New Approaches on Cancer Immunotherapy

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Metastasis, which occurs when cancer cells disseminate from the primary tumor site to other parts of the body, is the primary cause of mortality in patients, and the recurrence of multiple metastatic tumors is an obstacle to eliminating cancer. Recent clinical studies demonstrated that patients who respond to immunotherapy have longer survival rates with lower metastatic relapse, suggesting that immunotherapy may be one of the solutions to overcome cancer metastasis. Indeed, various host immune cells not only shape the tumor microenvironment but also participate in multiple stages of metastasis. Therefore, to improve clinical outcome, it is critical to understand the immunological events associated with tumor development and progression. In this article, we summarize those events that are involved in tumor progression and discuss immunotherapies that can potentially target cancer metastasis.

Over the last two decades, various therapeutic approaches have been developed based on remarkable findings in the field of immuno-oncology. For example, cancer vaccines and chemokine therapy have been shown to increase the sensitivity and activity of immune cells against tumor cells (Papaioannou et al. 2016). In addition, antibodies targeting cancer immune checkpoints, including cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), programmed death-ligand (PD-L1), programmed death-1 (PD-1), lymphocyte-activation gene 3 (LAG-3), galectin-9 (Gal-9), and

T-cell immunoglobulin and mucin-domain-containing-3 (TIM3), have demonstrated promising results by restoring the patients' depleted immune system (Pardoll 2012). Moreover, adoptive cell transfer (ACT), such as dendritic cell (DC) vaccine, chimeric antigen receptor T (CAR-T) cell therapy, and CAR-natural killer (NK) cell therapy (Liu et al. 2017; Harrer et al. 2018), has emerged as another effective cancer treatment modality. Currently, two CAR-T cell therapies are approved by the U.S. Food and Drug Administration (FDA) for lymphoma: axicabtagene ciloleucel

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(Yescarta) and tisagenlecleucel (Kymriah) (Dwivedi et al. 2019).

Although the clinical trials for the above-mentioned immunotherapies have revealed significant antitumor effects against certain types of cancer, such as metastatic melanoma, B lymphoma, and non-small-cell lung cancer, they are limited in most solid tumors (Armand et al. 2013; Antonia et al. 2016; Seidel et al. 2018). Interestingly, patients with high response to immunotherapy have longer survival rates with reduced recurrence (Grossmann and Margolin 2015; Gauci et al. 2019), suggesting that immunotherapy may also target undetectable disseminated cancer cells in addition to primary tumor cells and reduce metastasis. Below, we discuss the immune response related to cancer malignancy during tumor development and progression, summarize the findings of current major immunotherapies, and offer some additional insights into the advances in immunotherapy with respect to cancer metastasis.

Immune Response in Tumor Development

The development of tumor occurs in multiple stages: initiation, promotion, and progression (Fig. 1A; Armitage 1985). During the initiation stage, DNA damage induced by carcinogenic factors, for example, genetic mutations, chemical carcinogens, radiation, and viral infection, up-regulates oncogenes and inhibits tumor suppressor genes, leading to the transformation of the initiated cells (Fig. 1A; Basu 2018). The growth factors and survival signals then activate oncogenes to facilitate the formation of neoplasm by inducing amplified cell proliferation of the initiated cells (Fig. 1A). In addition, such uncontrolled growth greatly increases the likelihood of mutations (Hanahan and Weinberg 2011). Meanwhile, cancer cells struggle fiercely to survive against the immune response. This dynamic process, also known as immunoeediting, consists of three phases, elimination, equilibrium, and escape (Fig. 1B; Dunn et al. 2002; Kim et al. 2007). Cancer cells that overcome immune defense can develop into tumors, and as they grow in the progression stage, interactions with various types of host cells occur to

establish a protumor microenvironment that fosters invasion and metastasis (Fig. 1A; Wang et al. 2017).

