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Cancer immunotherapy: Immune-checkpoint blockades

Liệu pháp miễn dịch trong chữa trị ung thư: Phong tỏa kiểm soát miễn dịch

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Abstract

Our immune system has its self-regulation mechanisms. Leukocytes can regulate their functions via control of the gene expression and/or the secretion of cytokines. In this review, we resumed the way T-cells are regulated between active and inactive states. The discovery of two receptors for the transduction of inhibitory signals, the cytotoxic T-cell receptor and the programmed cell death receptor, is presented in the second part. Disrupting the backward process of T-cells by blocking these receptors triggers the actions of T-cells against cancer cells and brings hope for cancer patients. In the third section, we discussed the developments of three groups of antibodies for anti-cancer purposes and their potentials in cancer treatments.

Keywords: Cancer immunotherapy; CTLA-4; PD1; PD-L1; Immune-checkpoint inhibitors.

Tóm tắt

Hệ thống miễn dịch của chúng ta có các cơ chế tự điều hòa. Các tế bào bạch cầu có khả năng tự điều hòa hoạt động của chúng thông qua việc kiểm soát biểu hiện gen và/hoặc sự tiết các cytokine. Trong bài tổng quan này, chúng tôi tóm lược phương thức các tế bào T được kích hoạt và sau đó trở lại trạng thái nghỉ. Quá trình khám phá ra hai thụ thể truyền tín hiệu ức chế, thụ thể của tế bào T độc và thụ thể gây chết tế bào theo chương trình, được trình bày trong phần thứ hai. Sự phá vỡ quá trình bất hoạt các tế bào T bằng cách phong tỏa các thụ thể này mang lại khả năng chống ung thư nhờ hoạt động của các tế bào T và mang lại hy vọng cho bệnh nhân ung thư. Trong phần thứ ba, chúng tôi thảo luận về sự phát triển của ba nhóm kháng thể kháng ung thư và tiềm năng của chúng trong điều trị ung thư.

Từ khóa: Liệu pháp miễn dịch ung thư; CTLA-4; PD-1; PD-L1; Các nhân tố ức chế kiểm soát miễn dịch.

1. Introduction

Human carries on a great battle against cancer, but it is still the most miserable fight and not likely to end shortly. Many therapies involving the use of hormones, drugs, radiotherapy, or surgery have been developed and applied in cancer treatment. In the last few decades, an alternative and promising therapy

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using our immune systems to fight cancer called the cancer immunotherapy had emerged [1].

In the mid-1990s, T lymphocyte-associated antigen 4 (CTLA-4) and programmed death 1 receptor (PD-1) caught scientists' attention since their functions as immune brakes were observed [2]. Details in their mechanisms of actions were increasingly studied and targeted for inhibition of tumor growth [3]. More importantly, new data has revealed that the inhibition of their signaling pathways recruits the T-cell into the attack on tumors [3]. Furthermore, blocking their two distinct signal pathways would result in better therapy [4, 5].

Different antibodies were produced and tested for blockade purposes on CTLA-4 and PD-1 receptors [6]. After years of many clinical trials, several monoclonal antibodies were approved by US Food and Drug Administration (FDA) including an anti-CTL-4 antibody: ipilimumab (BLA 125377, Mar. 2011); two anti-PD-1 antibodies: pembrolizumab (BLA 125514, April 2014) and nivolumab (BLA 125554, Dec. 2014) and three anti-PD-L1 antibodies: atezolizumab (BLA 761034, May 2016), avelumab (BLA 761049, Mar. 2017), durvalumab (BLA 761069, May 2017) for treatments of different cancer types [6]. The present review article covers an overview of signaling pathways of the two receptors, CTLA-4 and PD-1, on T-cells as well as the development of monoclonal antibodies against these two receptors.

