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Development of Cancer Immunotherapy Targeting the PD-1 Pathway

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Immune checkpoint inhibitors are causing a paradigm shift in cancer treatment. Immune checkpoint molecules such as programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) dampen T cell activation to avoid autoimmunity and the destructive effects of an excessive inflammatory response. Immune checkpoint signaling can be exploited by tumors to escape host immune surveillance, and immune checkpoint inhibitors enhance antitumor immunity by releasing the brakes on the immune system. PD-1 was identified in 1992 by Honjo and colleagues at Kyoto University. Studies in animal models revealed that PD-1 blockade can inhibit tumorigenesis and tumor metastasis. In addition, PD-1 blockade showed fewer adverse effects than CTLA-4 blockade. Based on these findings, a humanized monoclonal antibody against human PD-1 called nivolumab was developed. Since PD-1 blockade targets lymphocytes rather than tumor cells, the therapeutic effects last longer, even if mutations occur during tumorigenesis. Furthermore, because it does not depend on specific tumor antigens, PD-1 blockade can be applied to various kinds of tumors.
(J Nippon Med Sch 2019; 86: 10–14)

Key words: cancer immunotherapy, immune checkpoint inhibitor, PD-1, PD-L1, nivolumab

History of Cancer Immunotherapy

Although its usefulness has long been questioned, immunotherapy has recently emerged as the fourth pillar of cancer treatment besides surgical therapy, chemotherapy, and radiation therapy.

Cancer immunotherapy dates back to 1891, when William Coley, a New York surgeon, discovered that patients could recover from osteosarcoma after developing a form of cellulitis known as erysipelas[1]. He subsequently treated these patients by injecting the causative bacteria into the tumor. Only recently has this mechanism been understood and it took almost a century for the discovery of dendritic cells and elucidation of the mechanisms of pathogen recognition.

Cancer immunotherapy can be categorized as either specific immunotherapy, which induces an immune response against specific cancer antigens, or nonspecific immunotherapy, which enhances immune responses without targeting specific cancer antigens. Examples of the former include peptide therapy and dendritic cell therapy, whereas the latter include cytokine therapy and lymphokine-activated killer (LAK) cell therapy. One reason why immunotherapy has not been particularly effective thus far is the existence of "immune checkpoints", whose role in suppressing the immune system was not discovered until recently.

The immune system features both accelerators (activating costimulatory molecules) and brakes (coinhibitory molecules). Historically, cancer immunotherapy has focused on accelerators. In contrast, immune checkpoint inhibitors accelerate an immune response by releasing the brakes on the immune system. Immune checkpoint inhibitors such as programmed cell death protein 1 (PD-1) antibodies have profoundly changed the overall perception of cancer immunotherapy into that of a promising therapeutic method[2].

This review focuses on the immune checkpoint molecule PD-1, outlining how cancer escapes from the host's immune system and how PD-1 blockade exerts its anticancer effects.

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What is an Immune Checkpoint?

To optimally protect the organism, the immune system has to maintain a careful balance between elimination of foreign pathogens and tolerance of self-antigens. Costimulatory and coinhibitory molecules expressed on T cells play an important role in balancing immune response.

To activate T cells, costimulation (the second signal) is required in addition to antigen stimulation (the first signal). The second signal is transmitted by binding of the B7 molecule (CD80/B7-1 and CD86/B7-2) on antigen-presenting cells to CD28 on T cells. If this second signal is absent, T cells become unresponsive to the antigen (T cell anergy).

In recent years, several new members of the B7/CD28 family of molecules have been identified. CD28 family molecules include those that promote T cell activation (costimulatory molecules) and those that inhibit it (coinhibitory molecules), with the former including CD28 and ICOS and the latter including cytotoxic T lymphocyte antigen 4 (CTLA-4) and PD-1. Coinhibitory molecules such as CTLA-4 and PD-1 function as "immune checkpoints" that suppress inappropriate immune responses and excessive inflammatory reactions against the "self" and play an important role in protecting the body from tissue injury.

