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REVIEW – CANCER RESEARCH

The role of PD-1/PD-L1 axis and macrophage in the progression and treatment of cancer

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Abstract

Purpose During the past decades, PD-1/PD-L1 axis blockade has become a remarkable promising therapy which has exerted durable anti-tumor effect and long-term remissions on part of cancers. However, there are still some patients which do not show good response to the PD-1/PD-L1 targeted monotherapy. Till now, the widely accepted anti-tumor mechanism of PD-1/PD-L1 blockade is rejuvenating T cells, there is lack of studies which focus on other components of the tumor environment in the treatment of cancer with PD-1/PD-L1 blockade, especially the complicated relationship between macrophages and PD-1/PD-L1 pathway during the progression and treatment of cancer.

Methods The relevant literatures from PubMed have been reviewed in this article.

Results Even though the widely accepted anti-tumor mechanism of PD-1/PD-L1 blockade therapy is rejuvenating T cells, latest studies have demonstrated the complicated relationship between macrophages and PD-1/PD-L1 pathway during the progression and treatment of cancer and their engagement has serious implications for the therapeutic effect of PD-1/PD-L1 blockade agents. We focus on the dual regulation mechanisms between PD-1/PD-L1 axis and macrophages, and further clarify the mechanisms of resistance to PD-1/PD-L1 inhibitors related with macrophages.

Conclusion The combination of PD-1/PD-L1 blockade and macrophage-targeted therapy will exert synergetic anti-tumor effect and shape the future of cancer immunology and immunotherapy.

Keywords PD-1 · PD-L1 · Macrophage · T cell · Combination therapy

Introduction

The 2018 Nobel Prize in Physiology or Medicine was rewarded to James P. Allison and Tasuku Honjo "for their discovery of cancer therapy by inhibition of negative immune regulation". Their work on the programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) immune checkpoints revealed that these pathways serve as "brakes" on the immunity, and illustrated that immune checkpoint blockade could reactivate exhausted T cells to eradicate cancer cells more effectively. More and more studies during the recent years have revealed the remarkable therapeutic effect of immune checkpoint inhibitors.

PD-1/PD-L1 axis

PD-1 (also known as CD279) is an inhibitory receptor mainly expressed on activated T cells, certain B cells, natural killer cells (NKs), dendritic cells (DCs), and macrophages (Sharpe and Pauken 2018; Lim et al. 2015; Huang et al. 2009) PD-1 expression on macrophages increases over time and with disease progression (Gordon et al. 2017). PD-1 has two ligands: PD-ligand 1 (PD-L1, also termed CD274 and B7-H1) and PD-L2 (also termed CD273 and B7-DC). In addition to tumor cells, PD-L1 is widely expressed on different cells, such as haematopoietic cells, including T cells, B cells, DCs, macrophages and non-haematopoietic cells, like stromal and vascular endothelial cells, pancreatic islet cells,

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keratinocytes, and placental syncytiotrophoblasts (Sharpe and Pauken 2018), while PD-L2 is restrictively expressed on DCs, macrophages and B cells. PD-L1 is more commonly expressed even though both of them can be expressed on tumor cells (Sharpe and Pauken 2018). When engaged with PD-L1 or PD-L2, PD-1 is phosphorylated at tyrosine residues, inducing binding of protein tyrosine phosphatases (PTPs) which can dephosphorylate kinases, affecting downstream pathways such as phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt), phospholipase Cγ (PLCγ), extracellular-signal regulated kinase (ERK), VAV and RAS pathways (Riley 2009; Parry et al. 2005; Yokosuka et al. 2012; Hui et al. 2017; Patsoukis et al. 2012), thereby causing immunosuppression through inhibiting T-cell activation, proliferation, survival, and cytolytic function and contribute to cancer progression.