2. TCLA-4 and PD-1 and their potentials in cancer immunotherapy

CTLA-4 (also known as CD152) was first discovered in 1987 as a member of the immunoglobulin superfamily expressed in activated T lymphocytes [7]. Knocking down CTLA-4 gene in mice revealed that it functions as an immune checkpoint receptor [8, 9]. CTLA-4 is a homolog of CD28, a well-known receptor on surfaces of T-cells. B7 receptors stimulate both CD28 and CTLA-4 on the membrane of antigen-presenting cells (APCs), but they trigger two opposite pathways inside the activated T-cells [2, 10]. CD28 induces the production of interleukin-2 and proliferation of T-cells, while CTLA-4 inhibits these actions [10]. Besides studies for treatments of autoimmunity diseases, CTLA-4 was first investigated by James Patrick Allison as a potential target for cancer treatment by blocking this receptor, opening a new trend for cancer immunotherapy researches [3].

PD-1 (also known as CD279) was first reported by Tasuku Honjo [11]. It was restrictedly expressed in the thymus of mice and related to the programmed cell death of some cell lines [11]. Later, the PD-1 coding sequence was found on 2q37.3 in the human genome [12]. PD-1 is one member of CD28 family that is expressed and presented on surfaces of activated B and T lymphocytes upon stimulation [13, 14]. Similar to CTLA-4, PD-1 receptor has a negative regulation on activated T-cells but through different downstream signaling [15, 16]. Blocking PD-1 noteworthy inhibited hematogenous spreads of cancer cells [17]. This finding started the decades of antibody development for blocking PD-1 in combination with CTLA-4 antibodies and/or anti-cancer agents. The works of James Patrick Allison and Tasuku Honjo on these antibodies and their application in cancer immune therapy have been well appreciated and awarded a Nobel Prize in Physiology or Medicine in 2018 [18].

3. Inhibitory actions of CTLA-4 and PD-1 on activation of T-cell

T-cell is a type of lymphocytes which keeps an indispensable role in immune responses. When any "non-self" agents such as microbial subjects penetrate our body, receptors on the T-

cell surface will recognize them and initiate T-cell-mediated immune responses. Activation of T-cell is a complex process that requires more than one signal (Fig. 1A). The primary signal comes from the binding of the T-cell receptor (TCR) to its antigen on the major histocompatibility complex (MHC) molecule that is presented by an APC [19]. The activation of T-cell will be further adjusted by regulatory signals that arise from the binding of regulatory receptors on T-cell to other receptors on APCs, including CD28. Bindings of B7 receptors on APC such as B7-1 (also known as CD80) or B7-2 (also known as CD86) with CD28 receptors on the T-cell will give fully functional T-cell [19, 20]. This process provokes the proliferation and mobility of activated T-cells [19]. The expression of CTLA-4 is induced in activated T-cell, which, in turn, will harm the function of activated T-cell [21]. CTLA-4 strongly binds to B7 receptors in competition with CD28, and therefore, directly prevents stimulatory signals [22, 23]. When the linkages of CTLA-4/B7 formed, the complexes also trigger inhibitory signals that counteract the accelerator functions of TCR/MHC and CD28/B7 binding [24] (Fig. 1B).

Furthermore, CTLA-4 is also quickly endocytosed and degraded [25] due to a trans-endocytosis process that is triggered by itself. In the trans-endocytosis, CTLA-4 captures its ligands, B7 receptors on APC, and takes the ligands with it into endocellular vesicles of T-cell. As a result, B7 receptors are also degraded inside T-cells, and ligands for CD28 receptors are cleared [26]. In other words, CTLA-4/B7 linkages will stop activated T-cells from carrying on its functions [23, 27, 28].

