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Immunotherapy of cancer: CAR-T and PD-1 and/PD-L1

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Abstract. Gastric cancer, one of the lethal cancer, accounts for quantities of death in recent years. This article discusses the application of in the treatment of cancer by immunotherapy, including its advantages, shortcomings and prospects. In detail, two types of strategies. PD-1/PD-L1, as a pathway of immune checkpoint, could maintain the immune response under a physiological standard. While, for cancer cells, they evolve to utilize this mechanism to escape from immune response. So, by interfering with the PD-1 involved immune checkpoint, the T cell proliferation could be stimulated to prevent immune evasion. Chimeric antigen receptor T-cell (CAR-T), another immunotherapy, making the goal of precision cancer treatment by engineering human T-cells to specifically target and remove tumour cells. If CAR acts as an artificial receptor, it can discover and capture disease-causing proteins against cancer cells, based on the activation of the host immune system. Low specificity and low drug resistance are challenges for the application of both therapies, but new advances have been made, such as combining immunotherapy with other approaches and CAR-T-based dual antigen therapy.

Keywords: immunotherapy, PD-1/PD-L1, CAR-T.

1. Introduction

Gastric cancer accounts for 4.85% of the global cancer population and 660,175 deaths from gastric cancer in 2023.Gastric cancer has an insidious onset, often missed in the early stage due to no obvious symptoms, easy to metastasise and recur, and has a poor prognosis. It is easy to metastasise and recur, with poor prognosis. The incidence rate of gastric cancer in China is high, and its mortality rate accounts for the first place of various malignant tumours. In recent years, the World Health Organization has classified gastric cancer into: adenocarcinoma (papillary adenocarcinoma, tubular adenocarcinoma, mucinous adenocarcinoma, mixed adenocarcinoma, hepatoid adenocarcinoma), adenosquamous carcinoma, medullary carcinoma, imprinted cell carcinoma, squamous cell carcinoma, and undifferentiated carcinoma. The mainstay clinical treatment options for cancer are surgery, radiotherapy, chemotherapy, and targeted therapies, except for these, immunotherapy, as an emerging technology, also achieved great advancements in recent years. In 2013, it was named the most important scientific breakthrough of the year by Science magazine because of its outstanding efficacy and innovation. However, immunotherapy has significant complexities and uncertainties as it can also cause serious adverse effects due to an overactive immune system. The basic mechanism of immunotherapy is activating or promoting the host immune response to fight against the pathogens For example, under native state, the immune response is stringent regulated by a mechanism called immune checkpoint

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which is conducted by PD1/PD-L1. The PD-1 was discovered in 1992 by Dr. Yoshitomo Motosuke, a Japanese medical scientist, a foreign member of the National Academy of Sciences of the United States of America, a member of the Japanese Academy of Bachelor's Degrees, and a member of the German Society for Natural Sciences. In the case of some pathogens, they have evolved to put the host into this state of immune inactivation, thereby achieving immune evasion. Based on this theory, a therapeutic approach to targeting immune checkpoints, such as antibodies that can disrupt interactions, e.g., between PD1 and PD-L1, has been created. Theoretically, compared to other technology, the immunological checkpoints based therapy should be more effective and have fewer adverse effects . By dynamically stimulating the immune system or modulating the immune microenvironment, the ability of the immune system to attack and eliminate the cancer cells is improved. In clinic, this therapy has been shown to be effective when combined with a variety of immune checkpoint inhibitors or conventional oncology treatments, but more research needs to be invested in this area. The use and market demand for this type of medicine is also expanding as a result of steadily advancing research into antineoplastic drugs, thus giving a significant boost to the development of tumour immunotherapy. This article will introduce the advantages and disadvantages of immunotherapy at this stage, and describe the use of two types of immunocellular therapies, CAR-T and PD-1/PD-L1 in gastric cancer, and speculate on their future prospects.