The Elimination Phase

During the elimination phase, most cancer cells are removed via immunosurveillance by both innate and adaptive immunity (Fig. 1B; Swann and Smyth 2007). The primary effectors in the innate immune response are NK cells and immunostimulatory macrophages (Bellora et al. 2010). NK cells recognize cancer cells with low levels of nonclassical major histocompatibility complex (MHC) I and secrete cytotoxic perforin and granzymes to lyse them (Marcus et al. 2014). Meanwhile, proinflammatory cytokines in the tumor, such as TNF- α and IFN- γ , secreted by NK and Type 1 Helper T (TH1) cells instruct monocytes to differentiate into immunostimulatory macrophages to phagocytize cancer cells (Mosser and Edwards 2008; Braumüller et al. 2013). Activated macrophages also support adaptive immunity by secreting interleukin-12 (IL-12), which can stimulate the proliferation and activity of T cells, for example, TH1 and cytotoxic T lymphocytes (CTLs) (Mosser and Edwards 2008). Moreover, phagocytic macrophages and DCs present tumor antigen to TH1 cells and CTLs in the lymph node drainage. The educated T cells with antigen-specific long-term memory then home to the tumor site to kill the remaining cancer cells (Gajewski et al. 2013).

The Equilibrium Phase

A small number of cancer cells that survive the elimination phase undergo repeated proliferation and elimination, allowing them to enter the equilibrium phase and survive without tumor formation (Dunn et al. 2002). In clinical experience, the equilibrium phase may be the longest of the three phases of the immune response in tumor development and could last for many years (Kim et al. 2007), during which cancer cells may accumulate various resistant mutations against immunosurveillance (Fig. 1B).

Figure 1. Immune response during tumor development. (A) The processes of tumor development. (B) The processes of immune response in tumor formation. (NK) natural killer cell, (M ϕ) macrophages, (TH1) type 1 helper T cells, (CTL) cytotoxic T lymphocytes, (DC) dendritic cells, (MHC) major histocompatibility complex, (APM) antigen processing and presentation machinery, (gMDSs) granulocytic myeloid-derived suppressor cells, (ECM) extracellular matrix, (TME) tumor microenvironment, (TME) tumor microenvironment of metastasis, (TNF α) tumor necrosis factor alpha, (INF γ) interferon gamma, (TGF- β) transforming growth factor beta, (IL-12) interleukin-12, (T reg) regulatory T cells, (PD-L1) programmed death-ligand 1, (Gal-9) galectin-9, (CD80) B-lymphocyte activation antigen B7, (CD86) B-lymphocyte

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The Escape Phase

In the escape phase, the equilibrium between the cancer cell growth and its elimination by immunity becomes favorable toward cancer cells, and various genetic and epigenetic alterations that may have accumulated during the equilibrium phase provide cancer cells the stealth ability to evade immune recognition. It has been reported that select cancer cells harbor abnormalities in MHC levels and the related-antigen processing and presentation machinery (APM) that plays a role in displaying cancer antigen peptides to CTLs and NK cells (Leone et al. 2013). Most tumor cells exhibit down-regulation or loss of classical MHC class I molecules (HLA-A, HLA-B-, HLA-C) and APM, hindering their recognition by CTLs. Moreover, tumor cells also increase the levels of nonclassical MHC class I molecules (Kochan et al. 2013; Halenius et al. 2015), thereby blocking attacks by NK cells. Eventually, those that escape from the immune system form tumors (Fig. 1B, left).

The Tumor Microenvironment and Cancer Malignancy

As tumors grow, the tumor microenvironment (TME) is established through complex interactions between cancer cells and activated host stroma (Fig. 1B, right). In a protumoral microenvironment, the presence of inhibitory immune checkpoints, hypoxia, inflammatory cytokines, immunosuppressive tumor-associated macrophages (TAMs), regulatory T cells (T regs), and granulocytic myeloid-derived suppressor cells (gMDSCs) (Balkwill et al. 2012; Barcellos-Hoff et al. 2013), facilitates metastasis by providing favorable conditions before and after cancer invasion, and suppressing antitumor immunity (Quail and Joyce 2013; Mascaux et al. 2019).

Immune checkpoint molecules are abundantly expressed in the TME and regulated by adaptive signaling and intrinsic oncogenic pathways (Topalian et al. 2015). Hypoxia and cytokine signaling, such as TNF- α , and IFN- γ , are the dominant adaptive signaling pathways that up-regulate immune checkpoint molecules (both mRNA and proteins). The intrinsic oncogenic

pathways, including epigenetic alternations, epidermal growth factor receptor (EGFR), MYC, KRAS, and AKT, increase the RNA levels of immune checkpoint molecules (Parsa et al. 2007; Akbay et al. 2013; Casey et al. 2016; Coelho et al. 2017).

