An introduction to cancer vaccine, chimeric antigen receptor (CAR) T-cell and immune checkpoint blockade

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Abstract. Cancer immunotherapy has been a hot topic of cancer therapy discussion for over decades. Several successful cancer immunotherapies have already existed for about 30 years, however it is just in the past decade that immunotherapy has achieved broad breakthrough on patient survival in multiple high-incidence cancer indications. Immunotherapy, as a promising therapy depending mainly on the mechanism that immune cells work to eliminate cancer cells, has three hot topics recently. Cancer vaccine is a therapeutic vaccine that typically involves exogenous administration of selected tumour antigens to activate dendritic cells (DCs), or even DCs themselves in order to initiate and stimulate immune response to tumour cells, regain their control over tumour growth, induce existed tumour regression and eradicate minimal residual disease. Chimeric antigen receptor (CAR) T-cell therapy uses a patient's own T cells, but genetically engineered to express a synthetic receptor that binds to a tumour antigen more precisely and efficiently, to serve as more effective army against tumours. Immune checkpoint blockade (ICB) depends on blocking certain receptors and their ligands involved in pathways that attenuate T cell activation — for example, cytotoxic T lymphocyte antigen 4 (CTLA4), programmed cell death 1 (PD1) and its ligand, PDL1 — to restore T cells' activity and prevent acquired peripheral tolerance to tumour antigens. This review gives a brief introduction to how human immune system works and a basic overview on the principles of cancer vaccine, chimeric antigen receptor (CAR) T-cell and immune checkpoint blockade (ICB).

Keywords: immunotherapy, cancer vaccine, chimeric antigen receptor T-cell, immune checkpoint blockade.

1. Introduction

Our body is facing all kinds of threats from both inside and outside our body. It is the fundamental responsibility of immune system that every part of our body is completely under its surveillance. When certain normal cells mutate into cancer cells, it's our immune system that immediately eliminates them. However, sometimes cancer cells find their way to escape surveillance from immune system. That's how cancer happens.

As mentioned above, the immune system retains the capacity to kill cancer cells itself. The core principle of immunotherapy of cancers, which has made a broad impact on cancer treatment these years, is to restore the activity of immune cells against cancer cells or enhance the pre-existing immune response through external intervention. This review mainly focuses on three hot topics in this

field, that is cancer vaccine, chimeric antigen receptor (CAR) T-cell and immune checkpoint blockade. Cancer vaccine uses cancer-specific antigen as a stimulation to boost immune system to find and destroy tumours. It's only used in person who already has tumours so that it's called therapeutic cancer vaccine. CAR-T cell uses a kind of T cells that more precisely target on cancer cells. This special kind of cells are programmed from normal T cells, researchers load chimeric receptors onto the surface of the T cell to make it more precise and effective. Immune checkpoints are referred to some suppressers in the immune system, which is originally used to attenuate excessive immune response, however exploited by cancer cells to escape immunity. Many researches try to find those molecules called immune checkpoint inhibitors to block this checkpoint and restore immunoactivity, which works upon blocking certain checkpoint-associated proteins from binding with their receptors.

2. A brief introduction to human immune system

Human immune system is an army inside our body, mainly functioning in preventing foreign pathogens from infecting our cells and clearing those infected or aging or other abnormal cells like cancer cells. It mainly consists of all kinds of physical barriers like skin or mucous membranes, immunological molecules like antibodies and lysozymes and immune cells like T cells, B cells, phagocytic cells, dendritic cells, natural killer cells and so on.

When an invasion of pathogen happens or an abnormal cell appears, the immune system immediately detects it respond to it. The response can be divided into innate immune and adaptive immune. Innate immune exists inborn, acting as a protective and clearing agent against all kinds of pathogens without specificity. This is the first defense line and the second defense line of our body. The first line of human immune system consists of skin and mucous membranes, which provide physical barrier and secretions like gastric acid to kill pathogens. The second line consists of Phagocytic cells and lysozyme, "wandering" inside human body, detecting and killing pathogens without specificity. These two lines are called innate immune, because it's born with nearly everyone and is not against to any specific pathogen.