2. PD-1/PD-L1

Different stimuli also interact with each other through competitive binding of transcription factors, such as by NFAT, a transcription factor of the T-cell receptor (TCR) , and STAT5, a transcription factor run by IL-2 They have the same binding site on the PDCD1 gene, but the opposite for PDCD1. Inhibition of the PD-1 pathway also affects these transcription factors in turn, which in turn further controls immune status and PD-1 expression.

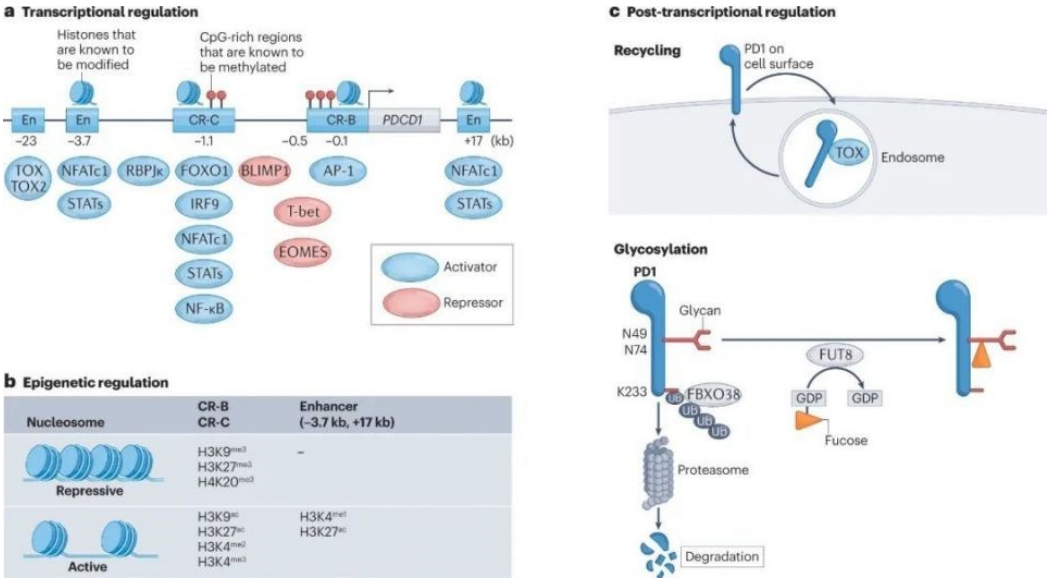

Figure 1. Schematic representation of the mechanisms regulating PD-1 transcription and expression[1]

The expression of PD-L1, as a ligand of PD-1, is regulated by two types of factors: extracellular signals related to the immune response, such as the classical interferon-gamma pathway and cytokines such as IL-6 and TNF; and intracellular regulation driven by signals from oncogenes, such as genes or signalling pathways such as EGFR and PI3K/AKT. In addition, effects at the post-transcriptional and post-translational epigenetic level need to be considered.

The signalling process of PD-1 combined to PD-L1 has been clarified in a series of studies at the beginning of this century, e.g., the binding of the two recruits SHP2 phosphatase to dephosphorylate

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signalling molecules downstream of the TCR through the intracellular immune receptor tyrosine inhibitory motif (ITIM) and immune receptor tyrosine motif switch motif (ITSM) structural domains, thereby inhibiting the T-cell-mediated immune response. Similarly, PD-1 regulates the CD28 co-stimulatory molecular pathway.

Notably, in addition to binding to PD-1, it also binds to CD80 on the surface of antigen-presenting cells, which reduces the amount of PD-1 that can be bound, thus affecting the inhibitory effect of the PD-1/L1 pathway. In addition, PD-1 and PD-L1 are involved in other signalling pathways, in particular non-dependent intracellular signalling, which is used as PD-L1-mediated PD-1, which may have an important impact on cancer cell and immune cell phenotypes, and has the to a new target for anticancer therapy.