Previously, Xu et al. reported that intratumoral hypoxia promotes epithelial-mesenchymal transition (EMT) of cancer cells by up-regulating EMT-inducible factors, for example, transforming growth factor-beta (TGF- β) (Xu et al. 2009), which possess strong immunosuppressive effects (Yang et al. 2010). Furthermore, hypoxia increases the release of chemoattractants, such as colony stimulating factor 1 (CSF1), endothelial-monocyte-activating polypeptide II (EMAP II), endothelin 2 (ET 2), and vascular endothelial growth factor (VEGF) (Murdoch et al. 2004), resulting in the recruitment of TAMs that are critical to cancer cell dissemination. Various chemoattractants released from the TME recruit T regs to help suppress other types of immune cells by secreting immunosuppressive cytokines, for example, TGF- β , IL-10, and IL-35 (Chaudhary and Elkord 2016). T regs can also inhibit the activity of effector T cells through coinhibitory checkpoint signaling and IL-2 consumption. In addition, T regs can indirectly neutralize adaptive immunity by reducing the antigen-presenting activity of DCs (Schmidt et al. 2012). In the tumor microenvironment of metastasis (TMEM) consisting of macrophages, vessel endothelium, and cancer cells, angiopoietin receptor-positive (Tie2⁺) TAMs have been shown to enhance vessel leakage and remodel the extracellular matrix (ECM) by secreting high levels of VEGF and matrix metalloproteinases (MMPs) (Rohan et al. 2014). High levels of coinhibitory immune checkpoint ligands, such as PD-L1, programmed death-ligand 2 (PD-L2), and costimulatory ligands, CD80 and CD86, expressed on TAMs inhibit NK cells and CTLs (Noy and Pollard 2014; Cassetta and Kitamura 2018).

Chronic inflammation of advanced tumor induces differentiation of myeloid precursor cells into gMDSCs, a major type of immune suppressive cells in the TME. gMDSCs not only differentiate into TAMs but also enhance

immunosuppressive macrophages polarity of nearby TAMs by secreting IL-10 (Kumar et al. 2016). In addition, gMDSCs play a critical role in depleting T cells in the TME and interfering with T-cell trafficking into tumors by cleaving L-selectin, an important adhesion molecule in T-cell extravasation (Hanson et al. 2009). Furthermore, various coinhibitory checkpoint molecules, in particular, Gal-9, which induces apoptosis of TIM3⁺-exhausted T cells, are highly expressed in gMDSCs (Anderson 2014). In total, such accumulation of malignant properties and immune malfunction in the TME allows cancer cells to disseminate from the primary tumor site and those that survive in the circulatory system to settle in a metastatic niche, eventually forming metastatic tumors.

Current Cancer Immunotherapy

The establishment of an antitumor immune response and memory not only eliminates tumor cells and prevents recurrence in the primary region but also inhibits metastasis to distant sites. Increasing the systemic immune response and normalizing immunosurveillance within the TME are two major strategies for the development of cancer immunotherapy (Mellman et al. 2011; Pardoll 2012; Mahoney et al. 2015; Sharma et al. 2017; Sanmamed and Chen 2018). To date, four different immunotherapies are approved by the FDA or are in clinical trials, all of which focus on boosting immune response and immune cell activities in the TME and have demonstrated profound clinical benefits in cancer patients. Below, we briefly describe each of these strategies.

Cytokine Therapy

In the TME, multiple cytokines are required for immune cells to maintain their growth, activation, and ability to infiltrate into the tumor regions. In particular, the administration of IL-2 induces significant antitumor effects and reduces metastatic tumor progression in murine tumor models (Rosenberg et al. 1985). IL-2 therapy was subsequently approved by the FDA in 1992 for the treatment of renal cell carcinoma and mela-

noma, achieving a 15%–29% response rate in different studies (Table 1; Rosenberg 2014).