But there are threats that this non-specific innate immune cannot handle. A kind of cells called antigen presenting cells (APCs) in our body can present antigens and initiate adaptive immune against specific pathogen. Higher efficiency and specificity are its features. This is the third line, also the most important line in our body, with participation of all kinds of immune cells.

2.1. Discrimination

Immune system needs to tell the differences between normal cells and cells infected by pathogens or those with 'sickness' like cancer. The way they use differs between innate immune and adaptive immune. The innate immune system recognizes pathogens by their conserved features through a sort of receptors called pattern-recognition receptors (PRRs), the most representative recognition receptors in innate immune, which are highly conserved in evolution. The features that PRRs detect include pathogen-associated molecular patterns (PAMPs), such as bacterial and fungal cell-wall components and viral nucleic acids, or damage-associated molecular patterns (DAMPs), such as heat shock proteins and IL-1α. This nonspecific detection of PAMPs and DAMPs by PRRs leads to the induction of inflammatory responses like pyroptosis and release of IL (interleukin), and further innate immune responses. Especially the sensing of microbes by PRRs expressing on APCs, particularly dendritic cells (DCs), directly leads to the activation of adaptive immune responses [1].

The recognition of pathogens by the adaptive immune system is mainly mediated by APC and major histocompatibility complex (MHC). MHC is a protein family located on animal cell surfaces, always combined with cell antigens. Cells infected by pathogens will present abnormal MHC molecules recognized by immune cells, mostly APCs, so the immune system knows what's wrong with those cells. Then they digest and process pathogens' antigens into short peptides, exposing and presenting them to other immune cells like T cells.

Common APCs include phagocytes, B cells and dendritic cells (DC). etc. Among all kinds of APCs, DC is the most efficient in presenting antigens to other immune cells. An activated DC acts as the core

of priming and maintaining adaptive immune response through induction of effective and persistent immune cell activity in participation with CD4+ T helper cell and cytotoxic T lymphocyte (CTL) responses, recruitment of T cells and B cells and durability and maintenance of response [2]. After antigen presentation, it's T cell's and B cell's work to attack pathogens.

2.2. T cell response

T cells mature in thymus where the name comes from. In a healthy person's body, these T cells act as military that keep moving between lymph nodes and the blood and the APCs were tested by T cells in case of signs for damage or infection [3]. After recognizing antigens presented by APCs depending on the T cell receptors (TCR), T cells initiate division and differentiation into different effector T cells, including cytotoxic T cells, helper T cells and regulatory T cells.

Cytotoxic T cells (also called killer T cells) attack infected cells directly through direct contact and cytokines released to initiate apoptosis. Helper T cells promote maturation and function of other immune cells like B cells differentiation and antibody secretion. Suppresser T cells can inhibit other immune cells' functions, preventing abnormal immune response to normal cells. They are the most important attacker against tumours.

2.3. B cell response

B cells can also act as one kind of APCs. After stimulated by antigens and helper T cells as followed, B cells go through differentiation and become plasma cells and memory B cells. Plasma cells secret a special protein called antibody, which originally starts as a cell-surface receptor [the B-cell receptor (BCR)]. so that it has high specificity to certain antigen and tightly combines with it, inhibiting its activity and assisting T cells to attack pathogens. And also these antibodies are able to function normally in tissues where T cells do not. Once they are produced by B cells, they are able to bind to pathogens that escape from the first two barriers. Another function that is easily ignored of antibodies is to neutralize soluble poisons (toxins) from some attackers, which shows importance in many cases, for example, in the response to diphtheria. After the response the antibodies keep existing in the blood for a period of time, also found within the mucus that lines our gastrointestinal organs and in interstitial tissue fluids, which is related with immune system's memory [3].

