs) (9). The existing suppressive signal transduction in the immune system could be utilized by tumor cells, including PD-1, ligand programmed death-ligand 1 (PD-L1), CTLA-4, cytotoxic T lymphocyte activation gene 3 (LAG-3), T cell immunoglobulin domain 3 mucin domain protein 3 (Tim-3) and cluster of differentiation (CD)160 (10), which may gradually result in T cell exhaustion (11); therefore, the inhibited function of TILs in the tumor microenvironment results in tumor immunosuppression. Tumor cells can also secrete immunosuppressive factors, including transforming growth factor- β (TGF- β), interleukin-6 (IL-6), IL-10, vascular endothelial growth factor (VEGF) and matrix metalloproteinase (5,9), in order to cause tumor-infiltrating immune cells to inhibit their antitumor effect.

Furthermore, abnormal metabolism of tumor cells can also enhance the immunosuppressive effect of the tumor microenvironment. Normal cells acquire energy primarily through oxidative phosphorylation, and a limited number use glycolysis, which can be inhibited under aerobic conditions; however, the method by which tumor cells acquire energy is different, and is termed aerobic glycolysis or 'the Warburg effect' (12). In this condition, tumor cells maintain an increased rate of glycolysis even in the presence of adequate oxygen. This was initially considered to be a strategy to adapt to hypoxia, but it is now widely accepted that this shift of energy metabolism is not only to produce the necessary resources for the biosynthetic activities of tumor cells, but also to generate numerous acidic products to form the acidic tumor microenvironment, which results in immunosuppression (13). In addition to aerobic glycolysis, in order to rapidly proliferate, tumor cells are also required to increase the demand for amino acids. Among them, glutamine, methionine, tryptophan, arginine and leucine

are essential for the tumor cells as metabolic regulators in supporting cancer cell growth (14), and the tumor cells are more competitive for these metabolic resources compared to tumor-infiltrating immune cells. Additionally, indoleamine 2,3-dioxygenase (IDO), which is highly expressed by tumor cells, is a rate-limiting enzyme of tryptophan metabolism that has regulatory effects on T cells resulting from tryptophan depletion in tumor microenvironments (15). In addition, the tryptophan metabolites, including 3-hydroxyquinolinic acid via the kynurenine pathway, can also directly inhibit T effector cells (16). Furthermore, the metabolic interplay between tumor cells and immune cells can contribute to the exhaustion of TILs and immunosuppression (17).

Effect of CAF on the tumor microenvironment. Fibroblasts are the dominant component of the tumor stroma (18). The important functions of fibroblasts include deposition of ECM, regulation of epithelial differentiation, regulation of inflammation and participation in wound healing. Activated fibroblasts, termed CAFs, are also critical for the formation of the tumor microenvironment, particularly for solid tumor types (19). Fibroblasts are a critical determinant in the tumor malignant progression and represent an important target for cancer therapies.

Fibroblast activation protein α (FAP α) is selectively expressed on the surface of CAF, and the majority of epithelial tumor types exhibit high expression of FAP α (20). FAP α has a dual role as a protease and in signal transduction. The former refers to its involvement in remodeling the construction of the microenvironment stroma by degrading fibronectin and changing the structure of collagen, in order to enhance the invasion ability of tumor cells along the fibers (21). The latter refers to its involvement with TGF- β , VEGF, stromal cell-derived factor-1, platelet-derived growth factor, hepatocyte growth factor and other cytokines, which could conduct signals to promote tumor growth, prevent immune cell recruitment, inhibit the function of tumor-infiltrating immune cells and enhance ECM proliferation for the formation of a tumor biological barrier (22). Furthermore, the desmoplastic stroma could then surround the tumor cells and prevent access of antitumor drugs (23). Kraman *et al* (24) confirmed that depleting FAP-expressing cells could reduce the occurrence of hypoxic necrosis *in vitro* and permit the immunological control of growth *in vivo*; therefore, FAP-expressing cells are an important immunosuppressive component of the tumor microenvironment.