2.1. Physiological functions of PD-1

2.1.1. Establishment of an "activation threshold" for T-cells

The fate of activated cytotoxic T lymphocytes (CTLs) varies, with some maintaining their original function, while many others enter a state of depletion or apoptosis due to over-activation. In order to increase their activation threshold and avoid continuous potent activation, CTLs can regulate this process by expressing PD-1. However, in antitumour immunity, this mechanism apparently suppresses the number of CTLs activated by tumour neoantigens. Inhibition of the PD-1 pathway reduces the threshold for CTL excitation by TCR signalling, expands the pool of anti-tumour T cells, and enhances the persistence of T-cell anti-tumour immune responses through three mechanisms:

1. Inhibition about PD-1 promotes proliferation of T cell clones that have a lower affinity for tumour neoantigens (i.e. suboptimal clones), increasing the number of T cells. As newborn cells may replace T cells that infiltrated into the tumour site earlier and become an effective anti-tumour immune response force [3].

2. Cross-reactivity between TCR and different antigens. A large number of suboptimal clones that inhibit PD-1 activation may cross-react with a variety of different antigens of the tumour, lead to a continuous attack on tumour.

3. Suboptimal clonal T cells typically express fewer immune checkpoint molecules and are therefore more likely to remain persistently activated and less susceptible to depletion when activated following PD-1 inhibition, which facilitates the achievement of long-term effects.

2.1.2. PD-1 and T-cell depletion

The concept of T-cells depletion is widely accepted and will not be repeated here. Depleted T cells can actually have 2 different phases: some are in a "pre-depleted" state and can still be activated by PD-1 inhibition, whereas the other part of PD-1-positive depleted T cells cannot be recovered [3].

2.1.3. PD-1 and the generation of memory T cells

Memory T cells are critical for a durable response to immunotherapy, but current studies have not yet determined the exact function of PD-1 signalling in this process. The effects of inhibiting PD-1 vary across disease models and at various stages of this kind of T cell formation. In addition, tissue-resident T cells (TRM cells) may expand and differentiate memory t-cells ,following PD-1/L1 inhibitor treatment [4].

2.1.4. Effects with PD-1 on regulatory T cells and intrinsic immune cells

Recent studies have demonstrated that the PD-1 pathway maintains the metabolic status and immunosuppressive function of regulatory T cells infiltrating into the tumour site, and that stopping the pathway of PD-1 , attenuates their suppressive function [5]. However, a small number of PD-1-deficient regulatory T cells show strong suppressive properties, which is considered to be the main cause of "hyperprogression" after immunotherapy [6-7], which may be related to the down-regulation of the activation threshold and the role of CTLA-4.

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A variety of intrinsic immune cells, such as intrinsic lymphocytes (ILCs), also express PD-1, which modulates the immune response to pathogenic infections and even tumours. Current studies have shown that inhibition of the PD-1 process generally positively regulates the anti-tumour activity of intrinsic immune cells, however, more precise mechanisms and implications require more research.

2.1.5. Regulation of microorganisms by PD-1

PD-1 also regulates gut microbes in the body by affecting specific immune cell subsets. For example, PD-1 deficiency leads to malfunction of follicular regulatory T cells, which in turn causes abnormal plasma cell function in the gut, allowing gut microbes to penetrate the immune barrier and trigger a systemic pro-inflammatory immune response.

2.2. Immunological combination therapy involving inhibitors of PD-1/L1

The era of inhibitor monotherapy is about to pass, and immune combination therapy has become a mainstream trend. In this review, Professor Toshiyuki Motosuke adopts a classification method different from the conventional one, and divides the combination therapy regimens containing PD-1/L1 inhibitors into three major categories: 1) in combination with other immune checkpoint inhibitors; 2) in combination with other drugs that lower the threshold of T-cell activation; and 3) in combination with drugs targeting the metabolic pathway of T-cells.