A majority of cancer patients do not respond to PD-1/PD-L1 targeted therapy

Blocking antibody targeting PD-1 and PD-L1 is called checkpoint blockade therapy which can partially reactivate the biological effects of PD-1$^+$ T cells (Wong et al. 2007; Ahmadzadeh et al. 2009; Fourcade et al. 2010; Matsuzaki et al. 2010). During the past decade, monoclonal antibodies (mAbs) that target PD-1, such as nivolumab and pembrolizumab, together with avelumab, atezolizumab, and durvalumab which target PD-L1 have exhibited remarkable clinical responses in a broad spectrum of cancer patients, including non-small-cell lung cancer (NSCLC), melanoma, Hodgkin lymphoma, renal cell carcinoma (RCC), head and neck squamous cell carcinoma, bladder cancer, and pediatric solid tumor (Sharpe and Pauken 2018; Page et al. 2014; Topalian et al. 2015). The clinical outcomes are promising, however, the majority of patients do not exhibit durable remission, and some tumors have been completely resistance to checkpoint blockade (Sharpe and Pauken 2018). Although the mechanisms of regulating T-cell suppression by PD-1/PD-L1 pathway are fully demonstrated, little is known about the influence of this axis on other components of the tumor microenvironment, such as macrophages. Act as the important regulators of homeostatic tissue and tumor microenvironment, macrophages can phagocytose tumor cells effectively (Alvey et al. 2017), whereas accumulating evidence have demonstrated macrophages could repress the antitumor effects of therapeutic antibodies through immunosuppression and their presence correlated with poor prognosis in cancers (Ruffell and Coussens 2015). The latest study has proved that PD-L1 expression on tumor-infiltrating immune cells, especially macrophages, was more closely related with clinical responses to anti-PD-L1 therapy than PD-L1 expression on tumor cells (Zou et al. 2016), and the suppression of tumor metastasis induced by anti-PD-1 antibodies (anti-PD-1) could result from macrophage polarization.

Macrophages

Macrophage polarization

As antigen-presenting cells (APCs), macrophages express lots of adhesion molecules for T cell (Peranzoni et al. 2018), and the macrophages that infiltrate into tumor islets are called tumor associated macrophages (TAMs). Depending upon their microenvironment, macrophages mainly polarize toward "M1" (classically activated macrophages) or "M2" (alternatively activated macrophages) phenotype (Martinez and Gordon 2014; Xue et al. 2014). Induced by microbial factors and Th1 cytokines, M1 macrophages (M1s) can secret TNF-α, IL-12, nitric oxide and promote pro-inflammatory response, mediate antimicrobial defense, tissue destruction and anti-tumor resistance (Martinez and Gordon 2014; Sica and Mantovani 2012). While M2 macrophages (M2s) include different functional populations: M2a induced by IL-4 or IL-13, M2b induced by immune complexes, M2c induced by glucocorticoids or IL-10, and M2d induced by IL-6-like cytokines. M2s secret arginase, IL-10, TGF-β, display Th2-type immunoregulation properties, mediate resolution of inflammation and play vital role in wound repair, angiogenesis, tumor progression and resistance to parasites (Martinez and Gordon 2014; Sica and Mantovani 2012). Macrophages can polarized toward different functional phenotypes in response to physiological (e.g., pregnancy and ontogenesis) and pathological state (e.g., infection, allergic and chronic inflammation, cancer, and tissue repair), playing detrimental or beneficial role in these processes (Sica and Mantovani 2012). Besides, macrophages can alter their phenotypes over time in the development of disease and different M2 stimuli can change immune functions in the re-polarizing macrophage (Bosco 2019).

TAMs inhibit T-cell immunity through PD-1/PD-L1 axis

TAMs can inhibit CD8$^+$ T-cell immune response against cancer via directly interacting with T cells through the PD-1/PD-L1 pathway or by secreting immunosuppressive molecules, like TGF-β and IL-10 (Noy and Pollard 2014; Alderton 2014). Wu et al. have found the expression of PD-L1 on Kupffer cells (KCs) was upregulated in tumor tissues compared with neighboring normal tissues in HCC patients and correlated with poorer survival; PD-L1$^+$ KCs and PD-1$^+$ T cells were colocalized in the HCC stroma and the PD-L1$^+$ KCs effectively impaired tumor-specific T-cell immune response and promote tumor growth (Wu