Effect of the tumor abnormal vascular structure on the tumor microenvironment. Tumor angiogenesis is an important process in the tumor microenvironment. Emerging evidence indicates that angiogenesis and immunosuppression frequently occur simultaneously in response to different stimuli (25). Tumor neovascularization is primarily leaky, tortuous, dilated and saccular. The structural and functional abnormalities of tumor blood vessels result in the impaired blood supply and interstitial hypertension or high interstitial fluid pressure (IFP) (26). The perfusion of tumor tissues is further hampered by the formation of hypoxia and high IFP in the microenvironment of malignant tumor types. The imbalance between the promotion and inhibition of angiogenic factors contributes to

the abnormal structure of the tumor vasculature (27). Among a whole range of pro-angiogenic factors that participate in physiological or pathological angiogenesis, VEGF is the most important and also a potent angiogenic factor that can increase the density of tumor blood vessels (28). The sufficient expression of VEGF depends on the oxygen concentration in tissues. There are a variety of transcription factors in tumor tissues, including hypoxia inducible factor (HIF), which can upregulate the VEGF under hypoxic conditions (29).

Therefore, combined with the abnormal metabolism of tumor cells, the suitable microenvironment for tumor survival is characterized by low pH, hypoxia and high IFP, which is considered to aid in rendering tumor microenvironments hostile to the immune cells. The low pH or acidic microenvironment can accelerate the differentiation of regulatory T cells (Tregs) and the development of myeloid-derived suppressor cells by promoting the production of IL-2, inhibiting the infiltration of T cells and inducing their apoptosis, and activating tumor-associated macrophages (TAMs), in order to secrete a large number of cytokines to promote tumor angiogenesis (30). Hypoxia can promote the formation of tumor blood vessels by upregulating the expression of pro-angiogenic factors such as HIF-1, VEGF, IL-6, TNF α , and tyrosine kinase receptor Tie2 (31), and increase the malignancy and trigger tumor metastasis by inducing epithelial-mesenchymal transition (EMT) (32). High IFP can prevent immune cells from recruiting to the tumor tissue and interfere with drug delivery. Additionally, high IFP and pro-angiogenic factors can also evoke lymphangiogenesis, which is the important physiological basis of lymphatic metastasis (26). Collectively, these vascular abnormalities result in a complex immunosuppressive microenvironment, which may promote the survival and metastasis of tumor cells.

3. Heterogeneity of the tumor microenvironment

The formation of the tumor immunosuppressive microenvironment is a dynamic and complex process. In addition to the significant heterogeneity of tumor cells, heterogeneity of stroma components and immune cells can also increase the complexity of the tumor microenvironment (8). Additionally, tumor progression, pathological stage, treatment efficacy and prognosis are also associated with the tumor microenvironment, which determines the antitumor response and remains a notable obstacle for the treatment of cancer (33). Therefore, due to the presence of tumor microenvironmental heterogeneity, the degree of the antitumor immune response in different individuals is variable.

The heterogeneity of stroma components in the tumor microenvironment is common. In pancreatic, breast and prostate cancer, and other solid tumor types with a high content of CAF, the formation of high-density ECM will increase the tumor IFP, and hinder the absorption of chemotherapy drugs and the infiltration of immune cells (19). Additionally, tumors with different types, locations and stages also exhibit tumor vascular heterogeneity (34). For instance, pancreatic ductal adenocarcinoma (PDA) is a stroma-rich cancer type and its tumor environment has been demonstrated to consist of an abundance of stroma containing numerous cells types, but predominantly pancreatic stellate cells (PSCs) (35). The