2.4. Memory

Memory is another representative feature of immune system. It means individuals who get rid of a specific pathogen at the same time gain the ability to resist reinfection with the same pathogen for a period of time. It just seems that the immune system builds memory about the pathogen after one infection. This marvelous capacity can save resources for our immune system, and it is also the key to vaccination.

Immune system uses many ways to keep memory about once-met antigens. Following an infection (or a vaccination, using antigens to stimulate the foundation of immune system's memory), antibody levels rise at first as immune response to antigens, and then fall off to lower levels that are higher than in the naive state however and keep themselves in that way for a long period of time [3]. This indicates that some antibodies remain to respond to a new infection about to happen in the future.

Besides antibodies, other important differentiations occur in different populations of antigen-specific lymphocytes like T cells and B cells. Part of B cells after stimulated differentiate into memory B cells which do not actively secrete antibodies immediately, but remain in the body long-term retaining the 'potential' to produce antibodies when a new infection occurs. It also has been proved by experiment that T cells which has 'met' the same antigen before are easier to activate, initiating response to the same pathogen.

3. Therapeutic cancer vaccine

Just like its defense function against foreign pathogens, immune system retains the ability to kill tumour cells, so there exist vaccines for cancer. Cancer vaccines according to its treatment aim can be

divided into preventative cancer vaccines and therapeutic cancer vaccines. Preventative cancer vaccines are vaccines that can protect healthy people against certain cancers, mainly caused by certain kinds of viruses. This type of vaccine only works when the person has gotten the vaccine before they are infected, just like normal "vaccines" you have heard. There are already 2 types of vaccines that prevent cancers have been approved by the U.S. Food and Drug Administration (FDA): HPV vaccine and Hepatitis B vaccine.

Therapeutic cancer vaccines are used for people who already have tumours, working to promote immune system to fight cancer. Different treatment vaccines deal with tumours in different ways. They can also stop a tumour from growing or spreading, keep destroying cancer cells still surviving after treatment and prevent the cancer from coming back. For most types of cancer vaccines, CD8+ cytotoxic T cell-mediated cellular immunity is especially significant in eliminating malignant cells for cure [4].

3.1. Principle
Most of cancer vaccines typically refer to the application of selected tumour antigens just like other vaccines against viruses do. Moreover, it is generally combined with adjuvants that are used for activating DCs, or even DCs themselves, the aim of which is to stimulate DCs' function that recruits, activates and maintains T cell response against specific tumour antigens, finally regaining control over tumour growth. The main basic concerns needed for successful therapeutic vaccination against tumours include delivery of antigens with both large amount and high quality to DCs, optimal DC activation, DC functioning in promoting strong and sustained T cell responses and durability of response and maintenance of effection [4].

3.2. Antigen selection
We can use the term 'neoantigen' to refer to those mutated tumour antigens that only express in tumour tissue. Neoantigens are typically not germ line encoded. Therefore, theoretically, the patient possesses no central tolerance towards these antigens, which means the existence of neoantigens is probable to arouse a robust T cell response [5]. Recently several clinical trials using neoantigens have indicated that patient survival increases, which can be taken as evidences of their immunogenicity. For example, a single-arm study uses monocyte-derived DCs loaded with personalized neoantigen short peptides on patients with melanoma demonstrating that neoantigen vaccines are able to induce a T cell-specific immune response [6]. Besides neoantigen vaccine, shared-antigen vaccine may be a better choice for those not suitable for the former. Some researchers attempt to include neoantigens along with shared antigens to expand the application scope of neoantigen vaccination [4].