IL-2 is also required for the expansion and promotion of activated T cells, and a high dose of IL-2 has been shown to induce an antitumor immune response in a certain population of cancer patients (Jiang et al. 2016). However, some side effects of high-dose IL-2, including significant toxicity in multiple organs and tissues, hypotension, and capillary leak syndrome, have been reported (Atkins et al. 1999; Rosenberg 2014). In 2016, Jiang et al. (2016) described a low-dose IL-2 treatment coupled with ACT therapy (see later section) with promising clinical benefits in cancer patients. To avoid the side effects of IL-2, orthogonal IL-2 cytokine-receptor complexes in which IL-2 interacts only with the matched IL-2 receptor on CAR-T or CAR-NK cells and induces less toxicity in other organs with normal IL-2 receptor have been proposed as the next-generation ACT therapy (Sokolosky et al. 2018).

Cancer Vaccines

The recognition of abnormal or transformed cells by immune cells is a key step in cancer immunosurveillance (Hollingsworth and Jansen 2019). T-cell tracking, infiltration, and activation via the TCR signaling pathway are required to identify tumor neoantigens expressed on cancer cells (Chen and Mellman 2013). However, during tumorigenesis, cancer cells silence the neoantigen exposure to reduce immunogenicity, which attenuates the presence of immune cells in the TME (also referred to as cold condition). By administering vaccines based on tumor antigens, immunosurveillance can be reactivated by increasing infiltrated immune cells at the tumor regions (also referred to as hot condition) (Galon and Bruni 2019).

Ideally, the antigens used in cancer vaccines are designed to achieve four goals: those that are (1) specifically expressed by cancer cells; (2) present on a broad range of cancer types; (3) required for cancer cell survival; and (4) highly immunogenic (Hollingsworth and Jansen 2019). Not all antigens can meet these goals but can still significantly activate the anticancer immune re-

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Table 1. Current immunotherapies approved by the FDA

Approach	Target	Name	Company	Diseases
Cytokine (IL-2)	IL-2R	Proleukin (Aldesleukin)	Prometheus Laboratories Inc.	Renal cell carcinoma, and melanoma
DC vaccine	PAP	Sipuleucel-T (Provenge)	Dendreon Corporation	Prostate cancer
Immune checkpoint blockade	CTLA-4	Ipilimumab (Yervoy)	Bristol-Myers Squibb	Melanoma, renal cell carcinoma, and colorectal cancer
		PD-1	Nivolumab (Opdivo)	Melanoma, non-small-cell lung cancer, renal cell carcinoma, Hodgkin's lymphoma, head and neck cancer, urothelial carcinoma, colorectal cancer, hepatocellular carcinoma, and small cell lung cancer
	PD-L1	Pembrolizumab (Keytruda)	Merck	Melanoma, non-small-cell lung cancer, head and neck cancer, Hodgkin's lymphoma, urothelial carcinoma, gastric cancer, cervical cancer, hepatocellular carcinoma, and Merkel cell carcinoma
		Cemiplimab (Libtayo)	Regeneron	Metastatic cutaneous squamous cell carcinoma
		Atezolizumab (Tecentriq)	Genentech	Bladder cancer, non-small-cell lung cancer, triple-negative breast cancer, and small cell lung cancer
		Avelumab (Bavencio)	Merck Serono and Pfizer	Merkel cell carcinoma, urothelial carcinoma
		Durvalumab (Imfinzi)	AstraZeneca	Urothelial carcinoma, non-small-cell lung cancer
		CD19	Axicabtagene ciloleucel (Yescarta)	Large B-cell lymphoma
CAR T-cell therapy	CD19	Tisagenlecleucel (Kymriah)	Novartis	Acute lymphoblastic leukemia, and large B-cell lymphoma

Data from FDA website (before 2019 April).

DC, dendritic cells; PAP, prostatic acid phosphatase.

sponse in cancer patients. To date, tumor-associated antigens and oncogenic viral antigens are the major candidates for cancer vaccines. Tumor-associated antigens, which are self-proteins but abnormally expressed by cancer cells (Hollingsworth and Jansen 2019), include overexpressed surface oncogenes, for example, HER2 (Disis et al. 2009), cell lineage differentiation antigens, for example, prostate-specific antigen (Correale et al. 1997), and other common mutations, for example, *BCR-ABL1* gene fusion (Finn 2018). In addition to tumor-associated antigens, oncogenic viral antigens are also molecular driv-

ers of oncogenesis that are highly immunogenic. Furthermore, around 16% of human cancers are related to viral infection (de Martel et al. 2012), implying that oncogenic viral antigen-based vaccines can prevent tumorigenesis. For example, hepatitis B virus (HBV) infection is one of the major risk factors contributing to the development of HCC, and the implementation of the HBV immunization program has reduced the occurrence of hepatocellular carcinoma (HCC) prevention in many Asian countries (Chang et al. 2009; Llovet et al. 2016). Likewise, vaccines against the human papilloma virus (HPV) also

provide protection against HPV infection and associated precancerous lesions (Ault and Future 2007; Paavonen et al. 2009).