vasculature in PDA is notably influenced by the excessive desmoplasia caused by the secretion of PSCs and finally leads to hypovascularity and perfusion impairment (36), which indicates that the role of tumor angiogenesis in the progression of pancreatic cancer is less notable compared with that of other hypervascular tumor types, including liver cancer. Kashiwagi *et al* (37) reported that murine melanoma cells were intracranially and subcutaneously implanted into mice, and the results determined that the vascular density of the intracranial tumor types was increased compared with that of subcutaneous tissues, but that the diameter was reduced. Compared with early renal cell carcinoma, advanced renal cell carcinoma exhibits an increased endothelial cell proliferation fraction, while presenting with a reduced microvessel density, which indicates that the heterogeneity in angiogenic activity is associated with tumor stage (38). Additionally, these structural abnormalities of tumor vasculature contribute to the spatial and temporal heterogeneity in tumor blood flow, and solid pressure generated by proliferating tumor cells compresses intratumor blood and lymphatic vessels, which further impairs not only the blood flow, but also the lymphatic flow (39); therefore, the heterogeneity in the stroma of tumor microenvironment requires consideration regarding the efficacy of immunotherapies.

The recruitment, differentiation and location of immune cells in the tumor microenvironment are variable among different tumor types, and their heterogeneity is also affected. Chevrier *et al* (40) conducted mass cytometry for high-dimensional single-cell analysis in order to produce an in-depth human atlas of the tumor immune microenvironment in patients with clear cell renal cell carcinoma, and this demonstrated the immune cell diversity in the tumor ecosystem and the fact that a number of specific immune signatures could function as biomarkers associated with progression-free survival. Additionally, the innate immune landscape in early lung adenocarcinoma indicates that the heterogeneity of immune cells may begin to form at an early tumor stage and evolve with the progression of the tumor stage, gradually compromising the antitumor immunity (41). Furthermore, the dominant types of immune cells infiltrated in tumor microenvironments are also different. TAMs are among the most frequently located cells in the pancreatic tumor microenvironment, while the majority of other tumor types are primarily dominated by TILs (42); therefore, the usage of agonist CD40 monoclonal antibody in PDA can activate and recruit a large number of macrophages, which are tumoricidal and could facilitate the depletion of the tumor stroma (43). The tumor microenvironment includes a complex network of immune T-cell subpopulations, and the state of activation, the location of infiltration and the density of the tumor stroma could be different (44). Therefore, due to the heterogeneity of immune cells in the tumor immune microenvironment, Chen *et al* (45) divided it into three phenotypes according to the distribution of immune cells, as follows: i) Immune-inflamed phenotype, where intratumor infiltration of CD4⁺ and CD8⁺ T cells, and parenchyma and stroma can be observed in a large number of immune cells; ii) immune-excluded phenotype, where immune cells cannot penetrate the parenchyma and only exist in the stroma; and iii) immune-desert phenotype, which a paucity of tumor-specific T cells are located in the parenchyma or

stroma. Among them, the first two phenotypes are associated with non-inflamed tumor types. This classification explains the heterogeneity of immune cells in the tumor microenvironment, indicating that the tumor microenvironment can be modified by changing the immune phenotype, and providing a theoretical basis for personalized immunotherapy.

4. Immunotherapies targeted to the tumor microenvironment

The complexity and heterogeneity of the tumor immunosuppressive microenvironment increases the difficulty of cancer immunotherapy and is an important reason for the variable efficacy in immunotherapies. Unlike directly targeting the tumor cells, the tumor microenvironment represents an increasingly popular therapeutic target with a decreased risk of resistance and recurrence due to the genetically stable stromal cells (46). Recently, the immune checkpoint inhibitors have provided new hope and have become a focus of current cancer immunotherapy (47). The antitumor immunity points of the tumor microenvironment also have other beneficial targets, and application together with conventional cancer therapies can also provide a survival benefit for increased numbers of patients with cancer. The association between the heterogeneity of the tumor microenvironment and the main microenvironment-targeted therapies is demonstrated in Fig. 1; therefore, the combination or 'cocktail' therapy for cancer provides an increased number of advantages compared with monotherapy, and has become an important method to improve the efficacy of tumor immunotherapy (48).

