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Cancer Immunotherapy: Future Prospective with Wide Therapeutic Spectrum

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Abstract

Cancer cells acquired the ability to resist against host immune responses and establish a complex network of tumor microenvironment. Emerging trends to recapitulate host immune system through genetic manipulation has gained wide attention for therapeutic intervention of cancer. The innate and adaptive arm of human immune system plays a key role in the recognition of tumor-associated antigens expressed on cancer cells. Immune cells, such as natural killer cells, natural killer T cells, macrophages of innate immunity and CD4+ Th1, CD8+ T cells of adaptive immunity infiltrate to the site of tumor microenvironment, but failed to induce anti-tumor response due to high levels of pro-tumorigenic factors. Importantly, certain subsets of immune cells, such as regulatory T (Tregs) cells minimizes T-cell mediates immune response by expressing immune suppressive transcription factor FOXP3. Recent advancement of knowledge and identification of immune-modulatory molecules still expanding the therapeutic spectrum of cancer immunotherapy.

Key words: Tumors; T-cells; Tregs; Cytotoxic T lymphocyte; CTLA4; PD1; FOXP3.

Abbreviations

APCs: Antigen presenting cells;

PD1: anti-programmed cell death protein 1;

ECMs: Extracellular matrix;

CARs: Chimeric antigen receptors,

CAFs: Cancer-associated fibroblasts;

CTLA4: cytotoxic T-lymphocyte-associated protein 4;

MHC: Major histocompatibility complex;

NK: Natural killer;

TME: Tumor Microenvironment;

TAAs: Tumor associated antigens;

TILs: Tumor-infiltrating immune cells;

TAMs: Tumor-associated macrophages,

Tregs: Regulatory T cells;

TCR: T cell receptor.

Introduction

The incidence of rising cancer cases worldwide is progressively becoming a major socio-economic burden, and efforts have been made to design therapeutic strategies for a successful treatment. The tumor cells orchestrate an immunosuppressive mechanism to evade the recruitment of tumor infiltrating leukocytes (TILs) and shape a hostile microenvironment that favors metastatic growth. Transformation of normal cells to establish a tumor mass is a complex mechanism [1]. Various complex signaling pathways are activated and maintained as a network in cancer cell growth that favors the tumour microenvironment (TME) [2]. The TME is composed of cancer cells, tumour-infiltrating immune (TILs) cells, cancer-associated fibroblasts (CAFs) and the extracellular matrix (ECM) that collectively impedes host immunity to favour tumour growth [3,4]. Importantly, a host body continuously checks the unwanted cellular outgrowth and tends to control through distinct signalling cascades [2]. Indeed, the human immune system constituted of innate and adaptive arms continuously monitor and identify tumor antigens and eliminate them from the main body stream [5]. Notably, cancer cell resistance to the immune system employed multiple factors such as suppression of regulatory immune cells, regulated cell death, immunosuppressive cytokines and chemokines to support TME for cell proliferation [6]. Current cancer treatments including surgical intervention, radiation therapy and chemotherapy have limited efficacy, severe side effects and a high mortality rate. Some recent development of novel therapeutic approaches based on engineered adaptive immune components has gained significant attention to treat tumor cells growth and proliferation [7]. A promising strategy to genetically manipulate a subset of immune cells has the potential for sustained immune response and anti-tumor effect. Indeed, cancer immunotherapy

has a great potential and advantage of manipulating patient's own immune system to recognize and remove tumor cells. Importantly, cancer immunotherapy strategies have limited risk of side effects and possess high target specificity. For instance, the antitumor cytolytic T lymphocytes (CTLs) present in the blood of cancer patients has been attributed as the most effective type of immune cells that recognize and kill tumor cells [8]. Gershon et al., described the concept of the immunosuppressive function of CD8+ T cell [9] An earlier study reported that thymectomy in a three day old mice generated autoimmunity by suppressing CD4+ T cell population [10]. However, T-cell mediated immunotherapy generates a weak T cell response as most of the tumor-associated antigens (TAAs) are recognized as self-proteins. Therefore, specific antigen receptor for T-cells activation is required to trigger a robust immune response against growing tumor cells. Schietinger et al., has shown that during Tumorigenesis cancer cells express tumor-specific neo-antigen to escape from CD8+ T cell encounter [11]. Tumor regression mediated through antitumor T cells (CTLs) to conquer the unsympathetic effects of tumors in the host is still challenging [12]. In this review, we will highlight the mechanisms employed by the immune system to control the outgrowth of cancer cells and the future strategies helpful to improve the cancer immunotherapy.