Mechanism of Immunosuppression by PD-1

The PD-1 gene was cloned in 1992 in the Honjo Laboratory at Kyoto University[3], and its function was subsequently determined by the same group (**Fig. 1**). PD-1 is an immunoinhibitory receptor belonging to the CD28 family that is expressed on activated T cells to regulate T cell proliferation and effector function (e.g., cytokine production and cytotoxic activity)[4]. The immunosuppressive action of PD-1 was revealed using PD-1-deficient mice[5,6]. Depending on their genetic background, PD-1-deficient mice develop a variety of autoimmune diseases, but their autoimmune symptoms are late onset and relatively mild compared with CTLA-4-deficient mice. PD-1-deficient mice develop lupus-like glomerulonephritis and arthritis on a C57BL/6 background, dilated cardiomyopathy with deposition of autoantibodies on a BALB/c background, and type I diabetes on an NOD background. Thus, PD-1 plays an important role in autoimmune tolerance.

PD-1 is expressed on antigen-specific effector T cells and memory T cells at a relatively late stage of the immune response. Expression is also observed on T cells "exhausted" by chronic stimulation. Regarding regulation of PD-1 expression, in the former cell type, the transcription factor NFATc1 is induced by antigen stimulation via T cell receptors (TCR) and costimulation via CD28, whereas in the latter, binding of IRF9 stimulated by inflammatory cytokines (IFN-α) to the promoter region of the PD-1 gene has been reported to promote the transcriptional activity of PD-1[7,8].

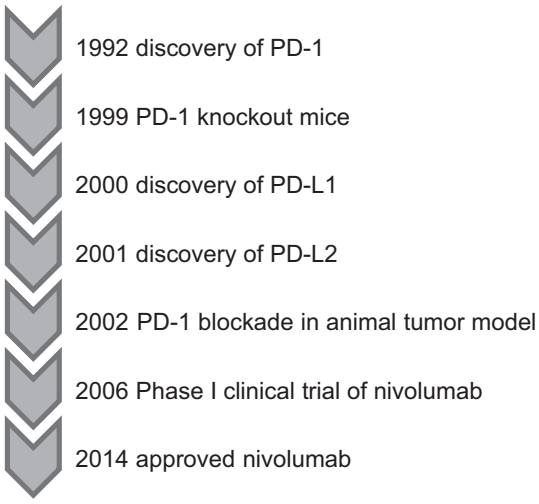

Fig. 1 History of PD-1 research

In 2000 and 2001, PD-L1 (B7-H1, CD274), and PD-L2 (B7-DC, CD273) were identified as ligands of PD-1, revealing the molecular mechanism of immunosuppression by PD-1[4,9]. The cytoplasmic domain of PD-1 contains an immunoreceptor tyrosine-based switch motif (ITSM). When physiological ligands such as PD-L1 and PD-L2 bind to PD-1, the ITSM is phosphorylated, allowing its association with the dephosphorylating enzyme SHP2. SHP2 dephosphorylates and inactivates the important TCR signaling adapter molecule ZAP70, suppressing T cell activation[2,10]. Consequently, PD-1 signaling suppresses T cell proliferation, production of cytokines such as IFN-γ, and cytotoxic activity.

Functional Differences between CTLA-4 and PD-1

PD-1 and CTLA-4 are also expressed in different cells as well as at different times. CTLA-4 is expressed constitutively in regulatory T cells and transiently in a wide range of T cells in the early stage of activation (within 24–48 h). In contrast, PD-1 is expressed in effector and memory T cells at a later stage of activation (after 72 h), in which selection and maturation by antigen affinity has progressed, and on T cells "exhausted" by chronic stimulation. Due to such differences in expression, PD-1 and CTLA-4 control different aspects of the immune response. CTLA-4 mainly controls antigen presentation in

lymphoid tissues, whereas PD-1 regulates the cytotoxic activity of killer T cells at the site of inflammation.

A further significant difference is revealed in the phenotype of the respective knockout mice. The autoimmune symptoms in CTLA-4-deficient mice develop at an early stage and are systemic, severe, and lethal[11]. In CTLA-4-deficient mice, T cells infiltrate the body organs, causing graft-versus-host disease (GVHD)-like symptoms and death at a young age. On the other hand, autoimmune symptoms in PD-1-deficient mice are delayed, organ-specific, and relatively mild[5,6]. The side effects of antibody administration resemble the phenotype of knockout mice, with monoclonal antibody (mAb) against CTLA-4 inducing a higher frequency and severity of adverse events than mAb against PD-1[12,13].