et al. 2009). Meanwhile, blocking KCs PD-L1 engagement with PD-1$^+$ CD8$^+$ T cells by neutralizing antibody reactivated effector T-cell function (Wu et al. 2009). In addition, B7-H4, like PD-L1, is another identified B7 family molecule of T-cell costimulatory molecules, also is a negative regulator of T-cell responses through inhibiting T-cell proliferation, cytokine production, and cell cycle (Sica et al. 2003; Chen 2004). B7-H4$^+$ TAMs could suppress T-cell immunity and inhibiting B7-H4 reactivated the T-cell stimulating ability of the macrophages and resulted in tumor regression, therefore, in addition to PD-L1, B7-H4 represents another important checkpoint in effecting host responses in ovarian cancer (Kryczek et al. 2006). Blocking B7-H4 or depleting B7-H4$^+$ TAMs may stand for novel approaches to enhance T-cell immunity in cancer treatment.

Macrophage upregulates PD-L1 expression on tumor cells

The overexpression of PD-L1 was associated with TAMs infiltration in HCC tissues and the PD-L1 expression on HCC cells (SMMC-7721 and BEL-7402) was upregulated at both mRNA and protein levels after cocultured with macrophages, moreover, the increased expression of PD-L1 mediated by macrophage was suppressed through inhibiting NF-κB or STAT3 pathways (Chen et al. 2012). These above results have suggested that the overexpression of PD-L1 in HCC may be mediated by inflammatory microenvironment involving macrophages. Likewise, PD-L1 expression on pancreatic ductal adenocarcinoma (PDAC) cells was also positively related with macrophage infiltration in tumor stroma and tumor-infiltrating macrophage could produce TNF-α to increase PD-L1 expression on PDAC cells via NF-κB pathway (Tsukamoto et al. 2018). TNF-α could lead to the stabilization of PD-L1 on tumor cells by COP9 signalosome subunit 5 (CSN5)-mediated de-ubiquitination (Lim et al. 2016).

PD-1/PD-L1 modulate the function and phenotype of macrophages

The role of PD-1/PD-L1 axis in adaptive immunity has been well investigated so far, however, their effect on innate immunity has seldom been characterized. Whether and how PD-1/PD-L1 affect the function and phenotype of macrophages attract more and more attention in recent years.

PD-1 affects the phagocytosis of macrophages

The morphology of FACS-sorted TAMs from tumors have shown apparent differences: PD-1$^+$ TAMs were foamy and large while PD-1$^-$ TAMs appeared more monocytic and smaller. And the foamy appearance of PD-1$^+$ TAMs was partially because of the accumulation of plentiful, uncleared phagocytic material, and lysosomes in the cytoplasm (Gordon et al. 2017). Gordon SR et al. speculated that PD-1 could affect TAM phagocytosis and to confirm this hypothesis, they sorted PD-1$^+$ and PD-1$^-$ TAMs to conduct an in vitro phagocytosis assay using GFP$^+$ *Staphylococcus aureus* bioparticles. PD-1$^+$ TAMs showed impaired phagocytosis of *S. aureus* compared with the PD-1$^-$ TAMs, suggesting that the PD-1$^+$ TAMs were in a phagocytically inhibited state, and this hypothesis was demonstrated in vivo as well (Gordon et al. 2017). The PD-1 expression on TAMs can negatively regulate their phagocytosis against tumor cells.

PD-1 promotes macrophages polarize towards M2 phenotype

PD-1$^+$ and PD-1$^-$ TAMs have been found to express similar levels of F4/80 and CD11b, but PD-1$^+$ TAMs expressed more M2-associated scavenger receptor CD206, more CD11c, and less MHC class II (Gordon et al. 2017), suggesting that PD-1 could promote macrophage polarize towards M2 phenotype. Besides, PD-1$^{-/-}$ mice have generated severe peritonitis with prominent infiltration of M1s, accompanied with upregulation of pro-inflammation molecules (Chen et al. 2016). Meanwhile, PD-1 deficiency prompted macrophages polarize towards M1 phenotype and exacerbated inflammation induced by zymosan through enhancing the phosphorylation of STAT1/p-NF-κB p65. PD-1 engagement followed by zymosan treatment might inhibit the phosphorylation of tyrosine residue in PD-L1 and the recruitment of SHP-2 to PD-L1, inducing the reduced of M1 macrophages cytokine production (Chen et al. 2016).