Function as another immune brake, PD-1 expression is increased in activated T-cells and transduces negative signals on these cells [29]. Its two ligands stimulate PD-1, programmed cell death ligand 1 and 2 (PD-L1 and PD-L2), the two membrane proteins [30] (Fig. 1C). PD-L1 (also called B7-H1 or CD274) is widely expressed on different cell types such as leukocytes, endothelial cells, reticular fibroblasts, keratinocytes [30-32]. It is also commonly expressed in cancer cells [33-35]. PD-L1-highly-expressing-cells can trigger apoptosis on activated T-cells, helping them to escape from cytotoxic T-cells [36]. Furthermore, PD-L1-positive-tumor lowers the penetration of lymphocytes into the tumor and becomes tolerant of immune cells [18]. Blocking PD-L1 increases tumor-infiltrating of lymphocytes, and thus it has become a target for anti-cancer therapy [33]. PD-L2 (known as B7-DC or CD273) is expressed in a few cell types [37], including APC and macrophages [38]. It is also expressed in different tumors like pancreatic adenocarcinoma [36], osteosarcoma [39], head and neck squamous carcinoma [35]. Similar to tumors with high PD-L1 expression levels, PD-L2-high-expressing-tumors give poor prognosis for carriers [40].

The balance of stimulatory and regulatory receptors is essential in normal physiological conditions. Regulatory pathways of CTLA-4 and PD-1 immune checkpoints can alter the tolerance of peripheral T-cells to prevent autoimmunity [41].