2.2.1. PD-1/L1 inhibitors in combination with other immune checkpoint inhibitors

In addition to the approved CTLA-4 inhibitors and LAG3 inhibitors, there are many investigational immune checkpoint inhibitors against targets such as TIGIT and TIM3. However, the synergistic mechanism of CTLA-4 inhibitors and PD-1/L1 inhibitors is relatively more clear at present: CTLA-4 inhibitors mainly act at the early stage of the immune response to activate T cells with different TCR affinities, thus effectively lowering the activation threshold of T cells.

2.2.2. PD-1/L1 inhibitors in combination with other drugs that lower the threshold of T-cell activation

Downstream molecules of the TCR signalling pathway likewise affect the activation threshold of T cells. For example, the E3 ubiquitin ligase CBL-B inhibits the phosphorylation of key signalling molecules, thereby acting as a negative feedback regulator in the TCR pathway. Knockdown of CBL-B significantly reduces the activation threshold of T cells, enhances their activity, and induces the conversion of T cells into memory T cells, making CBL-B an ideal therapeutic target. The PD-1-SHP2 axis composed of SHP2 phosphatase and PD-1 mentioned in the paper, as well as similar regulatory molecules such as CD45 and PTPN22, can be used to rise the activation threshold to T cells through negative feedback regulation. Designing targeted new drugs for these loci is a very feasible strategy.

2.2.3. PD-1/L1 inhibitors in combination with drugs targeting T-cell metabolism and mitochondrial status

The metabolic state of T cells has an important impact on their anti-tumour activity and even determines their activity to a large extent. It is currently believed that cytotoxic T lymphocytes (CTLs) mainly rely on glycolysis for energy supply, and inhibition of the PD-1 pathway can effectively promote glycolysis, but it may also lead to the over-activation of CTLs, which may cause them to enter the depletion state or apoptosis prematurely, and thus reduce the number of CTLs that are actually involved in the anti-tumour process. T-cell clones that expand after PD-1 inhibitor treatment, as well as long-lived memory T cells, which are essential for the lasting effects of anti-immunotherapy, are more inclined to rely on oxidative phosphorylation or fatty acid metabolism involving mitochondria. Therefore, intervening in the metabolic pathways of T cells on a case-by-case basis is essential, and effective interventions can even bring CTLs "back from the dead".

For example, in 2018, a study conducted by Prof Benjamin's team showed that the lipid-lowering drug benzofibrate could synergise with PD-1 inhibitors by activating mitochondrial function in cytotoxic T-lymphocytes (CTLs) and restarting oxidative phosphorylation or fatty acid metabolism [8].In 2022,

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Prof Benjamin's team discovered another dietary supplement with a similar function --polyamine spermidine [9].

In addition, considering that gut microbes and their metabolites can influence the function of T cells and other immune cells through a variety of pathways, interventions from the perspective of gut microbes, e.g., through faecal bacterial transplantation or supplementation with specific metabolites, are likewise a promising strategy for future immune-combination therapies.

3. CAR-T

CAR-T immunotherapy achieves the goal of precision cancer treatment by engineering human T-cells to the target and remove cancer cells. Although CAR-T immunotherapy shows great potential, there are still many challenges in its clinical application, such as insufficient specificity in recognising tumours, safety issues and that CAR-T cells cannot be maintained in the body for too long. Researchers are working on improvements to enhance the safety and anti-tumour effects of CAR-T in clinical applications [8].

3.1. The evolution of CAR-T

In CAR-T immunotherapy, CAR designing is a central element. CAR is a single-chain variable fragments (scFvs) incorporating monoclonal antibodies that specifically recognise antigens, including variable heavy chain regions (VH) and variable light chain regions (VL), and binds to the intracellular signalling domain of the T-cell receptor (TCR), and the T-cells expressing CAR are not restricted by the MHC. Currently, CAR has been developed to the fourth generation.

-First generation CAR:scFv integrates only the CD3ζ signalling domain and is capable of triggering tumour-specific cytotoxicity [9]. Some first-generation CAR-T cells continued [10], but have limited expansion and do not induce meaningful anti-tumour effects [11].