PD-1 modulates the panel of cytokine secreted by macrophages

In HCV-infected patients, the IL-12 produced by macrophages was suppressed while PD-1/PD-L1 were increased compared with healthy or HCV-resolved subjects. PD-1 negatively modulated IL-12 expression via inhibiting the phosphorylation of STAT-1 in macrophages during chronic HCV infection (Ma et al. 2011). Cho et al. have demonstrated that the production of IL-12 was significantly inhibited in LPS-stimulated RAW264.7 cells upon PD-1 interaction with PD-L1, and restored in response to anti-PD-1. PD-1 induced inhibition of IL-12 was mediated by suppressing Janus N-terminal-linked kinase (JNK) and PI3K/Akt pathway via the recruitment of SHP-2 to PD-1 (Cho et al. 2009). Besides, the engagement of PD-1 and PD-L1 also suppressed the expression of co-stimulatory molecules such as CD86, CD80, MHC class I and II in LPS-stimulated RAW264.7 cells (Cho et al. 2009). Moreover, PD-L1 generated negative

signal to macrophages to induce immunosuppressive phenotype, anti-PD-L1 administration reversed the phenotype and induced macrophage-mediated anti-tumor activity (Hartley et al. 2018).

The role of macrophages in PD-1/PD-L1 blockade treatment

PD-L1 blockade increases tumor infiltration with activated macrophages

Treating monocyte-derived macrophages and CSF-1 generated bone marrow macrophages with PD-L1 antibodies would increase macrophage survival, activation and proliferation. In addition, anti-PD-L1 treatment promoted tumor infiltration with activated macrophages in B16 melanoma tumor model (Hartley et al. 2018). PD-L1 antibodies increased costimulatory molecule expression on TAMs in tumor-bearing $RAG^{-/-}$ mice and suppressed tumor growth (Hartley et al. 2018). Anti-PD-L1 could activate mTOR pathway, and repressing this pathway might partially reversed the macrophage-activating effects of anti-PD-L1, so modulation of the mTOR pathway can be one of the mechanisms by which PD-L1 affects the functions of macrophage (Hartley et al. 2018).

PD-1 blockade induces M1s polarization and reactivates T cells

Mediavilla et al. have investigated the effect of PD-1 blockades (pembrolizumab and nivolumab) on the polarization of TAMs and activation of cytotoxic T cells in a 3D in vitro system of advanced non-small cell lung cancer (NSCLC). Both pembrolizumab and nivolumab upregulated the proportion of M1s and the secretion of TNF-α, IFN-γ, MIP1b and GM-CSF related with M1 phenotype. Meanwhile, PD-1 blockades reactivated $CD8^+$ T cells assessed by Ki67 and CD107a (Mediavilla et al. 2017). Their findings have illustrated that PD-1 blockade would induce M1 macrophage polarization and T-cell activation to exert therapeutic effect in NSCLC patients.

The role of macrophages in PD-1/PD-L1 blockade resistance

Although immune checkpoint blockade is a promising therapeutic treatment during these years, a majority of cancer patients do not respond to PD-1/PD-L1 antibodies and there is lack of full understanding of the mechanisms that relate with treatment efficacy and resistance. Since macrophage is an important component of tumor microenvironment and there is complicated relationship between macrophage and PD-1/PD-L1 axis, macrophages will exert crucial role in the resistance to PD-1/PD-L1 antibodies.

Macrophages inhibit T cells reactivated by PD-1 antibody to migrate to tumor islets

Extracellular stroma is important for tumor growth and progression and its main components including neovasculature, fibroblasts and TAMs. Tumor stroma can inhibit the infiltration and activation of T cells (Spiotto et al. 2002; Yu et al. 2004; Singh et al. 1992). To eradicate tumor cells, T cells must possess some abilities. First, they should accumulate and migrate efficiently to interact with tumor cells; Second, T cells must respond adequately to tumor antigens and other activation signals (Peranzoni et al. 2018). Plenty of studies have confirmed the exclusion of $CD8^+$ T cells from tumor nests always associated with poor clinical response. The main purpose of the current immunotherapies always are restoring the dysfunction of T cells (Anderson et al. 2017; Chen and Mellman 2017), but lack of studies aim at promoting T cells to migrate to tumor nests. Macrophages have been proved to promote the formation of extracellular matrix (Afik et al. 2016) and $CD8^+$ T cells poorly migrated and infiltrated into tumor islets resulted from long-lasting interaction with macrophages which could trap lymphocytes (Peranzoni et al. 2018). Therefore, macrophages depletion may reactivate $CD8^+$ T cells to migrate and invade tumor islets, and improve the therapeutic effect of anti-PD-1 treatment.