Immune checkpoint blockade. Immune checkpoint inhibitors are strategies for activating immune function and normalizing the tumor microenvironment. Immune checkpoint inhibitors have become an effective means of treating numerous tumor types (49). The anti-PD-1/PD-L1 monoclonal antibody has been successfully used in clinical application and has already been approved for use in numerous cancer types, including melanoma, non-small cell lung cancer, kidney cancer and bladder cancer (50). Clinical trials are currently being used to determine the success of the application of the anti-PD-1 antibody (nivolumab) for different malignant tumor types, and the objective response rate (ORR) has been found to be variable: 32% of melanoma, 29% of renal cell carcinoma, 17% of non-small cell lung cancer (33% of squamous cell carcinoma and 12% of non-squamous cell carcinoma) (51), and only 13.3% of head and neck cancer (52). The mechanism of inhibitor therapy is the activation of T cells, which requires an adequate number of TILs; therefore, the heterogeneity of the antitumor immune response is directly associated with the density of TILs. According to Teng *et al* (53), the tumor microenvironment could be stratified into four different types based on the presence or absence of TILs and PD-L1 expression, as follows: i) Type I (TILs⁺PD-L1⁺), where the tumor microenvironment is PD-L1⁺, with TILs driving adaptive immune resistance, indicating that it may benefit from a single-agent anti-PD-1/L1 blockade; ii) type II (TILs⁺PD-L1⁻), where the tumor microenvironment is PD-L1⁻, with no TILs, indicating immune ignorance; iii) type III (TILs⁻PD-L1⁺), where the tumor microenvironment is PD-L1⁺, with no TILs, indicating intrinsic induction and that the recruitment of

TILs is necessary; and iv) type IV (TILs⁺PD-L1⁻), where the tumor microenvironment is PD-L1⁻, with TILs, indicating the role of other suppressor pathways in promoting immune tolerance. For type II tumors, due to their inability to produce an antitumor immune response in the tumor microenvironment, the recruitment of T cells should be a priority. This stratification of the tumor microenvironment can predict the clinical efficacy of anti-PD-1/PD-L1 therapies and enable the optimal combination of cancer therapies tailored to target different tumor microenvironments (54). Furthermore, the latest research demonstrated that the expression of PD-L1 on the minimal residual disease would increase when the tumor recurs and acquires treatment resistance, while the proportion of effector cells could consistently express increased PD-1 and Tim-3 expression in the tumor microenvironment (55). This indicates that the expression of immune checkpoints should be monitored dynamically, and that the combination treatments may be valuable for improving efficacy and preventing recurrence in patients with tumors.

Recently, anti-PD-1 antibody Keytruda (pembrolizumab) has been approved by the FDA to treat solid tumor with microsatellite instability-high or mismatch repair-deficient (56). This approval confirms the important position of the immunotherapy-targeted tumor microenvironment in the cancer therapies and produces a foundation for cancer immunotherapy as a major part of the combination therapy strategy for different tumor types. With the success of PD-1/PD-L1 inhibitors in cancer immunotherapy, combination therapy with other immune checkpoints has received increasing attention in order to achieve greater clinical benefit. The combination therapies, including dual immune checkpoint inhibitors, are undergoing clinical trials. Clinical studies have demonstrated that the anti-PD-1/PD-L1 antibodies integrated with CTLA-4 inhibitors can increase the therapeutic efficacy and the percentage of responders in the treatment of advanced melanoma, indicating that the combination of immune checkpoint inhibitors can significantly enhance antitumor immunity (57). Currently, the indications and safety of PD-1/PD-L1 inhibitors in combination with CTLA-4 inhibitors for potential usage have also been investigated (58), and this combination was approved by the US Food and Drug Administration (FDA) for patients with BRAF V600 wild-type, unresectable or metastatic melanoma. Recently, a phase II clinical trial (CheckMate 069) indicated that the combination of first-line nivolumab plus ipilimumab could result in improved outcomes compared with first-line ipilimumab alone in patients with advanced melanoma, and the 2-year survival rates were 63.8 and 53.6%, respectively (59). Additionally, Wei *et al* (60) demonstrated the distinct underlying mechanisms of anti-PD-1 and anti-CTLA-4 checkpoint blockade therapies using a mass cytometry-based systems approach to identify different subsets of exhausted T cells, which could improve the understanding of why the combination checkpoint blockade therapies are more effective than monotherapy. In other words, this combination could overcome the heterogeneity of TILs. Additionally, anti-PD-1/PD-L1 treatment can be used in combination with the inhibition of other immune checkpoints. Notably, the inhibition of PD-1 can stimulate other immune checkpoints expressed on T cells and increase the resistance of anti-PD-1 therapies, including Tim-3 (61,62) and LAG-3 (63), providing a theoretical basis for

