Progress and Development of Cytokines Therapy

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Abstract. Cytokines are known as a group of protein messengers released by infected cells. This process takes place at the cell membrane: the cytokines bind with the specific receptors to present a signal to warn the existence of invaders and activate the defender to destroy the invaders and prevent further separation. After a long history of discovery and evolution, cytokines are now regarded as an effective cancer therapy and an important basis for new medicine development. Although the bottlenecks of the cytokines still exist, scientists are studying new developments to avoid some potential risks and problems, and achieve higher improvement on the existing basis. The possible future development in the field also has great expectancy. By combining various methods, the efficacy of drugs can be significantly improved, and some side effects can be avoided; by combining nanotechnology and light control technology, drugs can achieve specific and targeted treatment. This overview of the progress and development of cytokines includes the introduction of types and mechanisms of typical types of cytokines, application of cytokines in the therapy of specific cancer, the bottleneck of the present stage of cytokines, and possible directions of future improvement.

Keywords: Cytokines, Therapy, Cancer

1. Introduction

To treat cancer, scientists have developed a series of methods, including checkpoint inhibitors (avoidance of reproduction of the invaders), monoclonal antibodies, cancer vaccines, CAR-T cell therapy, and cytokines. In all of these treatments, cytokines are not so fancy or widely used as the vaccines, CAR-T cell therapy, and normally be regarded as that it already came to an end in this field, due to their huge side effects and limitations. However, cytokines have their specialties and are certainly underestimated by most people.

As a group of molecular proteins, which are smaller than other molecules, secreted by immune cells and some other cells, cytokines have the functions of immune regulation, promotion of differentiation and development of immune cells, and hematopoiesis stimulation.

The study that is based on or relative to cytokines had a long history. Cytokine biology started from the research about pus, a product made from the body of white blood cells and dead tissue debris. When the immune system senses that the body is invaded by pathogens, it is stimulated and sends the defender cells, like white blood cells, to the location to fight against the invaders. During the fight, defender cells and some tissue debris die because of the protease released by bacteria. A combination of cell bodies, tissue debris, and tissue fluid forms a visible thick yellowish-white liquid, which is called "pus"[1]. People noticed the symptom of forming pus after being infected before the invention of the microscope. Even though people were not able to invent the detailed components in the pus, scientists at that time were still keenly aware of the important role of the pus and the process of forming pus in some systems in the human body that can self-defend the invaders, like pathogens or virus.

As the significant improvement and exploration of ideas and technology sprang out, scientists got great advances in the mid1940s. One experiment of testing the releasing of cytokines (which were called "soluble factors" before the name was invented) from neutrophils in the peritoneal cavity of livestock, or rabbits to be precise raised the study of the immune system to a brand-new phage [2]. The results showed that polymorphic nuclear leukocytes (PMNs) could influence macrophages in the primitive postoperative period. This statement resulted in a clear relationship between PMNs and macrophages - PMNs modulate macrophage secret cytokines to allow them to enter the culture medium, and the cytokines will only function for a specific period. In other words, cytokines, the factors released by macrophages, will regulate the response of the host immune system to disease or invaders. These early-stage cytokines were proved to have biological functions, including abnormal rising in body temperature, resisting viral infections, increasing the number of white blood cells, and synthesizing acute-phase proteins. Also, it will lead to cancer cells' diminishing and inflammatory cells' metastasis. Since then, the study of the immune system and the role of cytokines has had an acute start.

After 30 years of progress and research, the soluble factors released by cells in the human body started to be regarded as regulators in the lymphatic system. gal Gery and Byron Waksman first described the factor as a "lymphocyte activating factor"[3]. Since then, the deeper and richer contents of cytokines had been more open to scientists. People categorized them depending on different sources, different targeted cells, and different main biological functions. The 6 most well-known subgroups of cytokines are interleukin (IL), colony-stimulating factor (CSF), interferon (IFN), tumor necrosis factor (TFN), and growth factor (GF). They have different functions in clinical treatment. Some can kill the tumor cell and some can cause inflammation. Recently, because of these biological functions, cytokines are developed as a special therapy for many diseases. However, the vigorous reactions and defending methods that cytokines bring out definitely lead to bad effects on the human body. For example, inflammations may result in arthrophlogosis. As a result, the study of the side effects of this field also has great expectancy. By combining various methods, the efficacy of drugs can be significantly improved, and some side effects can be avoided. By transforming the cytokine itself, inhibiting its damage to normal cells, or increasing its effect, it can play a role in a safer dose range. It also could be designed by combining nanotechnology and light control technology, drugs can achieve specific and targeted treatment. Another idea is to improve the non-specific nature of cytokine so that it can specially target tumor cells. Surely, the study of cytokines is not coming to an end, and more and more new things will be discovered and applied in the treatments of diseases.

This review focused on 3 parts. The first part is the use of cytokines as a therapy for certain types of cancers. In this part, types of cancers are the evidence, and they connect different functions and applications of cytokines in different therapy. The second is the bottlenecks of cytokines. This part contains the discussion and reflection on the shortages or disadvantages. The final part is about future development. This includes some thoughts that will overcome some side effects or increase the efficacy of the medicine.

2. Cytokine with specific cancers

With the development of cytokine therapy, many cytokines can be used to treat much cancer, including kidney cancer, melanoma, and so on. In this article, melanoma, renal cell cancer, and leukemia will be the main cancer to introduce.