Currently, the specificity and efficacy of immune cell activation by cancer antigen presentation rely on several platforms: cellular, viral vector, and DNA/RNA vaccines. For example, the FDA-approved cell-based immunotherapy sipuleucel-T vaccine (Provenge) provides a 4-mo increase in median survival of patients with metastatic castration-resistant prostate cancer (Table 1; Kantoff et al. 2010). Specifically, Provenge consists of autologous peripheral blood mononuclear cells (PBMCs) isolated by leukapheresis from patients and DCs activated by prostate antigen and granulocyte-macrophage colony-stimulating factor (GM-CSF) (Bol et al. 2016). Moreover, increased progression-free and overall survival were observed in several clinical trials using glioma tumor lysate-loaded DCs. These results suggested that DC vaccines are effective cell-based immunotherapy (Anguille et al. 2014; Van Gool 2015).

Immune Checkpoint Blockade (ICB)

Activation of immune cells is tightly controlled by ligand–receptor interactions between immune cells or immune cells and tumor cells. T-cell activation is initiated via neoantigen recognition by the TCR and regulated by costimulatory and inhibitory signals from immune checkpoint molecules (Pardoll 2012). The intricate balance between costimulatory and coinhibitory signaling is critical to prevent autoimmune responses and maintain immunosurveillance under normal physiological conditions. However, cancer cells can tip the balance by amplifying coinhibitory molecules signaling and/or reducing costimulatory signaling, which can hamper anti-immune response and provide a niche for cancer immune evasion (Pardoll 2012; Topalian et al. 2015). Targeting immune checkpoints by specific antibodies to restore immunosurveillance has demonstrated promising clinical outcomes in cancer treatment.

CTLA-4/B7-1 and PD-1/PD-L1 are two major immune checkpoints that inhibit T-cell activation during the priming and effector phase,

respectively (Chen and Mellman 2013). Mechanistically, the interactions between CTLA-4 and B7-1, and PD-1 and PD-L1 enhance the activities of protein phosphatase 2 (PP2A) and Src homology region 2 domain-containing phosphatases (SPH2), respectively, leading to down-regulation of the TCR pathway and inhibition of T-cell activities (Schildberg et al. 2016). As a result, several antibodies against those immune checkpoints, including ipilimumab (CTLA-4), nivolumab, pembrolizumab and cemiplimab (PD-1), and atezolizumab, avelumab, and durvalumab (PD-L1), were developed and are now approved by the FDA for the treatment of multiple solid tumors (Table 1). The response rates of a single immune checkpoint inhibitor are in the range of 10%–45% depending on the cancer types (Sharma and Allison 2015; Zou et al. 2016). Blockade of several coinhibitory receptors and ligands, such as LAG-3 (ligands: class II MHC and fibrinogen-like protein 1) (Li et al. 2013; Wang et al. 2019) and TIM-3 (ligand: Gal-9) (Li et al. 2012), have demonstrated significant antitumor effects in clinical trials. In patients with advanced melanoma receiving nivolumab-plus-ipilimumab, the 3-yr overall survival rate was 58% (Wolchok et al. 2017). This finding is highly encouraging and provides a great opportunity to improve therapeutic efficacy based on the current success of immune checkpoint therapy by elucidating the mechanisms underlying immune checkpoint biosynthesis.

Along the same line, impressive efficacy of combination therapies involving anti-immune checkpoints in clinical trials and preclinical studies have been reported. For example, the expression of coinhibitory molecule, for example, PD-L1 or PD-1, in tumor microenvironment is tightly controlled by posttranslational modifications, such as glycosylation, palmitoylation, and ubiquitination (Li et al. 2016, 2019; Lim et al. 2016; Cha et al. 2018; Hsu et al. 2018; Meng et al. 2018; Yang et al. 2019; Yao et al. 2019). Our most recent work identified another regulatory mechanism underlying PD-L1 protein stability via the IL-6/Janus kinase 1 (JAK1) signaling axis (Chan et al. 2019). Preclinical studies targeting posttranslational mod-