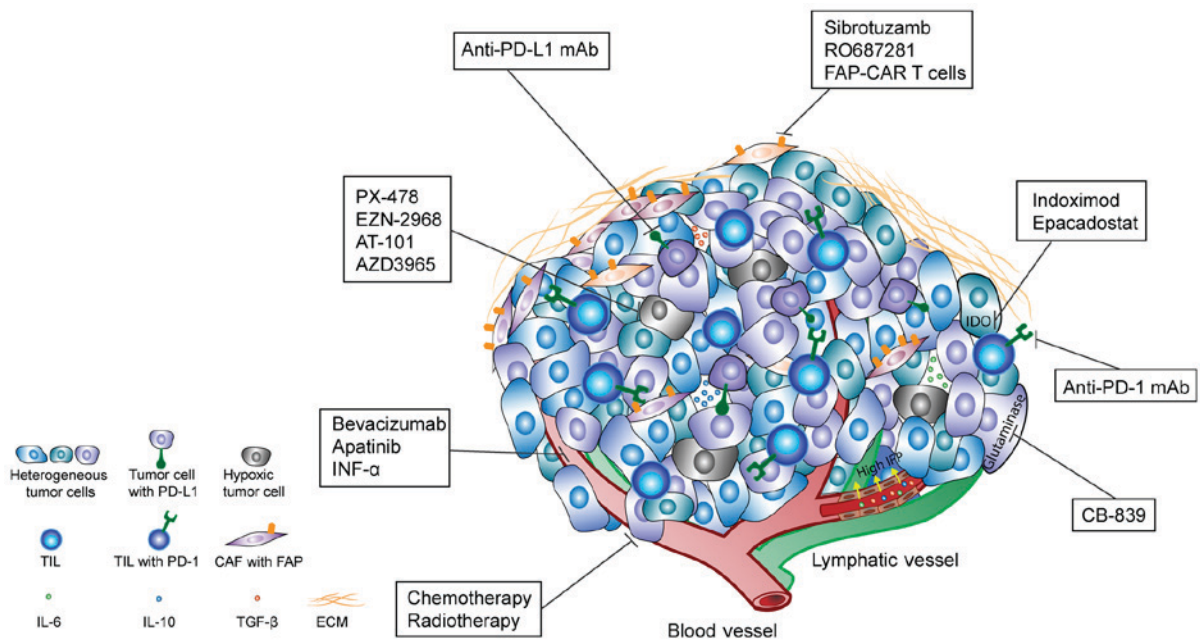


Figure 1. Heterogeneity of the tumor microenvironment and the main microenvironment-targeted therapies. Tumor cells, expression of biomarkers, oxygen concentration, pH, IFP, angiogenesis, metabolism, ECM and other intra- and extra-tumor characters exhibit notable heterogeneity. Tumor cells can secrete factors into the ECM, including TGF-β, IL-6 and IL-10, in order to inhibit the function of TIL and result in tumor immunosuppression. These corresponding therapies include: Anti-PD-1 and anti-PD-L1 antibody targeting the immunosuppressive microenvironment; IDO inhibitor (epacadostat and indoximod) and glutaminase inhibitor (CB-839) targeting the tumor abnormal amino acids metabolism; hypoxia inducible factor 1α inhibitors (PX-478 and EZN-2968), B-cell lymphoma 2 inhibitor (AT-101) and monocarboxylate transporter inhibitor (AZD3965) targeting the hypoxic tumor cells in the hypoxic tumor microenvironment; anti-angiogenic inhibitors (bevacizumab, INF-α and apatinib) and FAPα inhibitor (sibrotuzumab, RO6874281 and FAP-CAR T cells) targeting the regulation of tumor stroma; and combination therapies with chemotherapy, radiotherapy and other therapies. IFP, interstitial fluid pressure; TGF-β, transforming growth factor-β; IL, interleukin; PD-1, programmed death 1; PD-L1, PD-ligand 1; IDO, indoleamine 2,3-dioxygenase; INF-α, interferon-α; FAP, fibroblast activation protein; FAP-CAR, FAP-specific chimeric antigen receptor; TIL, tumor-infiltrating lymphocyte; CAF, cancer-associated fibroblast; ECM, extracellular matrix.

the combination therapy. Currently, all 5 clinical trials regarding the combined inhibition of Tim-3 and PD-1 are undergoing recruitment (NCT02817633, NCT03099109, NCT02608268, NCT03066648 and NCT02947165). Additionally, 8 clinical trials containing the combination of LAG-3 and PD-1 antibodies are also recruiting (NCT02658981, NCT01968109, NCT02061761, NCT03005782, NCT02676869, NCT02966548, NCT02488759 and NCT02060188) (<https://clinicaltrials.gov/>). Claudin-low breast cancer, an aggressive subtype that confers poor prognosis and exhibits a high expression level of EMT genes, has been reported to recruit Tregs to the tumor microenvironment, which inhibits an effective antitumor immune response (64), and this study indicated that future clinical trials should target the immunosuppressive elements in the tumor microenvironment in combination with immune checkpoint blockades in order to increase the efficacy.