Moreover, Quaranta et al. have found granulin might relate with the specific mechanism of inhibiting $CD8^+$ T cells to migrate to tumor nests mediated by macrophages (Quaranta et al. 2018). Granulin, an approximately 70 kDa glycoprotein which has been demonstrated to induce wound healing by promoting fibroblast migration and mediate fibrosis in tumor progression (Quaranta et al. 2018; He et al. 2003). In the context of metastatic tumor microenvironment, granulin was overexpressed on metastasis associated macrophages and could contribute to PDAC metastasis through activating resident hepatic stellate cells into myofibroblasts and stimulating periostin secretion (Elkabets et al. 2011). In addition, macrophages could secret granulin to exclude $CD8^+$ T cells from metastatic livers, thereby leading tumor progression. Genetic depletion of granulin reduced fibrotic stroma formation, thus permitting T cells migrate to metastatic site to exert anti-tumor effect (Nielsen et al. 2016).

Macrophages phagocyte PD-1 antibody

Arlauckas et al. have investigated the activity of PD-1 antibody in real time and at subcellular resolution by in vivo imaging. They observed anti-PD-1 quickly and effectively

bound PD-1$^+$ tumor-infiltrating CD8$^+$ T cells after administration. However, this binding was transient (Arlauckas et al. 2017). Anti-PD-1 were seized within minutes from the T cell surface by PD-1$^-$ macrophages and failed to reactivate exhausted T cells. The capture of anti-PD-1 by macrophage depended both on antibody's fragment crystallizable (Fc) domain glycan and the Fcγ receptors (FcγRs) expressed on macrophages (Arlauckas et al. 2017).

Macrophage secrets IDO to induce immunosuppression

Indoleamine 2,3-dioxygenase (IDO) is a crucial negative modulator of immunity, which could induce tryptophan degradation, produce immune modulatory tryptophan metabolites and inhibit T cell proliferation, resulting in immunosuppression eventually (Katz et al. 2008; Jürgens et al. 2009). Toulmonde et al. have found most soft-tissue sarcoma (STS) and gastrointestinal stromal tumor (GIST) which were resistant to PD-1 blockade were abundantly infiltrated by M2s and these macrophages expressed IDO (Toulmonde et al. 2018). Furthermore, previous studies have demonstrated the IDO expressed in tumor microenvironment would lead to immunosuppression and immune evasion mainly by two ways. First, deactivating effective T cells via IDO expressed by APCs in tumor-draining lymph nodes (Munn et al. 2004); second, the anti-tumor immune response is suppressed because of the IDO expressed by tumors themselves (Brandacher et al. 2006; Uyttenhove et al. 2003). Meanwhile, IDO could induce macrophage to polarize towards M2 phenotype. In conclusion, the IDO secreted by macrophages may lead to immunosuppressive microenvironment and will be a crucial mechanism of the resistance to PD-1 blockade (Wang et al. 2014).

Combination therapy

In consideration of lots of patients do not respond to monotherapy that targeting PD-1/PD-L1 and the clinical outcome may relate with macrophages, enhancing the anti-tumor effect of macrophages, modulating them toward anti-tumor phenotype together with PD-1/PD-L1 checkpoint blockade therapy will shape the future of cancer immunotherapy.

TGF-β inhibition

Act as one of the most important immunosuppressive cytokine secreted by M2s, TGF-β could induce the upregulation of PD-L1 and contribute to tumor metastasis and chemotherapy resistance (Newsted et al. 2019). Secretion of TGF-β and upregulation of PD-L1 are two pivotal contributors to immune evasion and cancer progression (Knudson et al. 2018). Mariathasan et al. used a mouse of mammary carcinoma which exhibited the immune-excluded phenotype, in this model, they proved that TGF-β pathway inhibited T cells infiltrate the tumor-associated stroma and dual blockade of TGF-β pathway and PD-1/PD-L1 enabled T cell migration into the tumor, thus resulting in improved anti-tumor effect (Mariathasan et al. 2018). Tauriello et al. have found that in mice which developed metastatic intestinal tumors, treatment with galunisertib, a small molecular TGF-β inhibitor induced anti-tumor response to prevent metastasis. However, once the metastases grew past a certain stage, galunisertib monotherapy could be noneffective, and the combination of galunisertib and anti-PD-L1 inhibited liver metastases in the majority of mice (Tauriello et al. 2018).