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ifications and coinhibitory molecule expression regulation in the TME by inhibitors have revealed promising combination therapies, such as CDK4/6 inhibitor plus anti-PD-1 (Goel et al. 2017) and metformin plus anti-CTLA-4 (Cha et al. 2018). Several recent studies demonstrated that exosomal PD-L1 suppresses T-cell activity and down-regulates antitumor immunity (Yang et al. 2018; Poggio et al. 2019; Yao et al. 2019). The authors of those studies further suggested that exosomal immune checkpoints establish a premetastatic niche in distant organs by suppressing systemic immunosurveillance and memory. Together, the blockade of immune checkpoint function is a reliable strategy to inhibit local tumor progression and systemic metastasis.

Adoptive Cell Transfer (ACT) Therapy

ACT was developed as cancer treatment based on the concept of using tumor-reactive immune cells to eradicate tumor cells expressing specific surface markers (Rosenberg and Restifo 2015). The T cell population is a major candidate for this type of precise cell-based immunotherapy.

Generally, there are two strategies for T-cell transfer therapy. First, tumor-infiltrating lymphocytes (TILs) are isolated from a patient's tumor and expanded ex vivo with T cell-activating cytokines, for example, IL-2. The reactivated TILs are then reinfused into the same patient along with cytokines to expand and promote activation of T cells against tumor cells (Rosenberg and Restifo 2015). One of the main limitations of using this approach is that a majority of cancer patients are already with lymphodepletion after radiotherapy or chemotherapy, causing insufficient T-cell population for ex vivo expansion (Rosenberg et al. 2011). Second, the isolated autologous T cells are engineered to express CARs that recognize tumor cells with a specific marker. CARs possess both the extracellular domain of antigen-specific single-chain variable fragment (scFv) and the intracellular domain of costimulatory receptor, such as CD28 and/or CD137 (Sanmamed and Chen 2018; O'Donnell et al. 2019). Therefore, CAR T cells are not restricted by neoantigen peptides

presented on the MHC for activation and recognition of cancer cells (O'Donnell et al. 2019).

In several clinical trials, CAR T cells that target CD19-expressing cancer cells have demonstrated considerable effectiveness on destroying hematological malignant cells, including B cell lymphoma, non-Hodgkin's lymphoma (Kochenderfer et al. 2010), and chronic lymphocytic leukemia (Porter et al. 2011). Other studies have also reported a 25%–90% response rate of CAR T-cell therapy in different hematological malignancies (Brentjens et al. 2013; Grupp et al. 2013).

Those encouraging results led to the accelerated approval of CD19 CAR T-cell therapy (Kymriah and Yescarta) in 2017 (Table 1). The efficacy of CAR T-cell therapy for solid tumors, however, warrants further investigation (D'Aloia et al. 2018). Unlike hematological malignancies, surface markers on solid tumors are highly diverse due to their origin, and this poses a major challenge in developing specific targeted CAR T-cell therapy.

CAR T-cell therapy also requires the collection and gene transduction of autologous T cells from patients, a process that can be time-consuming and inefficient. Furthermore, lymphodepletion also limits the number of patients eligible for this type of therapy (Li et al. 2018b). Different from T cells, NK cells mediate their tumor-killing effects without requiring matched human leukocyte antigen (HLA) (Morvan and Lanier 2016), suggesting the utility of NK cells from other sources for ACT therapy (Guillerey et al. 2016). In this regard, a recent study indicated that NK cells derived from human induced pluripotent stem cells (iPSC)s expressing CAR may be considered as another ACT therapy (Li et al. 2018b). Given that engineered NK cells can be generated without any limits through differentiation from iPSCs, this strategy may provide an affordable and timely cell-based cancer immunotherapy.

Perspectives on Cancer Immunotherapy

Several clinical trials have revealed a strong positive correlation between prognosis after ICB or ACT therapy and the number and activity of

TILs (Svane and Verdegaal 2014). The efficacy in eliminating the primary tumor and preventing metastatic recurrence has the potential to be greatly improved if an immune suppressed or “cold” tumor (low TIL population) can be converted into an inflamed or “hot” tumor (high TIL population). To this end, various approaches are ongoing to establish a TME that is friendly to T-cell immunity. Moreover, recent studies on gut microbiota suggested a new paradigm in antitumor immunity (Fig. 2).