However, the combinations of immunotherapies are not always successful. Two independent studies (65,66) demonstrated that the concurrent administration of the anti-PD-1 antibody and the agonist antibody to OX40, a tumor necrosis factor family costimulatory receptor that could promote the activation and expansion of T cells, had an adverse effect on the antitumor response of OX40 stimulation and resulted in poor outcomes in mice. Additionally, the antitumor effect of sequential anti-OX40 and anti-PD-1 combination is controversial between the two studies; therefore, the sequence and timing of immunotherapies are critical to the success of combination therapy, and require further investigation prior to clinical use.

The appropriate selection of immune checkpoints inhibitors or other immunotherapies for personalized combination therapy is an indispensable option and the underlying mechanism requires further investigation.

Tumor metabolism regulation. Improving the immunosuppression of the tumor microenvironment by regulating tumor metabolism is a popular research topic. Immunotherapy with inhibition of IDO to inhibit tumor metabolism has achieved notable results. Currently, there are two primary drug types directed against IDO: i) Highly potent IDO inhibitor that directly inhibits the degradation of tryptophan, such as the drug epacadostat (67); and ii) IDO pathway inhibitor that inhibits the degradation of tryptophan and also reverses IDO-mediated immune suppression, such as the drug indoximod (68). Additionally, the safety and clinical efficacy of these two drug types have also been confirmed in recent clinical trials (69,70). Significant breakthroughs in the studies of tumor cell metabolism have also provided novel options to combine with immunotherapies. IDO inhibitor epacadostat and anti-PD-1 antibody pembrolizumab have been demonstrated to have a promising clinical efficacy and safety for advanced cancer types in clinical trials improved objective response rate and disease control rate (71). Furthermore, a clinical trial has been initiated to evaluate the preliminary efficacy of indoximod combined with immune checkpoint inhibitors (NCT02073123); therefore, it is possible to conclude that IDO inhibitors have a potential synergistic effect with

immune checkpoint inhibitors. Additionally, due to the success of IDO inhibitors, other tumor-associated amino acids with abnormal metabolism are gaining increasing attention. For example, clinical trials of glutaminase inhibitor CB-839 alone (NCT02861300) and combined with nivolumab (NCT02771626) for the treatment of solid tumor types are also under recruitment.