Cancer cells plays hide and seek with Immune system

TME induced barriers to Immune system

The host immune system elicits limited and weak immune responses against tumors compared to bacterial and viral infections [13, 14]. It is important to note that, TME acquired several mechanisms to restrict or suppress T cell activation and preventing the infiltration of immune effector cells [15, 16]. Moreover, TME eventually establishes a protective mechanism to impede host immune response through provoking the expression of TGF-β [17] and indoleamine 2,3-dioxygenase (IDO) levels [18]. In fact, host factors such as genetic polymorphisms associated with proliferation regulatory pathways, mutation leading to loss of MHC class I expression and recognition of CD8 effector T cells, inactivation of NK cells costimulatory molecules CD58 also contributes to immune evasion [19]. The tumor cells has the potential to disrupt key T-cell mediated immune response such as T-cell activation and T-cell infiltration. Nevertheless, several factors intrinsic to a host have significant impact on the natural immunogenicity as well as responsiveness to immunomodulatory agents. Notably, the aggressive tumor environment mitigates inflammatory reach at the site and promotes tumor growth, now considered as one of the hallmarks of cancer [2].

There are distinct steps employed by the host immune system to eliminate cancer cells; first recognition of cancer antigens by circulating antigen presenting cells (APCs) to process and recruit T cells. Second, expression of co-stimulatory molecules such as B7.1 (CD80), B7.2 (CD86) on APCs that interacts with CD28 of the naive T-Cells [20-22]. Third, suppressing T cell inhibitory molecules such as CTLA-4 and PD-1 to improve the effectiveness of immune-clearance [23]. The physiological alterations in cancer cells have also caused reduced tumor immunogenicity and immune response: for instance, low oxygen supply (hypoxia) and glucose insufficiency impaired MHC-I surface expression of T-cells leading to reduced CD8 T cell activation. Notably, the transcriptional regulation of MHC-I through IFNγ-STAT1 signaling has been shown to be modulated by hypoxia [24]. In fact, mutation leading to impaired IFNγ-STAT1siganling propagate metastatic transformation in cancer patients [15, 25, 26]. In addition, hypoxia also favors metastatic transformation through the production of lysyl oxidase [27] and impeding natural killer (NK) cell functions [28]. Moreover, hypoxic tumor microenvironment also instigates CCL28 expression to recruit Tregs cells in ovarian cancer [29]. It is important to note that, dysregulated angiogenesis and aberrant tumor growth in cancer patients induces metabolic stress that may also lead to hypoxia and nutrient deficiency [30, 31]. Thus, TME surmounts or hijack cancer regulatory mechanisms through modulating signaling pathways to acquire proliferative capacity.

Immune modulators favor cancer cells for immune evasion

In the process of tumor development, cancer cells hijack host immune surveillance components and direct them to favor metastatic growth. Noteworthy, the tumor-associated macrophages (TAMs) have been considered as a major culprit in developing tumor-associated angiogenesis and metastasis [32]. Although, the exact mechanisms of TAMs mediated immunosuppression have not been fully Understood. Although some studies investigated that TAMs induces vascular endothelial growth factor (VEGF) and transforming growth factor beta (TGFβ) expression during angiogenesis to inhibit T cell mediated responses [33-36]. TAMs reportedly inhibit the T cell mediated immune outcomes [37]. Importantly, tumor cells suppress major histocompatibility complex (MHC) expression and processing to escape from anti-tumor T-cell response. Mechanistically, T-cell activation relies on co-stimulatory signals originated from antigen presenting cells (APCs) such as dendritic cells (DCs), however, tumor cells mitigate APCs stimulation leading to insufficient expression of MHC class I- and II-peptide molecules [38]. It is important to note that, TME contains NKGD2 ligands such as MIC-A/MIC-B to reduce the activation of effector T-cells, αβ T cells, gamma-delta T cells and NK cells [39]. Moreover, cancer cells induce the levels of nitric oxide, reactive oxygen species (ROS), arginase, IL-10 and TGF-β to suppress immune response. In addition, cancer associated fibroblasts secrete chemokines CCL2 and CXCL12 to promote the immune suppression [40].