3.3. Administration of antigen
So far, effective sources of tumour antigen delivery have included DNA, RNA and synthetic long peptides (SLPs) [4]. DNA vaccines can direct synthesis of antigen peptides, however requiring transcription, translation and process after injection. Certain advantages of DNA vaccines are that they are easy to manufacture, carrying built-in adjuvants and able to synthese large amounts of antigens [7]. RNA vaccines are similar, however without need for transcription and thus it directly goes to antigen protein translation and processing and presentation on MHC molecules. SLP vaccines can be directly recognized by DCs, the greatest advantage of which compared with short peptide vaccines is its requirement for DCs' processing before combining with MHC, thus increasing the treatment's efficiency.

3.4. Tumour resistance against vaccine
The resistance of tumour against therapeutic cancer vaccine can be divided into two mechanisms. One is tumour cell intrinsic mechanisms, mainly including resistance strategies conducted by those tumour cells themselves. Tumour intrinsic mechanism mainly include the downregulation or lack of tumour antigen expression [8], alterations in the antigen processing pathway and loss of HLA expression [9],

all of which to the end prevent recognition of tumour cells by T cells [4]. Another mechanism is tumour cell extrinsic mechanisms, mostly correlated with the tumour stromal components that downregulate T cell response. Tumour extrinsic mechanism include accumulation of immunosuppressive cells in TME (tumour microenvironment) such as Treg cells, MDSCs, tumour-associated macrophages (TAMs) that immune system has prepared in case of need for downregulation of T cell activation.

Because of the existence of immune escape in tumours, single vaccine hardly works. Use of combined therapy with cancer vaccines and other therapy like ICB or traditional chemotherapy are more common. Adjuvants are also introduced usually, roles of which include maximizing DC activation, promoting recruitment and activation of T cells, keeping activity of immune cells, etc.

4. Chimeric antigen receptor T cell (CAR t-cell)

As followed, immune cells inside our body originally have the ability to kill the tumour, among which T cells act as the main force when attacking tumours. Unfortunately, tumour cells have their methods to escape T cells, which called immune escape, a key mechanism of which is to reduce T cells' affinity for tumour antigens. This ability also influences the effectiveness of immunotherapy applied to the patient, leading to tolerance in the setting of cancer immunotherapy. The adoptive transfer of autologous T cells retrofited by genetic engineering to express more 'powerful' receptors targeting molecules expressed on malignant cells may have greater potential for cancer therapeutics compared to the approaches above [10].

4.1. Principle

As is known to all immune cells have the ability to kill tumour cells, but tumour forms when somehow they lose the power to recognize. To enhance TCR's affinity for a tumour antigen, researchers redirect and reprogram T cells with a special receptor called chimeric antigen receptors (CARs).

Chimeric antigen receptor is a kind of recombinant receptor which provide both antigen-binding and T-cell–activating functions. Over the past decade has reported a multitude of these so-called CARs, targeting an array of tumour antigens on tumour cell surface [11]. Typically, a CAR possesses a single chain variable fragment (scFv) originally from the antibody targeting the tumour antigen with affinities several orders of magnitude higher than normal TCRs, which gives these CAR T-cells the ability to be insensitive to most tumour escape mechanisms, mostly related to MHC loss.

4.2. Generation of CAR T-cells

Researchers typically generate CAR T-cells by the following steps: collecting cells from the patient by leukapheresis, removing myeloid cells by elutriation, enrichment of T cells, genetic engineering and ex vivo cultivation for expansion. The most critical constraint in cell manufacturing is isolation of T cells from leukapheresis samples. Typical use of positive and negative selection methods may inevitably cause T lymphocytes enriched to still be mixed with inhibitory cell types that may impede further CAR T-cell expansion in culture [10]. However, a recent case was reported that during T-cell sample processing an leukemia cell was unexpectedly transduced by the CAR transgene, resulting in these receptors binding to the CD19 epitope on the surface of the leukemia clone that had expanded massively in an ALL patient, making it unrecognizable by anti-CD19 CAR T-cells [12]. Such cases indicate that more efforts should be focused on the purification of T cells to better improve the safety and efficacy of this type of therapy.

