Cytokines in cancer immunotherapy

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Abstract: Cancer has always been the leading cause of death, with high mortality rate and low curability. Meanwhile, the incidence rate of various types of cancer is growing with time. Cytokine is a substance that regulate the activity of various immune cells. The usage of various cytokines to treat cancer had been studied in some research groups. This paper mainly attended to the history, mechanisms, and the effects of interleukin-2(IL-2) and interferon-alpha on various cancers as mono therapy or adjuvant therapy. This review further discussed the potential drawbacks, the development bottlenecks, and the future prospect of cytokine in immunotherapy.

1. Introduction

Immunotherapy is the advanced technology that use the artificial stimulated human immune system to fight against disease instead using medicine that directly eradicate the pathogens. Cancer is one of the major targets of this technology owing to the devastated side effects of traditional therapy. Various approaches were developed to treat cancer, majority of them are listed below.

Immune checkpoint is a self-defence mechanism that can supress the activity of some immune cell to prevent them from being too aggressive and attack normal cell. Cancer cells, however, have a checkpoint protein like CTLA-4 and PD-1 on their cell membrane that can bind to the immune checkpoint and inhibit the activities of various immune cells, which can help them to escape from the immune cells. Hence, drugs can be developed as the inhibitor of immune check-point to reverse the suppression.

These substances are designed to attack certain antigen presented on the pathogen and can be mass-produced in the laboratory. This technology has successfully been applied to fight against various virus or bacteria infected disease. Regarding the cancer treatment, normally the neoantigen of specific cancer is taken out to the lab to design the specific antibodies, which will be injected back to human body later to let the antibodies latch onto the cancer cells and attract other immune cells

In this type of therapy, T cells are typically extracted from the patient's own blood or tumor tissue, multiplied in the laboratory, and then returned to the patient to assist the immune system in fighting the cancer. Two categories are included: chimeric antigen receptor T-cell (CAR T-cell) therapy and tumor-infiltrating lymphocyte (TIL) therapy.

Though all the therapies mentioned above seem to be efficient in fighting cancer. Various limitations are still presence. For instance, majority of the immunotherapy can over-activate the immune system and cause some auto-immune diseases.

Cytokines are major regulators of the innate and adaptive immune systems that allow cells of the immune systems to communicate over short distances in paracrine and autocrine fashion. They control proliferation, differentiation, effector functions, and survival of leukocytes. In recent years, a number of cytokines, including interleukin (IL)-2, IL-12, IL-15, IL-21, granulocyte macrophage colony-stimulating factor (GM-CSF), and interferon (IFN)-α have been shown to have efficacy in preclinical murine cancer models.

Cytokine’s signal through a series of common and shared receptors, which have proven useful for a more functional classification of cytokines. The categories of cytokines are classified by their receptors. To date, there are several cytokine receptor families: Interleukin (IL)-2, IL-15, IL-21, granulocyte macrophage colony-stimulating factor (GM-CSF), and type 1 interferons (IFNs)

In the paper, the cytokine in cancer is sorted out to be effective in activating various immune cells to fight against cancer.

2. The development of cancer immunotherapy

In 1977, several researchers from the National Cancer Institute's Laboratory of Tumour Cell Biology in Bethesda, Maryland, presented a new paper explaining an experiment with an unexpected outcome. The researchers had been researching leukaemia, a type of disease that affects the blood and bone marrow. They'd attempted to cultivate disease cultures in the lab, but when they examined the vats, they discovered that they'd grown a large number of healthy human T cells instead. A subsequent examination revealed that the happy accident was caused by a chemical messenger called cytokine, which is produced by immune cells. Because the cytokine appeared to serve as a growth serum for T cells, it was dubbed "T-Cell Growth Factor" before becoming known as interleukin-2, or IL-2. For a T cell–focused researcher, IL-2 appeared to be the perfect fertilizer.