-Second- and third-generation CARs: integration of one or two co-stimulatory structural domains (usually CD28 or 4-1BB) upstream of CD3ζ, which confers a more potent anti-tumour cellular effect on T cells, as well as increased cytokine production and improved proliferation and persistence of CAR-T cells [12-13].

Fourth-generation CARs (also known as "armoured CARs"): based on second- or third-generation CARs, other stimulatory structural domains have been integrated to co-express key cytokines or co-stimulatory ligands aimed at improving the tumour microenvironment, such as the inflammatory cytokine IL-12 [14], IL-18 [15] or CD40L [16] secretion to enhance antigen cross-presentation and promote epitope spreading.

Taking an overview of these four generations of CAR, the first generation of CAR has relatively weak anti-tumour effects, while the third and fourth generations have enhanced the ability of T cells to kill tumours, but at the same time have brought more toxic side effects. In contrast, second-generation CARs are more widely used in tumour therapy and are relatively milder.

3.2. Three Challenges for CAR-T Cell Therapy

Challenge 1: Maintaining the persistence of CAR-T cells in vivo. In the context of chronic infection or cancer, prolonged antigenic stimulation leads T cells to enter a state of depletion as evidenced by the surface expression of multiple inhibitory receptors, such as PD-1, TIM-3, and LAG-3, etc., and these depleted T cells have poor proliferative capacity and low effector function [21]. Preclinical and clinical data have amply demonstrated that CAR-T cells are similarly susceptible to depletion, thereby limiting their therapeutic efficacy. Typical depletion markers on tumour-infiltrating CD19 CAR-Ts were higher in non-responders than in full responders [22]. Recent studies have shown that functional defects in c-Jun, a major gene that regulates cellular protein levels and whose dysfunction leads to an rise in proteins that stop T-cell activity, are a key factor in T-cell depletion [23]. How to enhance the persistence of CAR-T cells in vivo and effectively increase their antitumour potency is a major facyor that CAR-T technology is currently facing.

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Challenge 2: Overcoming the CAR-T off-target problem and improving specific recognition ability. To ensure that CAR-T cells attack only tumour cells without damaging normal tissues, their ability to specifically recognise tumour surface antigens must be enhanced. However, several studies have shown that tumours can evade recognition by the immune system through down-regulation or loss of surface antigens, e.g. after treatment of B-cell lymphomas with CAR-T targeting CD19, up to 60% of relapses were characterised by loss of CD19 antigen. One of the possible reasons for this is the mutation of the CD19 gene in exons 2 to 5, which results in the loss of the CD19 protein, thus making it unrecognised by CAR-T [17]. In solid tumours, its heterogeneity and complex tumour microenvironment make it more difficult to find therapeutic targets that are specific, safe and not easily off-target, while tumours are protected from CAR-T cells by tumour microenvironmental protection [18], all these factors lead to a more challenging application of CAR-T tumours.

Challenge 3: Ensuring safety applications. Safety is a prerequisite for the application of CAR-T technology. Significant toxicities.The development of CRS is closely associated with macrophages, which are a major source of inflammatory cytokines (e.g., IL-6, IL-1, and IFN-γ) and may be involved in endocytosis and self-expanding catecholamine loops, which directly activate macrophage production and cytokine release during CRS [19]. Recent studies have revealed the mechanism of this therapy, i.e. recognition of target cells by CAR-T results in the release of perforin and granzyme B, which activate the caspase3-GSDME pathway leading to cellular pyroptosis and triggering CRS [20]. Scorched cells stimulate macrophages to activate caspase1-GSDMD to produce IL-6 and IL-1β, which triggers CRS.The team also noted that, unlike cells targeted by CAR-T, cells targeted by natural T cells undergo apoptosis rather than scorched death. There are two possible reasons for this: firstly, CAR-T cells release more perforin/granzyme B with higher affinity than natural T cells; secondly, natural T cells induce only a small amount of GSDME cleavage without activating GSDMD [20]. The elucidation of these mechanisms provides new directions for optimising CAR-T therapies.