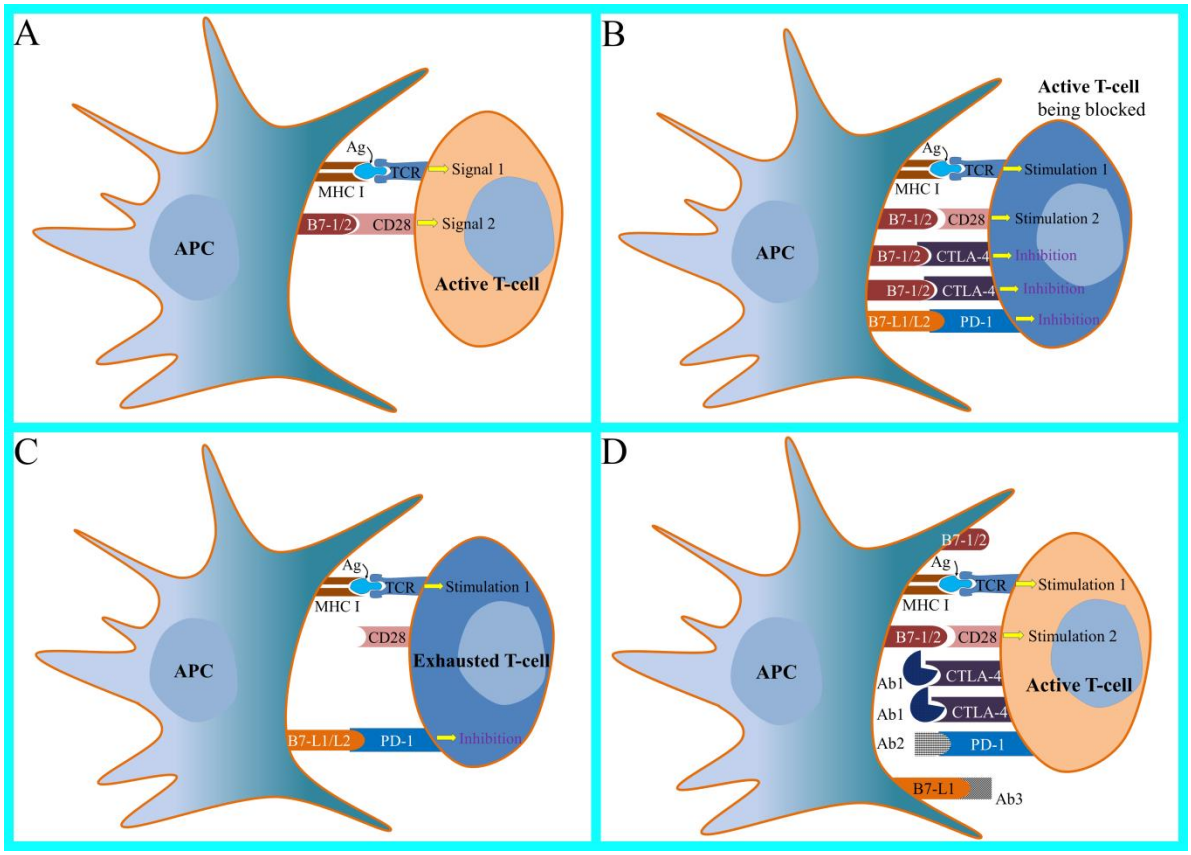

Figure 3.1. Regulation of T-cell activity and the blockade of antibodies against T lymphocyte-associated antigen 4 (CTLA-4) and programmed death 1 receptor (PD-1). A) T-cells are activated by two distinct signals, regulation signals inhibit one through T-cell receptor (TCR) and the other through CD28; B) Active T-cells from CTLA-4 and PD-1 receptors on their membranes; C) B7-1/2 is cleared, thereby, stimulation signal from CD28 is stopped and exhausted T-cells are back to rest; D) Regulation signals are blocked by three antibody groups: Ab1 (anti-CTLA-4 antibodies), and/or Ab2 (anti-PD-1 antibodies), and/or Ab3 (anti-PD-L1 antibodies), and thus, working duration of active T-cells are prolonged.

4. Development of monoclonal antibodies against CTLA-1 and PD-1

Although both CTLA-1 and PD-1 function as inhibitory components in the regulation chart in T-cell behavior, their downstream signals are distinct. CTLA-4 blocks the early stage of T-cell activation inside the lymphoid organs, whereas PD-1 inhibits T-cell activity at later stages of an immune response in peripheral tissues [30]. CTLA-4 has a higher affinity with B7 molecules comparing to CD28 [42] and completely reverses stimulation from CD28 [43-45]. The signals from PD-1 are slower and are not able to eliminate CD28-induced activation. For anti-cancer actions, CTLA-4 blockade will unleash the immune system to attack cancer cells, and blocking PD-1-normal-function can further enhance activities of the immune system against cancer cells [17, 46]. The blockades showed synergic efficacy and gave a breakthrough in cancer treatments [47]. Shortly after initial studies with remarkable results, different antibodies were developed (Fig. 1D). Clinical trials increased dramatically and achieved impressive results, leading to FDA-approved antibodies in cancer immunotherapy [6]. Some of those antibodies

are humanized antibodies, while others are fully human antibodies. Different from fully human antibodies that entirely encoded in human DNA, the humanized antibodies contain small but essential parts of non-human peptides that directly bind to targets of the antibodies.

Antibody for blocking CTLA-4

Ipilimumab (traded name: Yervoy) is a humanized, full monoclonal IgG1 antibody against CTLA-4. It blocks the inhibitory action of CTLA-4 on cytotoxic T-lymphocytes (CTLs) and frees the CTLs to attack cancer cells [48, 49]. In terms of structure properties, Ipilimumab is a high protein with a molecular weight of over 140 kDa [50]. The protein's sequence was published in 2017 and stored in protein database bank (PDB, https://www.rcsb.org/) with ID 5TRU [51]. The heavy chain of the antibody contains 225 amino acids, while its light chain has 215 amino acids [51]. Ipilimumab was developed by Bristol-Myers Squibb for the treatment of advanced metastatic melanoma (stage III or stage IV), the deadliest form of skin cancer, and showed positive effects on 487 recruited patients in 2007 [52]. In 2010, the outcomes of 676 advanced metastatic melanoma patients treated with Ipilimumab indicated that Ipilimumab had been noteworthy improved the patients' survival [53]. This data led to approvals by FDA (2011) [37], Canada, and the European Union (2012) for applying Ipilimumab in the treatment of melanoma. So far, Ipilimumab which is the only antibody against CTLA-4 has been approved. Since then, a large number of studies about Ipilimumab have been carried out, and more than a thousand papers were published (PubMed searched on 15/01/2020). Many clinical trials are going on with combinations of Ipilimumab and other anti-cancer agents. Among those studies, a combination of Ipilimumab and Nivolumab (an antibody described below) resulted in significantly better outcomes compared to the usage of each antibody separately [54]. This combination was approved by FDA in 2018 for the treatment of advanced renal cell carcinoma [54]. Clinical trials using other combinations are continuing for treatments of different metastatic cancers such as saliva gland cancer, gastric cancer, colorectal cancer, pancreatic cancer, etc (https://www.clinicaltrials.gov/ with ipilimumab).