M7824 is a bifunctional fusion protein comprising a monoclonal antibody against PD-L1, fused to the extracellular domain of TGF-β receptor 2 (Grenga et al. 2018). Latest researches have proved that M7824 decreased tumor burden and improved overall survival compared with targeting TGF-β alone in murine colon and breast cancer models (Knudson et al. 2018), and the efficacy of M7824 was also demonstrated in urothelial carcinoma cell lines (Knudson et al. 2018). A phase I research is ongoing to investigate the combined effect of anti-TGF-β monoclonal antibody NIS793 and PDR001 (NCT02947165), while another phase I/II study is assessing the combination of galunisertib and nivolumab (NCT02423343) in patients with advanced malignancies (Santoni et al. 2018). From the above, checkpoint blockade and targeting TGF-β pathways may be also a good combination therapy for cancer treatment.

Anti-CD47

As a cell surface molecule expressed on all types of cancers, CD47 promotes immune evasion by engaging signal regulatory protein-α (SIRPα) on the macrophages and DCs (Jiang et al. 1999; Li et al. 2018). Blockading the engagement of CD47 and SIRPα with anti-CD47 antibodies have induced macrophage-mediated phagocytosis of tumor cells, thus inhibiting CD47/SIRPα axis will be a promising immunotherapeutic approach for cancer treatment (Weiskopf et al. 2016). Gordon et al. have found that although both HAC (a anti-PD-L1 small protein) and anti-CD47 monotherapies could inhibit tumor growth equivalently, the combined therapy with HAC and anti-CD47 induced greatest reduction of tumor size and enhanced the survival rate in a human colon cancer xenograft mouse model with NSG mice (Gordon et al. 2017).

CSF-1R inhibition

CSF-1, also termed macrophage M-CSF, is a hemopoietic growth factor which is correlated with the survival, proliferation and differentiation of macrophages, monocytes and bone marrow progenitor cells (Stanley et al. 1997). CSF-1 modulates monocytes and macrophages through promoting phagocytic and chemotactic ability, and tumor-cell cytotoxicity (Nemunaitis 1993). Macrophages would polarize towards M2 phenotype upon CSF-1 treatment (Rőszer 2015). However, Peranzoni et al. have found that macrophages depleted with PLX3397 (an inhibitor of CSF-1 receptor) would promote $CD8^+$ T cell to migrate and infiltrate into tumor nests, while CSF-1R (CSF-1 receptor) inhibition alone had a minor effect on tumor progression, indicating that the increase of $CD8^+$ T cell that migrate to tumor islets was not sufficient to induce tumor regression (Peranzoni et al. 2018). Combine CSF-1R inhibition with anti-PD-1/anti-PD-L1 blockade will have synergistic therapeutic effect.

Granulin inhibition

Treating macrophages with CSF-1 could upregulate granulin expression and genetic depletion of granulin inhibited the formation of fibrotic stroma, allowing T cells to migrate to metastatic site; CSF-1R inhibition impaired granulin expression in macrophages and reduced desmoplasia; Moreover, granulin depletion sensitized metastatic tumor to anti-PD-1 therapy (Quaranta et al. 2018). These findings prove that inhibiting granulin may act as a promising therapeutic method to promote $CD8^+$ T cell infiltration in metastatic tumor which are refractory to PD-1/PD-L1 blockades. Although granulin depletion promoted $CD8^+$ T-cell infiltration in metastatic tumor, T-cell dysfunctionality still remained. Therefore, it is possible to combine macrophage inhibition targeting granulin with immune checkpoint blockade for metastatic caner treatment.