Hypoxic and acidic microenvironments are associated with the consequence of tumor metabolism, and reversing them is also being used as a strategy to regulate the tumor microenvironment. PX-478 is a selective inhibitor that can suppress hypoxia-induced HIF-1 α levels (72). In a previous clinical trial, patients with refractory solid tumor types were treated with EZN-2968, a locked nucleic acid antisense oligonucleotide against HIF-1 α (73). The number of cases was too small to veritably reflect the efficacy, but even so, there were a number of patients who responded to the treatment. Additionally, lactate can specifically upregulate B-cell lymphoma 2 (Bcl-2) through translational control and can promote resistance to the glucose starvation of tumor cells (74). In the clinical trial of abnormal lactate metabolism, it was determined that the use of cisplatin and etoposide in combination with Bcl-2 inhibitor AT-101 could enhance the antitumor effect (75). Furthermore, the clinical trial regarding the transport of lactic acid, pyruvate and other metabolites, and monocarboxylate transporter inhibitor AZD3965, which could prevent the release of lactic acid by hypoxic tumor cells and then inhibit their growth and survival, is also recruiting (NCT01791595) (76).

Tumor stroma regulation. Regulation of the heterogeneous stroma components in the tumor microenvironment could modulate its immunosuppressive conditions. Promoting normalization of tumor blood vessels and weakening the function of CAFs are the key roles in effectively transporting oxygen, drugs or immune cells and other components to tumor tissues, reducing the tumor proliferation and invasion (77).

The first anti-angiogenic therapy, Avastin (bevacizumab), was approved by the FDA in 2003. Considerable effort into the development of anti-angiogenic therapies has been undertaken, and a number of these inhibitory agents have been approved for clinical use against a number of cancer types; however, tumors can frequently escape the effects of these agents, causing the disease to eventually progress (78). Therefore, anti-angiogenic therapy may serve a role in vessel normalization, in order to increase immune cell infiltration and enhance the efficacies of immunotherapies (79). The combined treatment of bevacizumab and interferon- α has also entered phase 2 and 3 clinical trials and demonstrated improved clinical efficacy in metastatic renal cell carcinoma, confirming the clinical value of combined application of anti-vascular therapy and immunotherapy when compared with monotherapy (80,81). Furthermore, the clinical trials combined with VEGF receptor tyrosine kinase inhibitor apatinib and PD-1 inhibitor are currently recruiting, in order to evaluate the efficacy for the treatment of gastric cancer types (NCT03092895 and NCT02942329).

Additionally, there are several studies have also attempted to improve the immunosuppressive condition by modulating the function of CAFs in the tumor microenvironment. The humanized monoclonal antibody sibrotuzumab, which is directed against the specific antigen FAP α on

CAFs, could block its dual function of protease and signal transduction, then inhibit tumor progression, invasion and metastasis progression, and reduce its negative regulation of antitumor immunity. In phase 1 and 2 clinical trials using sibrotuzumab alone (82,83), only a limited number of patients achieved stable disease and the expected clinical response rate was not met; however, whether the efficacy of the treatment could be improved by combining it with other immunotherapies requires further investigation. RO6874281 is a bispecific antibody containing an IL-2 variant targeting FAP α . The IL-2 variant does not bind to Tregs, which could prevent the immunosuppressive capacity of the Tregs (84). By specifically targeting FAP α , the antibody could not only increase the local IL-2 concentration, in order to activate the immune effector cells in the tumor microenvironment, but also inhibit the deterioration of the tumor microenvironment by directly blocking FAP α . Due to the consistent expression of FAP in the tumor stroma, modified T cells that express a FAP-specific chimeric antigen receptor have also been engineered to inhibit the tumor proliferation and augment host immunity (85-87). Clinical trials regarding monotherapy (NCT02627274) and combination with other immunotherapies (NCT03063762) are currently being conducted to evaluate the safety, tolerability and preliminary therapeutic efficacy.