There are billions of T cells circulating throughout the human body, each of which is like a lottery ticket randomly tuned to every potential antigen-recognition combination. However, only a small number of them can be activated because they interact and match with the specific antigen. As a result, when a virus invades, only a few dozen T cells are activated.

In a normal situation, the likelihood of an activated T cell is relatively low. Furthermore, the duration of activation is relatively long. Thus, some scholars considered that how about to improve efficiency. Then, some scholars may have considered How about increasing the number of T cells? As a result, some researchers attempted to employ cytokines to accelerate t cell proliferation, which might greatly increase the likelihood of it matching with a pathogen, including cancer cells.

Following that, more research was conducted, and in 1977, Rosenberg met with the authors of the IL-2 study. Then, he tested it in his own lab, using a method he had borrowed for generating IL-2 from mice. Five days later, they discovered that the mass had grown to 1.2 million cells.

Later, with the increase of commercial activities, IL-2 was initially pushed to the market with its recombinant version, with the alanine removed from its N-terminal and residue 125 replaced with serine, by the Cetus corporation. This technology was than approved by various European country and US food and drug administration for renal cell carcinoma in 1992 [1].

3. Interleukin-2

3.1 Mechanism of interleukin-2

There are three subunits in the IL-2 receptor, include the γ chain. It complexes with a cytokine specific moiety to initiate intracellular signals through the coordinated activity of Janus kinases (JAK) 1 and 3 and signal transducer and transcription activator 5 (STAT5), which initiates transcriptional elements and regulates the expression of IL-2-inducible genes. Which may lead to the activation of the target cell. IL2 can stimulate the proliferation of antigen-activated CD8+T-cells, treatment with endogenous IL2 leads to an increase in the expression of CD25, IL2 receptor, which in turn stimulates the proliferation of CD8+T-cells. IL2 increases the expression of LAMP-1 on the surface of CD8+T-cells, decreases the expression of PD-1, an immunosuppressive receptor, thereby mediating the cytotoxic activity of CD8+T-cells (Figure 1).

3.2 Current situation of this technology

The major property of this chemical is its pleiotropy, which is single gene contributes to multiple phenotypic traits. Thus, the variety function brought by interleukin-2(IL-2) may contribute some unknown effects, either positive or negative. The uncontrollability of this chemical may be the bottleneck of the future development. Issues regarding to this technology also include the stimulation of regulatory T cell. Various studies found the capacity of iL-2 to increase the expression of FOXP3 in CD3+CD25+ T-cells [3]. This in term stimulate and maintain the formation of regulatory T cell phenotype, which functions as the immunosuppressant by inhibiting the proliferation of T cell, can

significantly decrease the immune responses. This might be negative when facing the cancer cell. Likewise, iL-2 also increase the expression of CTLA-4, which is an immune checkpoint, can also suppress the T cell and prevent the cancer cell from killing.

Like other majority of immunotherapy, treatment with interleukin-2 is accompanied by considerable side effects but dose-related. Common toxicity may cause the patients to have fever, chills, general malaise, nausea, vomiting, skin toxicity, hepatic and renal function disturbances, respiratory failure and haemodynamic changes resembling those observed during septic shock. Syndrome includes vascular leak syndrome. Peripheral or pulmonary oedema, weight gain, and ascites or pleural effusions are common symptoms of it. In high-dose case, the VLS has frequently been worsened by severe hypotension, oliguria, and respiratory failure. In exceptional situations, IL-2 might cause dizziness, disorientation, or visual hallucinations. Additionally, due to the diversity and the large quantity of distinct immune cell and vast variety of markers presented on the immune cell, the impact of several prospective targets on different immune cell types is yet unknown. The scenario is similar when it comes to surface CD markers [4].