Most importantly, certain subsets of T cells dampens immune surveillance and promoted tumor progression. The CD4+ CD25+ FoxP3+ regulatory T cells (Tregs) cells have been considered as a major immune suppressive subset that are highly resistant to immunotherapy [41]. Earlier studies have examined that, tumor-associated Tregs are highly immunosuppressive compared to normal Tregs in humans [42, 43]. The secretion of tumor associated chemokines infiltrates Tregs to the tumor microenvironment [44, 45]. The immunosuppressive activity of Tregs is chiefly governed by associated transcription factor FOXP3 that itself is considered as a highly immune suppressive molecule. Moreover, Tregs exerts inhibitory effect by inducing the

expression of inhibitory cytokines (IL-10, TGF-β) and inactivate PI3K signals in Tregs to persist tumor regression [46]. In fact, the tumour hypoxia condition secrets paracrine mediators such as adenosine that can suppress T-cell activation by enhancing Tregs expression [47]. In addition, Tregs participate in the suppression of DCs, T-cells activation, IL-10 producing cells and myeloid-derived mesenchymal stem cells [48, 49]. Moreover, the proportion of CD8+ T cells to Tregs are critical determinants of tumor progression, for instance the enhanced CD8+ T cell infiltration has been considered as a prolonged survival factor for patients earlier encountered with colorectal and breast cancer [50-52]. The sufficient amount of CD8+ T cells with reduced Tregs expression in the ovarian cancer has shown positive response to immunotherapy [44, 53]. In contrast, TAMs together with Tregs exhibits a poor immune response [44, 54]. Altogether, tumors acquired the ability to resist and escape from machineries associated with host immune surveillance for rapid proliferation.

Guards on duty: Immune regulation of tumor cells

Trackers on tumors -Tumor associated antigens (TAAs)

The success of cancer immunotherapy made over the last few decades unequivocally relies on identification and targeting Tumor associated antigens (TAAs) expressed in tumours. Importantly, to distinguish normal and malignant cells for effective immunotherapy immune cells largely depends on antigenic cell surface markers. Indeed, cancer cells express distinct surface markers due to metabolic and signalling alteration that can be recognised by circulating T-cells [55]. The circulating T-cells recognize and respond to pathogens or abnormal cells through antigen interactions with the target cells. Identification of TAAs that binds to T-cell receptors (TCRs) with optimal affinity are a major challenge in the development of adoptive T-cell therapy in cancer [56, 57]. The TAAs are broadly classified in three major groups: First group, consist of cancer-testis (CT) antigens, normally silent in non-transformed cells but transcriptionally active in tumor cells. CT antigens include MAGE-A1, NY-ESO-1 and SSX-2 [58]. Second group, consist of differentiation antigens, expressed in melanocytes Gp100, Melan-A/Mart-1, and cells of epithelial origin. Third group, consist of overexpressed TAAs that can be directly recognized by T cell and induces an immunological response [58]. The 3rd group has members of hTERT and tumor suppressor p53 [59]. Bruggen et al., have shown that cancer cell expressing antigen that can activate T cells and could be developed as tumor based vaccines [60]. Nemunaitis et al., have designed a cell based vaccine, which activates the tumor-specific CD4+ T cells in cancer patients [58]. Obenaus et al., has identified antigens which are having optimal affinity towards human T-cell receptor by identifying a CT antigen NY-ESO [61]. Lee et al. have found MART-1 or tyrosinase as TAAs specific for circulating CD8+ T-cell [62]. Thus, identifying highly tumor specific antigens and associated mechanism may provide a better understanding to design a successful therapy.