4.3. Structure of a chimeric antigen receptor

The structure of CAR is another key factor for enhancing affinity to tumour antigens and the following immune response. The first generation of CAR mainly consists of two parts. The extracellular part is tumour-antigen-combine domain originally from a scFv, and the intracellular part is CD8 and the CD3ζ signaling chain that mediate T cell activation just like how TCR does. The second generation, in order to further strengthen T cell function after antigen recognition, introduces a co-stimulatory

endodomain. Several ligands for immunoglobulin (Ig) super-family and TNF receptor family costimulatory receptor have been confirmed by experiments being able to functioning in the enhancement of T-cell expansion and cytokine secretion [13]. The third generation contains multiple co-stimulatory molecules (e.g. CD28). Such designs both allow the CAR T-cells to have a higher affinity to cancer antigens than normal T cells with TCRs and trigger a more rapid, long-lasting and stronger immune response.

4.4. Overcoming resistance to CAR T-cell therapy
Just like many other therapies applied, tumours could grow resistance to CAR T-cell therapy in some circumstances. Just take pediatric ALL for an example. The loss of the CD19 antigen or epitope may be one possible mechanism of therapy failure, thus leading the CAR to lose their target, despite adequate persistence of transferred cells. Combinations of CARs that aim at multiple targets on tumour cells are hopeful to eliminate the single protein loss like this. Another main reason could be failure of expansion and/or persistence of CAR T-cells, probably related to patients' pre-existing T cell quality or other immunosuppressive mechanisms [14, 15]. Researchers trying to bypass these factors have been using precision genome editing like zinc-finger nucleases to successfully develop a 'universal' CAR T-cell line, which collects T cells originally from healthy, allogeneic donors. The universal ones are believed to have the potential to overcome failures associated with autologous T-cell defects such as terminal differentiation [11]. In vivo the immune checkpoint blockade which will be mentioned below with concurrent CAR T-cell therapy may also be effective as a strategy against resistance caused by certain immunosuppressive mechanisms.

5. Immune checkpoint blockade
Just as the methods mentioned above, the existence of suppresser T cells shows the evidence that immune system possesses its own way to attenuate T cell activation, probably in order to prevent abnormal immune reaction to normal somatic cells, which is also called T cell tolerance. We use immune checkpoint to refer to those key nodes (ligands and their receptors) in the inhibitory pathways existing in the immune response. These checkpoints play an important role in normal operation of immune system, including maintaining self-tolerance and regulating the duration and extent of immune responses, primarily to minimize possible damages caused by immune cells against normal tissue [16].

5.1. How ICB works
Some of the tumour cells take advantage of certain immune-checkpoint pathways as their major mechanisms of immune resistance. Some key receptors and their ligands in this process, such as cytotoxic T lymphocyte antigen 4 (CTLA4) , programmed cell death 1 (PD1) and its ligand, PDL1, are used by cancer cells to attenuate T cells for immune escape. Basically most of the immune checkpoints are shown in the pattern of ligand–receptor interactions. Therefore, these checkpoints can be easily blocked by preventing this interaction, produced by competitive binding or recombinant of ligands or receptors, etc [16]. This therapy is called immune checkpoint blockade, or immune checkpoint inhibition, finally resulting in reactivation of T cells against tumours.

5.2. Principle of CTLA4 blockade
CTLA4, as a member of T cell transmembrane protein, has been proved to be an attenuator of T cell activation by Allison et.al [17]. Researchers found that CTLA4 and CD28 competitively bind to the co-stimulatory ligands T lymphocyte activation antigen B7-1 and B7-2, which typically express on APCs. Furthermore, CTLA4 realizes its function that attenuates T cell activation through multiple mechanisms: recruitment of phosphatase to inhibit TCR signal pathway; preventing CD28 binding by changing CD28 localization; blockade of B7 ligands by transendocytosis. Besides direct interference to the signal pathway, CTLA4 can also increase T cell motility and thus reduce the contact of T cells to APCs, resulting in decreased proliferation of them [17].