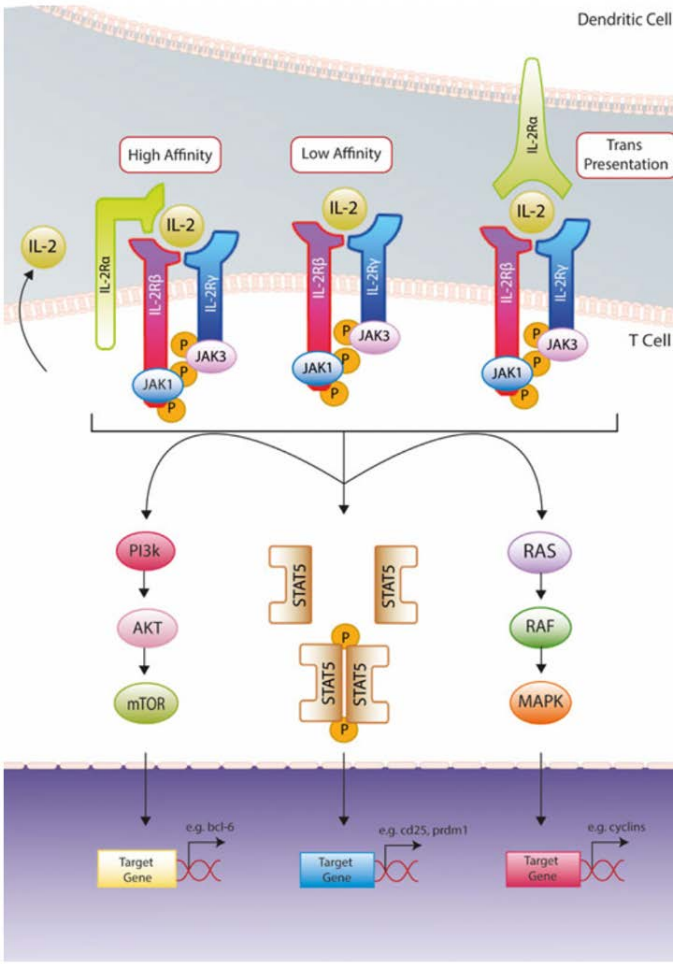

Figure 1. The IL-2 receptor and its complexes [2].

4. Interferon

4.1 Mechanism of interferon

The IFN receptors principally signal through receptor associated JAK1 to initiate the multiple STAT1 and STAT2 phosphorylation cascades. Hundreds of genes associated with antiviral and anti-proliferative functions are induced in response to different IFNs. Type I IFNs induce the expression of MHC class I molecules on tumour cells, mediate the maturation of a subset of dendritic cells (DCs),

have anti-angiogenic properties, activate B and T cells, increase cytotoxic cell numbers, and induce apoptosis of tumour cells (Figure 2).