Antibody for blocking PD-1

Pembrolizumab (trade name Keytruda) is another humanized monoclonal IgG that blocks the PD-1 receptor. It inhibits the binding of PD-L1 and PD-L2 to the PD-1 receptor and eliminates the inhibitory signals from PD-1 in the regulation of T-cell activities. Pembrolizumab was developed by Merck and first approved by the US FDA in 2014 for the treatment of metastatic melanoma with mutation of BRAF V600E [55]. The FDA also approved it for the treatment of metastatic non-small cell lung cancer [36]. This antibody was used in more than a thousand studies (PubMed search with "pembrolizumab" in "title" on 15/01/2020) in the last five years. Since then, it has been continuously used in clinical trials for the treatment of other cancer types. To date, it has been recommended for the treatment of various cancer types, including some in the deadliest list, such as esophageal carcinoma, lung cancers, and hepatocellular carcinoma [56].

Nivolumab (trade name Opdivo) is another anti-PD-1 monoclonal antibody. It was produced by spleen cells from transgenic mice that have a humanized immune system [57]. It is, therefore, different from pembrolizumab, a fully human antibody. Nivolumab stops the inhibitory signal of PD-1 on T-cells, free the T-cells to attack cancer cells. This antibody has

received FDA approval for the treatment of patients with unresectable or metastatic melanoma in 2014 [58]. Later, it has also been approved by FDA for the treatment of classical Hodgkin lymphoma (2016) [59], hepatocellular carcinoma (2017) [58], colorectal cancer (2017) [60], renal cell carcinoma (2018) [61], lung cancer (2018) [62]. Nivolumab is still one of the most appealing subjects in researches. A search with its name in the titles showed 610 reports in 2019 alone, and over 1700 reports in total (PubMed searched on 15/01/2020).

Also, only 62% similarity was observed in amino acid sequences in extracellular domains of murine PD-1 and human PD-1 [55]. Human PD-1 binds differently to murine ligands compared with murine PD-1 [56]. The binding pocket of human PD-1 to its ligands was not well documented and still being investigated [18]. Furthermore, different structures might trigger alternative signaling [63]. The structure data of PD-L1 may provide a helpful dock for small molecule screening [18]. The development of antibodies using an updated structure database of human PD-1 holds the potential for better clinical efficacy [64, 65].

Antibody for blocking PD-L1

A group of antibodies has been developed to block PD-L1 from binding to PD1 [66]. Blocking PD-L1 results in higher anti-tumor efficacy in comparison with anti-PD-1 antibodies [67]. Atezolizumab was developed by Genentech, Inc. (a member of the Roche group) to attack PD-L1 for the treatment of urothelial carcinoma. Similar to many other antibodies that have been used in immunotherapy, Atezolizumab is also a humanized monoclonal antibody, with a heavy chain containing 448 and a light chain containing 214 amino acids [68]. FDA issued an approval letter for Atezolizumab on May 18, 2016, with trade name Tecentriq [69]. A clinical trial phase 3 (identifier NCT02302807) had been completed in November 2018 on 931 patients with metastatic urothelial bladder cancer showing a considerable benefit for the treated patients [70]. Dozens of clinical trials were carried out and resulted in the approval of the FDA for the utilization of atezolizumab in the treatments of lung cancers and triple-negative breast cancer [71]. A meta-analysis that analyzed data from 14 different trials with 2496 patients confirmed the benefits for patients with relatively low risk [72]. Adverse drug responses (ADR) were somehow co-related with the efficacy of atezolizumab in clinical trials. Total ADR rate (at all levels) from meta-analysis, after injecting atezolizumab was 69%. The most common severe atezolizumab-related ADR were fatigue, anemia, and dyspnea, accounting for 6%, and atezolizumab associated death was shallow (0.17%) [72].