Differences in Expression between PD-1 and PD-L1/PD-L2

Expression of PD-1 is restricted in time and location. PD-1 is scarcely expressed in normal mice or in the peripheral blood of healthy individuals. Expression appears only after an immune response such as during infection or inflammation and is restricted to late-stage activated T cells. Particularly strong expression can be seen in effector T cells invading peripheral tissues at sites of inflammation.

In contrast to PD-1, PD-L1 is widely expressed in various cells and tissues. PD-L1 is constitutively expressed in normal peripheral tissues, antigen-presenting cells, and vascular endothelial cells of various organs, and its expression increases during inflammation[4,14]. During an immune response, most immune cells, including activated T cells and B cells, express PD-L1. In addition, during viral infections, not only vascular endothelial cells, but also parenchymal cells of affected organs express PD-L1. Even more interestingly, PD-L1 is expressed in many types of cancers such as hematologic tumors, skin cancer, lung cancer, ovarian cancer, and breast cancer[9,15]. In contrast, expression of PD-L2 is limited to antigen-presenting cells[9].

Antitumor Effects of Anti-PD-1/PD-L1 Antibody

After an immune response, activated T cells express PD-1 and inflammatory stimulation induces PD-L1 expression in various cells of lymphoid and peripheral tissues. PD-L1 binds to PD-1 and suppresses the function of T cells, resulting in immune tolerance, which suppresses an excessive immune response and protects living organisms from tissue injury. This is considered to be the inherent physiological role of PD-1/PD-L1 signaling. Cancer cells expressing PD-L1 suppress activation of T cells to escape host immune surveillance[16]. In the cancer microenvironment, PD-L1-expressing cells other than cancer cells, such as immunocompetent cells and vascular endothelial cells, may be involved in T cell suppression.

Overexpression of PD-L1 in tumor cells suppresses the proliferation, cytokine production, and cytotoxic activity of T cells via PD-1 expressed on T cells. Transplantation of tumor cells expressing PD-L1 into syngeneic mice significantly increases tumor size and promotes invasion or metastasis into other organs. This evidence led us to show that inhibition of PD-1/PD-L1 signals can impair the growth of tumors in an animal experimental model in 2002[16]. In this report, administration of anti-PD-L1 mAb in tumor model animals markedly suppressed tumor growth and concomitantly prolonged individual lifespan via T cell activation through inhibition of the PD-1 signal.

Although CTLA-4 blockade has already been reported to have antitumor effects against highly immunogenic tumors, it was ineffective against tumors with low immunogenicity such as B16 melanoma and viral infection[17,18]. However, PD-1 blockade shows antitumor effects against poorly immunogenic tumors in a metastasis model as a single agent[19]. In addition, PD-1 blockade shows protective immunity against viral infection[14].

From Basic Studies to Clinical Application

Based on the basic medical research described above, a fully humanized PD-1 mAb, nivolumab (also known as BMS-936558, MDX-1106, and ONO-4538), was developed for clinical application. Nivolumab was prepared from a humanized immunoglobulin gene-expressing mouse (XenoMouse), stabilized by mutant IgG4-S228P, and improved by minimizing cytotoxicity through a reduction in antibody-dependent cell-mediated cytotoxicity (ADCC).

Nivolumab phase I clinical trials started in the US in 2006 and in Japan in 2009. The response rate to nivolumab in advanced cancers was approximately 20% to 30% (i.e., 28%, 18%, and 27% for malignant melanoma, non-small cell lung cancer (NSCLC), and renal cell carcinoma, respectively) with a 14% frequency of adverse events of grade 3 and above. Nivolumab was approved as a therapeutic agent for malignant melanoma in Japan in 2014 and later for NSCLC in the US, EU, and Japan in 2015. It was subsequently approved for renal cell carcinoma in the US. Phase III clinical trials for various can-

cers are currently underway around the world. The agents for immune checkpoint blockade currently include nivolumab and pembrolizumab as PD-1 mAbs and atezolizumab and durvalumab as PD-L1 mAbs.