Among all immune cells, Treg cells typically express the highest level of CTLA4. Experiments have proved that functional CTLA4 loss downregulates B7 ligands organization of Treg cells, in turn proving that CTLA4 is strongly associated with immune suppression performed by Treg cells. Effective anti-CTLA4 antibodies are able to bind to activated FcγR receptors, thus depleting intratumoural Treg cells while sparing Treg cells in the periphery, finally resulting in an improved Teff/Treg cell ratio within the tumour [17]. Solely use of ligand blockade causes expansion and activation of Treg cells, as a role of negative feedback control, so agents that deplete Treg cells should be applied in combination.

5.3. Principle of PD1 blockade

PD1 is another widely-studied immune checkpoint to be targeted by ICB, with two inhibitory ligands PDL1 and PDL2 as known. PDL1 was previously reported to be an attenuator of T cell activation. In 2002, Chen and his colleagues reported the fact that PDL1 is observed to express in a variety of human cancer tissues. Using a mouse syngeneic tumour model, it has been proved by researchers that PDL1 expression could increase tumour cell proliferation and survival and increase T cell apoptosis, all of which could be neutralized by an anti-PDL1 antibody [18-20]. PDL2 is speculated to play a role in negative regulation of PD1 checkpoint, but it seems confused that solely blockade of PDL2 has not demonstrated any antitumour effects [21].

PD1 mainly expresses during 2 different cellular processes, that is precursor T cells' differentiation into Teff cells or memory cells and activation or reactivation of these T cells. High level of expression of PD1 will cause Teff cells to enter an "exhausted" phase, finally resulting in programmed death. PD1 blockade is proved to be effective in early state of this "exhausted" phase by reactivation, but ineffective in terminal state, which indicate PD1 blockade is "state shift" rather than "state reversion" [17]. PD1 expression is also detected on suppresser T cells (Treg cells), but its function remains for further study [22]. Experiment on mice and observation of an decrease of Treg cells in patients receiving PD1 blockade therapy imply that PD1 may be related to maintenance of Treg cell population [21, 23]. PD1 is also expressed on macrophages and dendritic cells, suppressing their functions and thus supporting tumour growth.

ICB drugs like ipilimumab and nivolumab have already acquire success in treatment for specific cancers. Current focus on ICB is how to enhance its efficacy, mainly by deeper research on molecules and cells related to activation and suppression of immune response. Combination of ICB and other strategies like traditional chemotherapy or newly introduced cell therapy is also promising. Except for PD1 and CTLA4, research on other immune checkpoints such as LAG3 and TIGIT can probably provide more details and availability of ICB efficacy.

6. Conclusion

Immunity works to respond to numerous threats from both outside and inside our body. During the last few decades, researchers have been studying how to make use of our understanding of the fundamentals and principals of immunology to reinforce, regulate or interfere with the immune response, in order to better protect our body from infection or assist immune system eliminating tumour cells. Thanks to these efforts, immunotherapy has made great contribution into human health, and we expect much more from it in the coming future. We use cancer vaccine to present selected antigens and activate DCs, in order to teach immune system to better find and recognize tumour cells. We engineer CAR T-cell to strengthen its' binding to antigens, and transport these stronger 'soldiers' into the immune system. And we use ICB to block the negative regulation of T_{eff} cells from tumour cells, thus reactivating T cell response against cancer.

Future research efforts and clinical research on immunotherapy should be focused on enhancing the efficacy, mostly depending on our further understanding of tumour immune escaping mechanism. The key is to figure out the connections between molecules and mechanisms which closely relate to suppression and reactivation of T cell response, and especially more attention should be directed towards immunosuppressive-related cells and molecules which attenuate immune response to tumours.

Furthermore, combination of these therapies with other successful strategies such as cell therapy or those more traditional like radiation and chemotherapy has more chances to overcome tumour resistance and enhance the efficacy.

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