Avelumab (also known as MSB0010718C) is a fully human IgG1 [73]. It was first used in a clinical trial in 2014 for the treatment of Merkel cell carcinoma [74] and had been approved by the FDA in Mar. 2017 with trade name Bavencio [75, 76]. FDA also approved Avelumab for the treatment of solid tumors of metastatic urothelial carcinoma [75, 77]. Furthermore, many other clinical trials are still on-going with avelumab, mostly in combination regimens for the treatment of different types of cancer. Among those, a combination of avelumab and axitinib significantly prolonged progression-free survival for patients with advanced renal-cell carcinoma [78, 79].

Durvalumab (trade name Infinzi), developed by AstraZeneca UK Limited, is another fully human IgG1 against PD-L1. It was designed to attack PD-L1 and block the binding of PD-L1 to PD-1 for the treatment of advanced urothelial

cancers (locally or metastatic form) and stage III unresectable non-small cell lung cancer (NSCLC). It was first approved by FDA in May 2017 [80] and last updated information in July 2019 [81]. The results showed that Durvalumab brought benefits for the treated patients [82]. A three-year-follow-up study showed that durvalumab improved survival rates of patients with stage III NSCLC after chemoradiotherapy [83]. It was also elucidated to be a benefit for patients with small-cell lung cancer [84]. Different clinical trials are now on-going for the treatment of other cancer types.

Overall response rates (ORR) to durvalumab are dependent on the expression levels of PD-L1 [85]. For example, clinical trial NCT01693562 showed that a group of patients, who had more than 25% of tumor cells expressing PD-L1, had higher ORR (21.8%) than that of the group with less than 25% of tumor cells expressing PD-L1 (6.4%) [86]. A meta-analysis by Xi Liu and his colleagues revealed that the efficacy of all PD-L1 inhibitors was always dependent on the expression levels of PD-L1 in many cases [87]. However, it is also confirmed that using PD-L1 inhibitors was a benefit for patients in both PD-L1 positive and PD-L1 negative groups compared to the controls [87].

Side effects in general

Similar to all kinds of drugs, there is a list of ADR for each antibody used in the treatment of specific cancer types that included in the drug labels. Overall, using immune-checkpoint inhibitors in cancer treatments can cause the discovery of a wide range of ADR but rarely cause life-threatening ADR. A retrospective study showed details of this issue in using antibodies against CTLA-4 and PD1 [88]. Another review showed more details on this problem of all three antibody groups [89]. They both suggested the need for monitoring patient case-by-case based on their risk profiles [88, 89]. Optimizing properties of antibodies and their formula can also offer higher efficacy and lower the risks [89, 90].

5. Conclusion

The cancer immunotherapy utilizing antibodies to block signals from CTLA-4 and PD-1, thus, preventing active T-cells from being recognized and inactivated, is currently been used as regiments for advanced-stage or metastatic cancers. They bring hope for patients after other therapies failed to improve their sickness. The good outcomes and manageable risks are making immune-checkpoint inhibitors the most exciting subjects in the development of anti-cancer drugs to date. Anti-CTLA-4 and anti-PD1 antibodies function via blocking receptors on T-cells. Thus, their efficacy is not dependent on protein-expression profiles of tumors. Anti-PD-L1 antibodies, on the other hand, are dependent on expression levels of the target. The up-coming studies on optimization and novel inhibitors will continue to change the battle of human beings against cancers.

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