Direct Enhancement of T-Cell Immunity

Because antigen presentation is a prerequisite of T-cell homing to tumor tissues, boosting the antigen-presenting cell (APC) activity is expected to increase the TIL population and activation. Indeed, cold tumors with low T-cell immunity exhibit lower APC activity (Bonaventura et al. 2019) compared with hot tumors. In an animal model of glioma, a major type of cold tumor, Lynes and colleagues found that the efficacy of current ICB/ACT therapy is substantially increased when APC activity is boosted by DC vaccines or oncolytic viruses (Lynes et al. 2018).

In addition to increasing the APC activity, TGF- β signaling is also an attractive target to enhance T-cell immunity. TGF- β is a driver of tumor progression and metastasis (Massagué 2012), and is considered as one of the determinants that prevents immunosurveillance and contributes to poor response to PD-1/PD-L1 blockade in cancer patients (Ganesh and Massagué 2018). Mechanistically, TGF- β released

from carcinoma-associated fibroblasts (CAFs) can limit TIL activity. Therefore, blocking TGF- β signaling by antibodies or inhibitors could induce cold-to-hot tumor conversion. Because TGF- β signaling is also critical for maintaining metastatic niche, Tauriello et al. investigated the combined inhibition of TGF- β and PD-L1. Indeed, the authors demonstrated that combined inhibition of TGF- β and PD-L1 eliminated established metastases in a preclinical mouse model of colorectal cancer (Tauriello et al. 2018). A bispecific antibody against PD-L1 and TGF- β receptor has since been developed to achieve this dual blockade (Lan et al. 2018).

Multiple studies by Li and coworkers revealed a critical role of N-linked glycosylation of immune checkpoints for the ligand/receptor interactions (Li et al. 2018a). Specifically, N-linked glycosylation of immune checkpoints, for example, PD-L1, is required to maintain their protein stability (Li et al. 2016, 2018a; Cha et al. 2018; Hsu et al. 2018). On the basis of those findings, inhibitors that block N-glycosylation modification on immune checkpoints in combination with immunotherapies may achieve better response rates than single agent alone. Furthermore, aberrant glycosylation of proteins on cancer cells enhances cancer immune evasion, EMT, tumor cell dissociation and invasion, cell–matrix interactions, and metastasis (Pinho and Reis 2015; Rodríguez et al. 2018). Notably, blocking the biosynthesis of specific glycan structure of adhesion molecules on tumor cells reduces cancer metastasis (Agrawal et al. 2017). Thus, if these molecular pathways

Figure 2. Perspectives in cancer immunotherapy. The conversion of “cold” tumor (low TIL population) to “hot” tumor (high TIL population) has the potential to improve the efficacy of current immune therapy. Various approaches to establish a TME that is friendly to T-cell immunity may be considered, such as by combining current ICB and ACT therapy.

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are also involved in cancer metastasis by regulating aberrant biosynthesis of glycosylated molecules, the combination strategy targeting glycosylation and immune checkpoints may also improve the clinical benefits and prevent metastasis in cancer patients.

Preventing Chronic Inflammation

Treatment of chronic inflammation before and/or after cancer therapy is important to maintaining T-cell immunity as chronic exposure to inflammatory cytokines increases the gMDSC population in the blood and tumor tissues (Wang and DuBois 2015). gMDSCs inhibit T-cell immunity not only directly through the expression of the coinhibitory checkpoints and inhibitory cytokines but also indirectly by increasing the population of the T-cell immunity suppressors, immunosuppressive TAMs and Tregs (Kumar et al. 2016). Such immunosuppression induced by chronic inflammation can promote cancer cell survival in the circulatory system and metastatic niche. Removal of primary lesion by surgery or eradication by cytotoxic chemotherapy are the most common approaches in cancer therapy; however, some patients exhibit increased distant metastasis right after surgery or chemotherapy (Smith et al. 2008; Al-Sahaf et al. 2010; Keklikoglou et al. 2019), which may be attributed to inflammation from the surgical wound and tumor-released extracellular vesicles, suggesting that the transient immunosuppressive state following these therapies creates a niche for cancer cell metastasis. In this regard, a regimen of nonsteroidal anti-inflammatory drugs (NSAIDs), such as aspirin, celecoxib, and ibuprofen, after surgery may have some benefits in maintaining antitumor T-cell immunity. Indeed, several meta-analyses indicated NSAIDs can reduce the metastatic recurrence of various cancer types (Elwood et al. 2016; Zhao et al. 2017). Furthermore, a recent study also indicated that preoperative administration of NSAIDs further enhances anticancer T-cell immunity unleashed by immune checkpoint blockade and eradicates micrometastases (Panigrahy et al. 2019). Therefore, the addition of NSAIDs to immunotherapy has the potential

to improve the therapeutic efficacy and reduce metastasis.