Therefore, studies regarding the stroma components in the tumor microenvironment will continue to be conducted in order to improve the effectiveness of the tumor immunosuppression and provide a novel alternative approach for personalized combination therapy.

Combination with chemotherapy and radiotherapy. Immunotherapy combined with traditional radiotherapy and chemotherapy has received increasing attention. Different chemotherapeutic drugs have different immunological mechanisms underlying the efficacy of the cancer therapy (88), including: i) Increased immunogenicity resulting in tumor cell apoptosis from drugs such as anthracycline, 5-fluorouracil (5-Fu) and oxaliplatin; ii) direct immunostimulation activating the tumor immunity of immune effector cells from such as gemcitabine, paclitaxel and pemetrexed; and iii) indirect immunostimulation inhibiting the immunosuppressive cells from drugs such as 5-Fu, cyclophosphamide and oxaliplatin. Additionally, radiotherapy can also influence the tumor immune response. Tumor cell death from irradiation can enhance the antitumor immunity by inducing antigen expression on tumor cells and activating lymphocytes (89,90), and by generating the abscopal effect (91). Chemotherapy or radiotherapy can eliminate a number of the tumor cells in advance, then expose a large number of the tumor antigens and neoantigen products in the microenvironment, which could recruit increased numbers of immune effector cells, and finally improve the immunosuppressive state of the tumor microenvironment. Currently, the rationale for combining immunotherapy with chemotherapy and radiotherapy has been verified (92), and preclinical studies have also been well investigated (93,94); therefore, it is plausible that combining immunotherapy with standard conventional therapies, including chemotherapy or radiotherapy, will provide synergistic antitumor effects (95), but

the most beneficial dose and the appropriate time requires investigation (89,96).

5. Conclusions and perspectives

The tumor immunosuppressive microenvironment is in a dynamic status and is coordinated by multiple immunosuppressive signals in the regulatory network. In the course of clinical cancer treatment, due to the tumor types, stages, histological features and other microenvironment-associated factors, heterogeneity of the tumor microenvironment will cause immunosuppression and then result in the differences in the efficacy of immunotherapies. Despite the success in targeting non-tumor cell components, including immune checkpoint blockade, focusing on a single immunosuppressive target is ineffective in the majority of patients with cancer. Even among the cancer types that do respond to the immune checkpoint inhibitors, including melanoma, non-small cell lung cancer and renal cell cancer, few patients exhibit objective control of tumor progression. Following blocking or inhibiting of one immunosuppressive signal, the tumor will compensate through other mechanisms to generate the resistance and reduce the efficacy of this immunotherapy. The association between heterogeneity of the tumor microenvironment and the immunotherapy response remains a significant challenge.

In the future, immunotherapy may be required to be tailored for each patient with cancer according to the tumor microenvironment. The application of novel immune biomarkers and the ability to monitor and evaluate the tumor microenvironment by novel strategies, in order to improve early cancer diagnosis and predict the therapeutic efficacy and prognosis, requires further investigation. Personalized immunotherapy based on individual genetic, molecular and immune profiling has the potential to produce the most optimized outcomes for patients with cancer, but healthcare costs must be kept in an affordable range. Notably, the combinations must be designed in a rational and safe manner, and further clinical trials should be conducted, in order to verify the combination therapies prior to progressing to clinical use.

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Authors' contributions

YY was responsible for the literature search and manuscript preparation. JC was responsible for study design, manuscript co-writing and correction. Both authors revised the article and approved the final version for publication.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

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Competing interests

The authors declare that they have no competing interests.

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