T-cell priming against TAAs

Mechanistically, the affinity-matured T-cell receptors (TCRs) cross-reacts with targeted antigens for optimal signaling response and clearance of tumors [63, 64]. Notably, T-cells recognize the antigens processed through MHC class I and II molecules. It is imperative to understand the mechanistic steps of the MHC antigen presentation that may provide a target site for developing T cell–mediated immunotherapy [65, 66]. Some earlier studies demonstrated that modulating expression of MHC class I & II molecules has been noticed in various forms of cancers [67-69]. Notably, CD4+ T cells have also been reported in anti-tumor activity mediated through MHC II presentation with APCs and production of cytokines [70]. Srivastava et al., [71], Thompson et al., [72] has demonstrated that "MHC II vaccines" are able to activate a novel repertoire of tumor-specific CD4+ T cells. Mechanistically, the CD8+ cytotoxic T cells (CTL) and CD4+ helper T (Th)1 cells mediates anti-tumor response through the production of interferon (IFN)-γ and cytotoxins, but can be suppressed by chronic inflammation to proliferate cancer [73, 74]. Pathangey et al., have identified MUC1 class peptide and glycosylated-anchor epitope which elicits lytic responses in breast cancer patients and kills the cancer cells [75]. Grenier et al., has developed a vaccine against melanoma by combining in vitro activated T cells and melanoma antigen gp100 (PMEL) that generates life-long CD8+ T cell responses [76]. Additional strategies for enhanced T-cell differentiation through exposing T cells to γ-cytokines such as IL-7 and IL-15 has been employed for prolonged response [77, 78]. Current efforts have been made to directly transfer genetically engineered CD4+ and CD8+ T-cell to overcome limiting therapeutic efficacy for enhanced outcome. In addition, there are certain population of tumor-antigen-specific T cells, also known as TILs isolated from cancer patients and genetically modified through ex vivo has the potential to induce anti-tumor effects [79, 80]. It is imperative to identify novel immunotherapies that precisely recognize TAAs and directed them to MHC modification for optimal immune regulation.

Cancer immunotherapy and challenges

Therapeutic strategies to enhance T cell mediated tumor regression

Immunotherapy has been used as a revolutionized approach to treat cancer patients in recent era. However, defined tumor-antigen targeted therapy with effective ability to clear the tumor is still challenging due to the rapid changes that occur in TME. The existence of intra-tumoral T cells shown as a predictive marker for cancer survival, therefore currently, tumor specific T-cells are utilized as an effective tool for cancer treatment. The various strategies employed to use the tumor-specific T cells include; (a) tumor-infiltrating T cells (TILs) (b) the generation of CTLs and (c) genetically engineered chimeric antigen receptor (CAR) T cells [8]. The major disadvantage associated with TILs and cytotoxic T cells is the "on-target, off-tumor" toxicity [81]. Zhang et al., [81] and Schepisi et al.,[82] have shown that utilizing dual-antigen specific CAR-T cells for prostate and pancreatic cancers that restrictsthe persistent T cell functionn to only antigen

expressing target cells without generating any cytotoxicity to nearby cells. Another approach to circumvent this problem is by using switchable CAR T cells. In this strategy, in the presence of Fab switch activation of T cells occurs against tumor antigen as well as peptide neo-epitope [83]. The CTLA-4 and PD-1 mediated immunotherapy are highly effective against various cancer types [84, 85]. The anti-CTLA-4 antibody (Ipilimumab) treatment was the first immune checkpoint blockade to be used clinically and showed prolonged survival in cancer patients [84]. The anti-PD-1 based immunotherapy (Nivolumab) is the most efficient against solid tumors with the prolonged survival of cancer patients [86]. Hsu et al., have shown that the Ibrutinib combination with checkpoint inhibitors enhances antitumor activity in solid tumors [87]. Inhibition of immunosuppressive enzyme indoleamine 2,3-dioxygenase (IDO) accelerates the antitumor T cell responses in gastrointestinal stromal tumor by using Imatinib [88]. Tumor regression of solid B16 tumors and enhanced cytotoxic T-cell (CTL) activity in mice has been shown by using poly-lactide-co-glycolide (PLG) cancer vaccine in combination with immune checkpoint antibodies, anti–CTLA-4 or anti–PD-1 [89]. Also, by using combined vaccination therapies the Tumor regression in humans is achievable. The recent emergence of dendritic cell based immunotherapy globally acclaimed as a potential tool to manipulate anti-tumor immune response. It was examined that the dendritic cell-tumor stressed lysate (DC-TSL) acts as a powerful inducer of antitumor immunity aligned with Laryngeal cancer in humans by activating dendritic cells [90]. In another way, some immune-sensitizing agents such as ONCOS-102 are a serotype 5 adenovirus-mediated treatment that induces PD-L1 and infiltration of CD8+ T cells to regress tumors that works as an immune-sensitizing agent along with checkpoint inhibitors [91].