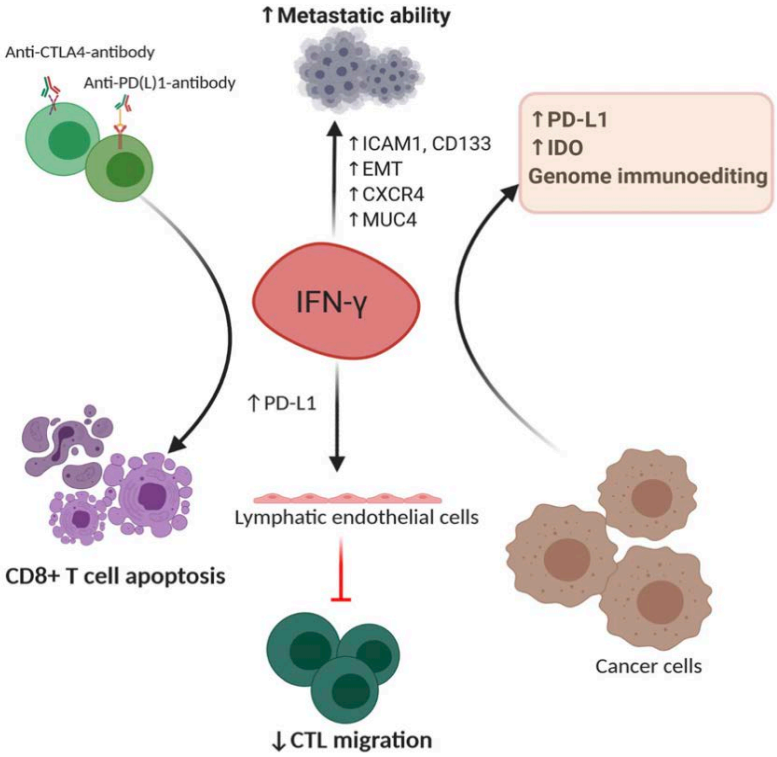

Figure 2. Interferon-mediated cancer promoting mechanisms [11].

4.2 Current situation of this technology

The toxicity of the use of interferon-alpha is dose-related, the side-effect is relatively mild that can be managed without interrupt the course of treatment. The constitutional symptoms involve fever, fatigue, headaches, gastrointestinal symptoms and myalgia. The frequency of occurrence of these symptoms are more than 80%. High-dose intravenous of this drug may consequently lead to increase in hepatic enzyme in some patients. Others like thrombocytopenia, leukopenia and neutropenia are common and can also be readily managed with dose reductions, or rarely, transfusions. Besides, in some cases, serious psychological disorder may be developed, including depression, mania, and confusion and so on. Injection interferon, obviously, may contribute to permanent alternation of immutability, involving the development of leucoderma and hypothyroidism, rarely combining with sarcoidosis, lupus, rheumatoid arthritis, polymyalgia rheumatica and psoriasis. Some other long-term side effect brought by the use of interferon also include anaemia, vision issue, thyroid issue and bleeding disorder.

5. Certain type of targeted cancer

Presently, using cytokine as an immunotherapy was thought to be an efficient adjuvant approach for treating cancer. Most types of cancer can be controlled by applying this the therapy contains interleukin-2 and interferon, some of which will be listed below.

5.1 Malignant melanoma

Melanoma is a lethal and common cancer caused by skin cells that begin to develop abnormally, which normally attribute to the over-exposure to the UV light. Melanoma is notorious for its high metastasis rate which is the biggest contributor to the high death rate [5].

According to the study done by Kirkwood et la, which particularly investigate the effect of interferon-alpha on metastatic melanoma, it is claimed that low-dose of interferon inject is nearly effortless. When high dose of interferon alpha are given to the patients, the survival rate within five

years showed a pattern of significant increase. Meanwhile, this effect comprises a 33% reduction in the risk of recurrence and a 28% reduction in the risk of mortality [6].

The treatment using interleukin-2 also showed promising effects. According to another experiment done by Kirkwood et al in 1991, il-2 alone could have strong effect on the malignant melanoma, but not as intense and significant as the therapy done by the interferon alpha.

5.2 Renal cell carcinoma

Renal cell carcinoma (RCC) is the most common kidney cancer, accounting for around 3% of all adult malignancies. Annual RCC incidence estimations show a steady rise, with more than a third of newly diagnosed patients having metastatic illness. The RCC is considered as one of the most lethal cancer with the survival rate within five years is less than 5%. The RCC is characterised by the loss of the Von Hippel Lindau (VHL) gene, which causes increased expression of genes such as vascular endothelial growth factor, platelet-derived growth factor, epidermal growth factor, hypoxia-inducible factors 1a and 2a, and constitutive activation of the mammalian target of rapamycin (mTOR) pathway. As a result, the following drugs have been developed to treat RCC: sunitinib, sorafenib, bevacizumab, temsirolimus, everolimus, axitinib, pazopanib, and erlotinib [7]. Numerous clinical trials demonstrated some significant and promising results of using il-2 and interferon as the adjuvant treatment of the drug mentioned above to treat the renal cell carcinoma.