FcγRs inhibition

The engagement of the Fc domain of many anti-tumor immunoglobulin and the Fcγ receptors (FcγRs) has been proved to be important for their therapeutic effect (Clynes et al. 2000). Dahan et al. have confirmed the presence of FcγR-binding capacity suppressed the anti-tumor effect of anti-PD-1, but promoted anti-PD-L1 anti-tumor activity (Dahan et al. 2015). Interactions of anti-PD-1 with TAMs seem to relate with drug resistance (Killock 2017), and the latest study have demonstrated anti-PD-1 was quickly removed from $PD\text{-}1^+$ $CD8^+$ T cells and shifted to neighboring $PD\text{-}1^-$ TAMs which didn't directly capture anti-PD-1 from T cells (Arlauckas et al. 2017). The capture of anti-PD-1 by macrophages was favored when the antibody was bound to PD-1 on T cells (while unbound anti-PD-1 was not captured by macrophages). The anti-PD-1 uptake by macrophages depended both on the Fc domain of the antibody and the FcγR expressed on macrophages (Arlauckas et al. 2017). In addition to modulating anti-PD-1 through Fc engineering or glycan modification, blockading FcγR on macrophage before anti-PD-1 treatment enhanced anti-PD-1 binding to $CD8^+$ T cells and inhibited the capture of antibody by macrophages, resulting in synergistic therapeutic efficacy (Arlauckas et al. 2017).

IDO inhibition

PD-1 blockade has been demonstrated to exert limited effect on advanced STS and GIST. The majority of these tumors were infiltrated with M2 macrophages, but the tumor-infiltrating $CD8^+$ T cells were significantly reduced (Toulmonde et al. 2018). These phenomena might resulted from the immunosuppressive tumor microenvironment mediated by macrophage infiltration and IDO pathway activation. IDO inducing macrophage to polarize towards M2 phenotype could be a probable mechanism that contributed to tumor progression and poor prognosis (Wang et al. 2014). Furthermore, the latest early phase clinical trial have showed that BMS-986205, an IDO inhibitor, in combination with nivolumab exerted promising response rates without increasing adverse effects in advanced cervical or bladder cancer patients (Tabernero et al. 2018), suggesting that the combination of IDO inhibitor and PD-1/PD-L1 blockade will be a promising option for cancer therapy.

Chemotherapy

Su et al. have found that neoadjuvant trastuzumab upregulated PD-L1 and IDO significantly in the TAMs of $HER2^+$ breast cancer patients, resulting in poor response, and combined treatment of anti-HER2 antibody with PD-L1 inhibitors enhanced therapeutic effect (Su et al. 2018). Meanwhile, Yang et al. have also demonstrated the low expression of PD-L1 on bone marrow stromal cells was markedly upregulated by doxorubicin in the treatment of lymphoma (Yang et al. 2017). Chemotherapeutic drugs stimulated stromal cells to release M-CSF which in turn activated ERK pathway leading to the upregulation of PD-L1 on TAMs, the drug-induced overexpression of PD-L1 on stromal cells could lead to significant dysfunctions of T cells and suppressing ERK pathway prevented chemotherapeutic agents-induced PD-L1 expression (Yang et al. 2017). Overall, these findings disclose a unrecognized mechanism through which chemotherapy induces tumor immune escape via upregulation of PD-L1 in bone marrow stromal cells, furthermore providing new proofs for the combined therapy of chemotherapeutic drugs with PD-1/PD-L1 blockade for cancer treatment.

Conclusion

Given the above, PD-1/PD-L1 blockade can regulate the proportion and phenotype of macrophages to exert anti-tumor effect and macrophages will affect the therapeutic effect of PD-1/PD-L1 inhibitors in turn. In consideration of the intertwined relationship between the PD-1/PD-L1 axis and macrophages during the progression and treatment of cancer, first, the proportion and the polarization of TAMs may become the biomarkers to predict the therapeutic efficacy of PD-1/PD-L1 blockade. Second, modulating adaptive and innate immune response by PD-1/PD-L1 blockade at the same time is important for cancer immunology. Last but not least, combined treatment of PD-1/PD-L1 blockade with macrophages targeted therapy will exert synergetic anti-tumor efficacy, especially for the patients who have failed treatment with PD-1/PD-L1 antibodies alone.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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