There are several advantages of PD-1 blockade: (i) PD-1 blockade can be applied to various types of tumors because it is not specific to tumor antigens; (ii) the effect of PD-1 blockade is durable even if the tumor cells mutate because it targets lymphocytes not tumor cells; (iii) PD-1 blockade can restore and enhance the functions of tumor-specific effector and memory T cells; and (iv) PD-1 blockade has less cytotoxicity and fewer adverse effects than conventional chemotherapy or CTLA-4 blockade. However, administration of PD-1 antibodies under inflammatory conditions might trigger autoimmune responses, so it is necessary to carefully consider combination therapies with other treatments (e.g., surgical therapy, radiotherapy, and chemotherapy) and the timing of PD-1 blockade.

Although its frequency is lower than CTLA-4 blockade and chemotherapy, PD-1 blockade can cause adverse effects such as autoimmune pneumonia, enteritis, hepatitis, thyroiditis, and pituitary inflammation. Therefore, follow-up monitoring and early detection of adverse events are extremely important. Urgent and appropriate procedures (e.g., discontinuation of PD-1 blockade, and steroid therapy) can inhibit autoimmune symptoms in most cases.

Recent Trends and Future Prospects

The focus of clinical trials of PD-1/PD-L1 inhibitors is shifting from malignant melanoma to lung cancer, other solid cancers, and hematological malignancies.

The therapeutic effects of immunotherapy targeting PD-1 depend on the type of cancer and tissue. The response rate is high for cancers with high immunogenicity such as malignant melanoma. In NSCLC, the response rate is high for squamous cell carcinoma (33% for squamous cell carcinoma, 18% for non-squamous cell carcinoma)[13]. Exome analysis of patients with NSCLC revealed a correlation between mismatch repair gene abnormality, smoking, and somatic mutations. Treatment with PD-1 inhibitors is more effective for tumors with a high somatic mutation frequency[20]. Animal models have shown that tumor-specific mutant antigens derived from genetic mutations (neoantigens) serve as targets for killer T cells activated by checkpoint inhibitors[21]. Stronger antitumor effects can be expected with a combination of a PD-1 inhibitor and vaccine therapy using neoantigens.

The response duration of PD-1 blockade is long and can be maintained for more than 2 years in many cases. PD-1 mAb therapy showed significantly better results than docetaxel as a standard therapy for patients with previously advancedsquamous cell carcinoma NSCLC in a phase III trial comparing these two therapies[22]. For example, the 1-year survival rate was 42% for PD-1 mAb and 24% for docetaxel; the response rate was 20% for PD-1 mAb and 9% for docetaxel; the response duration was still in progress for PD-1 mAb and 8.4 months for docetaxel. The trial also showed that PD-1 blockade had fewer adverse effects of grade 3 or more (i.e., 7% and 57% for PD-1 mAb and docetaxel, respectively). The estimated 5-year overall survival rate of patients with pretreated, advanced NSCLC who received nivolumab was 16%[23]. There are many cases in which the antitumor effect of PD-1 mAb persists for a long period even after antibody administration is stopped due to the occurrence of an adverse event. PD-1 blockade may recover the functions of memory T cells.

Conclusion

Immunotherapy targeting PD-1 is effective for approximately 30% of patients with cancer at most but not for the remaining 70%. Thus far, there is no consensus on the relationship between PD-L1 expression and the response rate in tumor tissues. PD-L1 is expressed not only in tumor cells, but also in various types of cells such as immunocompetent cells and vascular endothelial cells upon inflammation. Given that T cells receive inhibitory signals from a variety of cells in addition to tumor cells, it is important to establish a diagnostic method incorporating viewpoints to systemically and comprehensively evaluate immune and inflammatory responses. Therefore, from the perspective of medical economics, it is vital to urgently explore biomarkers that can predict treatment effect and establish reliable diagnostic methods.

Conflict of Interest: The authors declare no conflict of interest.

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(Received, May 15, 2018)
(Accepted, August 16, 2018)