Herein, they will be shown below. Initially, there was two groups of researchers investigate how interferon affect the RCC. In the experiment done by Quesada et al, is one of the pioneers of this field, examine how 19 patients with metastatic renal cell carcinoma react to the given interferon. The results were that five patients (26%) showed partial responses; two patients (10.5%), objective minor responses; three patients (16%), mixed effects (evidence of biological effect with regression of some lesions but concomitant progression); two patients (10.5%), disease stabilisation; and seven patients (37%), progressive disease. Conclusion could be made that interferon can positively influence the cancer treatment [8].

Another pioneer study, which is done by deKernion et al reported that in 48 patients with advanced-stage illness, the objective response rate was 16.5 percent. Hence, even in the early stages of interferon, positive and efficient results could be made, but still limited in size, which may threaten the overall generalisability of the experiment [8].

Over the past few years, more research have been done with greater participants size and the advanced interferon. These experiments have shown a more profound effect of interferon on treating renal cell carcinoma.

Another cytokine involved in the treatment of RCC is the interleukin- 2, which is also supported by several clinical trials. Experiment done by Fliglin et al in 1999, investigated the effect of il-2 on the renal cell carcinoma. The results were that three patients (7.9 percent) in the TIL/rIL-2 group had an ECOG PS of 0 and five patients (11.6 percent) had an ECOG PS of 1, for an overall response rate of 9.9 percent. Five patients (14.3 percent) in the rIL-2 control group had an ECOG PS of 0 and four patients (9.1 percent) had an ECOG PS of 1, for an overall response rate of 11.4 percent [9]. Using a logistic regression model, the difference in total response rate between treatment groups was not statistically significant, and ECOG PS was also not predictive of response. The odds ratios for treatment group were 0.851 and 1.07 for ECOG PS, demonstrating that the chance of response was equal regardless of TIL therapy or ECOG PS. Thus, it could be seen that, though the treatment regarding the il-2 do not have the same efficient results bought by interferon, it can still be considered as a well adjuvant treatment approach.

6. Future expectation of Cytokines in immunotherapy

The first and the most important point of future development is how to manage the toxicity of these drug to minimise the side effect brought by them. Then, as we mentioned above, il-2 is pleiotropy, which may cause anti-tumour and pro-tumour effect simultaneously. Thus, the management of the off-target effect show be focused. This might be able to be eliminated by combining two different types

of drugs together. For instance, the use of il-2 can be combined with immune checkpoint inhibitors while treating the cancer. Meanwhile, through the advancement in the assay technology, what specific immune cell could be affected by which particular cytokine could also be recognised [10]. The researchers could also focus not only on the efficiency of treating the cancer but could also investigate how to improve the drug to minimise the disturbance to the patients' normal life after the treatment.

The usage of the cytokine could not only be used in cancer but can also applied in other scenario which require the action of immune cell or involve the immunosuppression. Cytokine can be used to treat some bacteria-linked or some iatrogenic (things related to illness caused by the medical examination or treatment) suppression. For instance, various experimental models have demonstrated that some bacteria may cause faulty IL-2 production. Several staff have found that T-Cell no responsiveness resulted in high dosages of BCG (M Bovis) mycobacterium injected with mice. Colizzi has reported that this T-cell discrepancy can be reversed by relatively modest rIL-2 dosages. Although a lot more basic work is needed to evaluate whether rIL-2 might play a role in managing similar disease conditions in humans, it is not wrong to predict that systemic rIL-2 can be useful when in vivo IL-2 production is terminated. Meanwhile, il-2 is considered as an effective treatment for surgery-induced immunosuppression. Long known for its major surgery, both in animals and in humans, it is significantly reduced cell immunity. Clinical impacts may thus be significant in cancer patients, as operating procedures may adversely affect the post-operative development of a tumour that is otherwise medically curable [11].

In some of the viral infection, cytokine may be developed as a potential treatment. For instance, in HIV, which may cause the dysfunction of the immune cells, cytokine may be able to improve it. When adequate doses of IL-2 became available for clinical testing, HIV patients were among the first to be treated with it. HIV causes both qualitative and quantitative impairments in lymphocytes, notably CD4 (helper-inducer) cells. In HIV positive patients, IL-2 production and reactivity are reduced in the peripheral blood. Some of these impairments have been demonstrated to be corrected by IL-2 in vitro investigations [11].