Preventing Premetastatic Site Formation

Tumor metastasis relies on cancer cells (the “seeds”), which disseminate from the primary region by escaping immunosurveillance and specific organ microenvironment (the “soil”) (Fidler 2003). Moreover, the appropriate distant microenvironment is critical for attachment, growth, and colonization of metastatic cancer cells. Targeting either the seeds or soil provides an opportunity to block the progression of cancer metastasis. Interestingly, leukocyte composition at specific organ microenvironment and neutrophil-derived leukotrienes from premetastatic regions have been shown to favor the colonization of metastatic cancer cells (Wculek and Malanchi 2015). Recently, Albregues et al. revealed that neutrophil extracellular traps (NETs) promote lung metastases under chronic inflammation by awakening dormant cancer cells through NET-mediated ECM remodeling. Indeed, a therapeutic antibody targeting such ECM remodeling has been shown to effectively suppress lung metastases in a breast cancer animal model (Albregues et al. 2018). Consistent with these observations, Lee et al. (2019) also demonstrated that activated neutrophils form NETs and establish a premetastatic niche of ovarian cancer in the omentum. Together, those findings suggested that pharmacological inhibition of leukotrienes has the potential to abrogate prometastatic establishment and subsequent metastasis.

Beneficial Microbiota

There is accumulating evidence to support the association between antitumor immunity and gut microbiota. Recently, three different groups reported that the gut microbiota critically affects the efficacy of PD-L1/PD-1 therapies in metastatic melanoma and non-small-cell lung cancer preclinical models (Gopalakrishnan et al. 2018; Matson et al. 2018; Routy et al. 2018). The authors reported better PD-L1/PD-1 therapeutic efficacy in tumor-bearing mice transplanted

with feces from IBC responders than those with fecal implantation from nonresponders. Consistent with these observations, fecal microbiota transplantation (FMT) in IBC responders improved T-cell immunity, suggesting a critical role of the gut microbiota in adaptive immunity against tumors. Each group identified the specific beneficial germline that enhances immunotherapy: (1) *Faecalibacterium prausnitzii* (Gopalakrishnan et al. 2018); (2) *Bifidobacterium longum* (Matson et al. 2018); and (3) *Akkermansia muciniphila* (Routy et al. 2018). It would be worthwhile to further investigate the mechanisms underlying the beneficial effects of gut bacteria or FMT in immunotherapy.

CONCLUDING REMARKS

In recent decades, harnessing and renormalizing immune response in cancer patients have provided an enormous clinical impact and ignited the discovery of new cancer immunotherapies. Unlike targeting tumor cells directly, multiple processes, such as neoantigen recognition, immune cell activation, and immune cell infiltration, are required to establish a successful anti-cancer immune response. The immune system in a majority of cancer patients suffers from systematic destruction during tumor progression and the loss of balanced immunosurveillance. As described in this review, although single-agent immunotherapy has demonstrated promising results in certain cancer types, improvement of the response rates in others is necessary. Given that a multitude of factors influence anti-tumor immunity, enhancing or restoring one of the many immune response processes cannot compensate for malfunctions in others, which partly explains why single-agent immunotherapy has not been shown to sufficiently conquer cancer immune escape and overcome subsequent cancer metastasis. In this regard, combining immunotherapy with activation of different steps of immunosurveillance may restore anti-tumor immunity in cancer patients. The diversity of immune cell profiles in different cancer patients is another challenge in current immunotherapy. Defining the immune profiles in individual cancer patients by using biomarkers

indicative of immune evasion may reveal more efficient combination therapies. The knowledge of current immuno-oncology and those yet to be discovered will expand the frontiers of immunotherapy and benefit more cancer patients in the near future.

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