Future perspectives and challenges in cancer immunotherapy

The future of cancer immunotherapy relies on developing novel approaches and effective strategies to differentiate self vs non-self during the course of treatment. Noteworthy, once T cell activation happens a simultaneous process initiated by T cells to activate co-inhibitory receptors to minimize uncontrolled immune responses to normal cells in the region. It is anticipated that advancement in technique and our knowledge to identify and characterized TAAs may provide more accurate clinical targets. Nevertheless, and as we discuss, genetic manipulation of T-cell has enormous potential to target cancer cells, but the manufacturing cost and complexity in isolation from host body remains a challenge in immunotherapies. The restricted production of genetically engineered T-cells have limitation to reach a high proportion of cancer patients. Achieving remarkable success with T cell based immunotherapy needs broader clinical trials with higher vertebrates, however, still challenge persist to fulfil these conditions. Ongoing clinical trials show significant success in cancer immunotherapy, but feasibility of these trials is limited to small cohort that must be addressed. The anti-CTLA-4 antibody (Ipilimumab) treatment shows prolonged survival of cancer patients, and studies are under clinical trials [84]. The anti-PD-1 based immunotherapy (Nivolumab) are most efficient against solid tumors with prolonged survival of patients [86]. By using combined vaccination therapies, tumor regression in humans is also achievable. Several strategies are underway to enhance the T cell response by activating co-stimulatory receptors such as 4-1BB (CD137), OX-40 (CD134), GITR (CD357) and CD27 to induce broad anti-tumor effect [92]. Therefore, the future therapies are highly dependent on revealing the signaling mediators and pathways manipulated by cancer cells to resist immunotherapy.

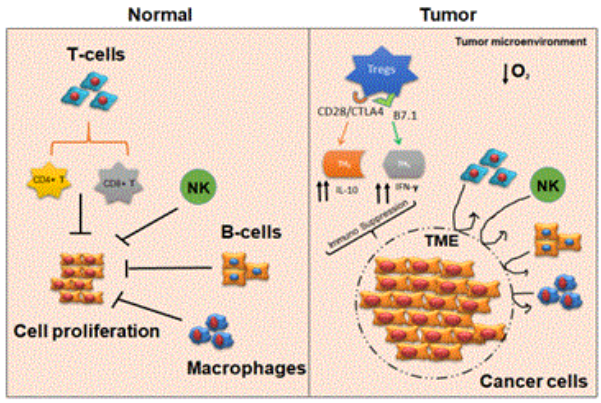

Figure 1: Comparison between normal to tumor cells and immune modulators in regulation of cancer cell proliferation.
The immune mediators T-cells, B-cells, NK cells and macrophage continuously check the outgrowth of cell proliferation to maintain cellular homeostasis. Perturbation to immune mediation lost control over cellular outgrowth and leading to cancer cells proliferation. Tregs and inflammatory cytokines impedes immune response to cancer cells and favor tumor microenvironment (TME) growth. Inhibiting CTLA-4 and PD-1 has the potential to resume cancer cell growth arrest.

Conclusion

Since the past decade, enormous efforts have been made to design strategies for the early detection and treatment of cancer. Conventional treatment approaches mediated through radiotherapy, chemotherapy and surgical removal has limitation of side effects, drug resistance and tumor re-occurrence respectively. Surprisingly, the advanced stage cancers dramatically failed to respond against these conventional therapies. Considering these conditions, some novel alternative therapeutic strategies have been evolved that usually derived from host and highly efficient to target cancer cells. Identification of human immunomodulatory molecules as an anti-tumor agent may hold promise for minimal side effect and highly targeted strategy. Strategies to engineer and redirect host derived immune mediators to suppress cancer progression are considered as a novel mechanism for cancer treatment. However, in some cases it was found that cancer cells simultaneously acquire mechanism to escape immunotherapy that

result in tumor relapse. For instance, anti-CTLA-4 and anti-PD-1 based immunotherapy has limitation to target a narrow range of tumor types. Designing immunomodulatory maneuvers to target immunosuppressive pathways needs combinatorial approach with chemotherapy or radiotherapy to become more effective against a wide range of cancers. In addition, unfolding the pro-tumor mechanisms acquired by cancer cells may provide some novel targets that help in designing new anti-tumor therapies. It is anticipated that accumulation of experimental evidences will help us to identify emerging obstacles in immunotherapy for better therapeutic strategy. It is also important to emphasize that current established immunotherapies have limitation of limited bioavailability and non-specificity. The emergence of personalized cancer immunotherapies has numerous advantage of improving efficacy and reduce toxicity. Altogether, identification of tumor biomarkers and strategies to target these antigens requires an immense effort, on the positive note research across the globe have gained tremendous success in treating advanced stages tumors through immunotherapy that gives a great hope for architecting future cancer therapies.

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Declarations

No conflict of Interest declared

Author Contributions

T.S.R.B.R, S.A.M., C.P. and K.R. conceived the idea of this review and wrote the manuscript.

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