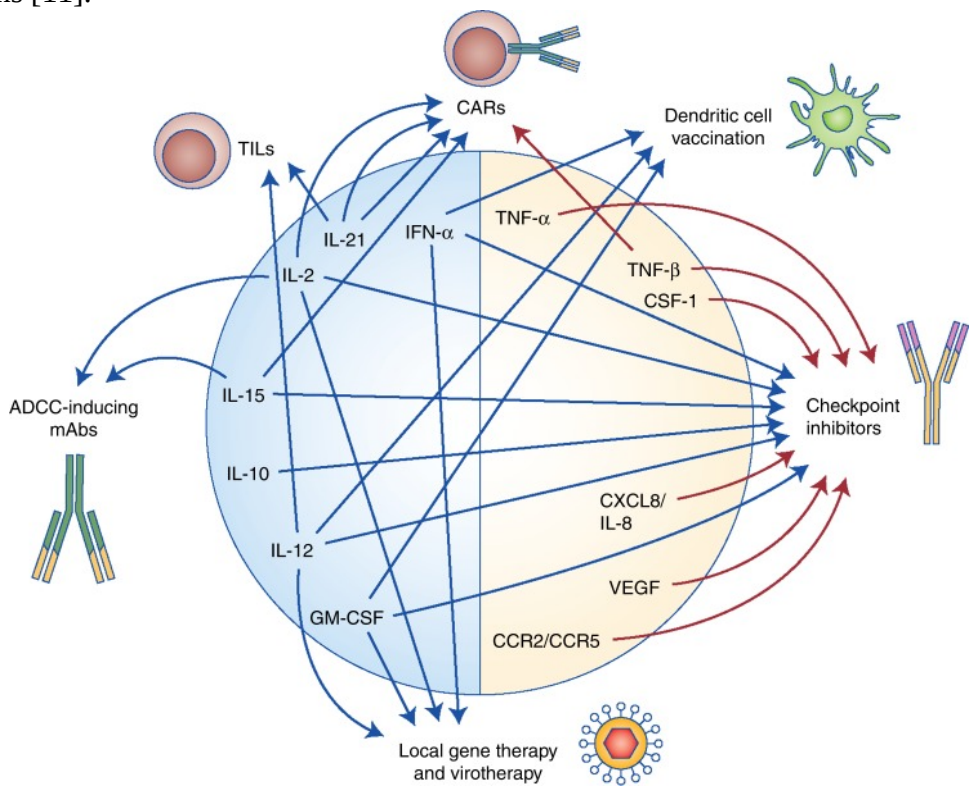

Figure 3. The interaction of different cytokine [12].

In future developments, two key aspects need to be considered: limiting the action of cytokines to the site of action to avoid systemic pro-inflammatory effects and incorporating these therapies into combined immunotherapy strategies. For the first point, it is possible to envisage a targeted drug-based

approach to TME or tumour-administered proteins or the genes that encode them. These tumour-targeting approaches are also associated with neutralization of immunosuppressive cytokines. Cytokines may be powerful partners for elegant synergistic strategies in the field of gene therapy, cell therapy and monoclonal antibody therapy (Figure 3), whose antitumor efficacy will only be revealed in the future.

7. Conclusion

Immunotherapy hold great potential to treat various diseases. It could also provide human researchers an alternative approaches and enricher insight when facing a disease, which is to focus on our own mechanism rather than rely on the dug. In this case, the usage of cytokines in the immunotherapy can be widely used in the field of cancer treatment. The challenges still remain. Like the toxicity and the uncontrollability of the cytokine in the cancer immunotherapy. Furthermore, it is important to be handled in the future. The development of cytokine will pave the way for clinical evaluation and increase the cure rate for cancer and other infective diseases in the years to come.

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