3.3. Optimisation and improvement of CAR-T technology

Enhancing the targeting specificity of CARs To cope with the antigen escape problem, scFv is optimised and usually designed to be able to target two antigens, with binding to either triggering activation of the CAR-T. One approach is to co-transduce two vectors encoding several CARs into a single T cell [24], or to construct a double cis-trans vector to transduce into cells so that each cell expresses two independent chimeric receptors [25]; another approach is to use a tandem bispecific CAR, where recognition of either pair of antigens by the two binding structural domains of the extracellular portion triggers effector function. The use of bispecific CD20/CD19 CAR-T for the treatment of patients with recurrent B-cell malignancies resulted in an overall remission rate of 82% after 28 days of treatment in a phase I clinical trial, and the infusion of non-cryopreserved bispecific CD20/CD19 CAR-T was found to be even more effective, with patients receiving the highest dose and being non-cryopreserved achieving a total remission rate of up to 100% at 28 days, which provides a new possibility for CAR -T offers a new possibility [26]. Other studies have promoted the engagement of endogenous non-engineered T-cells with tumour cells by secreting a bispecific T-cell engager consistingof two fused scFv. Choi et al [27] engineered an EGFR/CD3 BiTE-secreting CAR-T cell targeting EGFR-VIII to clear glioblastoma, demonstrating in a mouse model that 80% of mice showed complete remission after 3 weeks of treatment, with no tumour present in vivo. These strategies are a good solution to the difficulty of complete targeted clearance of glioblastomas due to their heterogeneity.

4. Conclusion

We must acknowledge that immunotherapy is a landmark advance in the field of cancer treatment, but it still has flaws.

Immunotherapy has certain limitations, which are mainly reflected in the inconsistency of efficacy, the possibility of pseudo-progression or hyper-progression, the possibility of immune-related adverse effects, and the need for individualised treatment.

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First, the efficacy of immunotherapy different in each people. Some patients may experience significant efficacy, whereas others may have no significant therapeutic effect. This is due to the temporal and spatial heterogeneity of tumours, i.e. the same patient may show inconsistent efficacy at different metastatic sites or at different stages of disease progression in the same patient. Therefore, the efficacy of immunotherapy depends to a large extent on individual differences in patients, and we cannot expect all patients to obtain the same therapeutic effect from immunotherapy.

Secondly, immunotherapy may be accompanied by problems of pseudoprogression and hyperprogression. 'Pseudoprogression' refers to the swelling of some tumours after immunotherapy due to infiltration and necrosis of inflammatory cells inside the tumour, which is known as "tumour inflammation". It is difficult to distinguish between 'pseudoprogression' and true disease progression with current imaging techniques, and therefore requires professional judgement by the doctor. If 'pseudoprogression' is diagnosed, immunotherapy should be continued to observe the subsequent efficacy of the treatment. 'Hyperprogression' refers to the fact that in some patients, the size of the lesion does not decrease after receiving immunotherapy, but rather grows faster. Both scenarios may lead to patients losing confidence in immunotherapy or even abandoning the treatment.

Again, immunotherapy may trigger immune-related adverse reactions. For example, allergic reactions or other immune-related side effects, such as rash, joint pain and fever, may occur. These adverse reactions may seriously affect patients' quality of life and may even threaten their lives. Therefore, when choosing immunotherapy, it is important to fully understand its potential risks and prepare accordingly.

Finally, immunotherapy requires individualised treatment. Each patient's condition and physical status is unique, so immunotherapy requires an individualised treatment plan based on each patient's specific situation. This requires an accurate assessment of the patient's condition and physical state in order to develop a treatment plan that is best suited to the patient.[28]

5. Discussion

If immunotherapy is not indicated, conventional therapy remains a sound option, and timely treatment will have a corresponding increase in the five-year survival rate of patients, with combination therapy being the current trend. At the same time, emotional counselling should be provided to the patient so that he or she trusts and cooperates with the medical team in order to achieve the desired outcome.

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