Melanoma is a highly malignant tumor resulting from melanocytic malignancy. In recent years, the incidence rate and mortality rate have increased. IFN-α acts as the most widely studied human cancer therapy cytokine and is the only adjunctive therapy approved to treat the patients who have melanoma during the second or third stage. Immune escape is achieved in BRAF mutations in melanoma. During this process, the expressing of differentiated antigens are inhibited [4]. However, by increasing MHC class I expression, which is know to all, IFN-α can join in the work of enhancing the recognition of tumor antigen. To treat metastatic melanoma, IL-2 is another important and useful

method. A new technology fusion molecular prototype recombinant human IL-2 is reported to bind partial fusion of multiple antigens, which is equivalent to local delivery of IL-2. It accumulates in the tumor and then gradually slows down the tumor growth process and induces an immune response. This local delivery will only enhance the response of effector T cells and increase tumor cells lysis in the region where the tumor is present, which is more efficient than systemic delivery of the same amount of IL-2 [5]. The recombinant fusion molecule has progressed in a series of clinical trials, at early stages, of adult melanoma. There are other cytokines that exhibit antitumor activity in the model, for example, IL-12. But it has not yet been applied to clinical experiments.

Renal cell cancer is also one of the most common tumors. Surgical treatment is the only possible cure for kidney cancer, and IFN and IL-2 are currently the main adjunctive therapy. IFN-α can be used as part of an anti-angiogenic regimen combined with some agents. To treat advanced renal cell carcinoma, high-dose of IL-2 is a possible choice. The researchers are now improving the regimen about IL-2, which is high-dose, in cancer treatment. In addition, scientists are working to develop modulators that can combine cytokines with other agents to achieve a better therapeutic effect [6]. Over-expression of IL-6 is common in many cancers. Therefore, one of the treatments may be the use of anti-IL-6 agents to inhibit IL-6 or the expression of IL-6. Therefore, anti-IL6 therapy may hold greater promise [4].

Leukemia is a malignant tumor of the hematopoietic system. IFN is particularly effective against hair-cell leukemia and chronic myelogenous leukemia. Recombinant GM-CSF is approved to have 2 main role: shortening the time for neutrophil to recover after induction chemotherapy and reducing the incidence of infection for the patients who have acute myelogenous leukemia. Recombinant viral expression of cytokines provides an alternative strategy to improve the efficacy of antigen-specific vaccines and locally deliver cytokines to the tumor microenvironment. Deleting pathogenic viral genes attenuate the virus, resulting in cemented viral replication in tumor cells, and increased antigen presentation in herpesvirus-infected cells ,which is achieved by a higher expression of the viral promoter. Then, the local GM-CSF enhanced the immune response [7]. Figure 1 shows the mechanism the virus works.

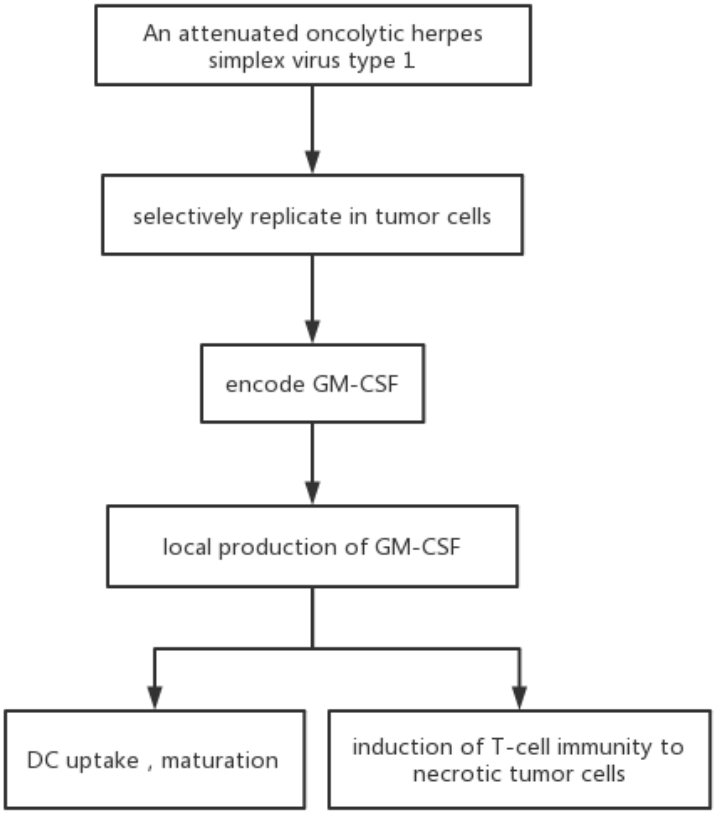


Figure 1. The mechanism of virus works [7].

3. The Bottleneck of Cytokine Therapy

As an effective cytokine, IL-2 can stimulate B cell proliferation; cytotoxic T cells and NK(natural killer) cells can also be activated by IL-2. But there are 3 main bottlenecks of IL-2. First of all, the cytotoxic lymphocytes which are in the tumor tissues can not be activated by IL-2, and IL-2 may be exhausted by a group of peripheral IL-2 sinks. Therefore, IL-2 has only a short half-life in the patient's body and that may influence the clinical treatment efficiency. Second, IL-2 has a higher affinity and tends to combinate with the high-affinity IL-2 α/β/γ complex, than the lower affinity IL-2 β/γ complex. Third, because IL-2 also has a high tendency to combine with the receptor complex, which is trimeric and with high-affinity, on immunosuppressive regulatory cells, only a high dose of IL-2 can activate the immune killing program of the cancer cells, which is mediated by CTL, can also lead to serious cytotoxicity [8].

IFN-β is a cytokine produced by innate immune cells, such as macrophages, dendritic cells, T cells, and NK cells. Some antiviral genes' expression, like 2',5' oligoadenylate synthetase (2',5' OAS), can be mediated by IFN-β. And the 2',5' OAS here can cleave viral RNA and prevent the translation of its viral protein. However, after prolonged administration, IFN-β can be immunogenic which has potential clinical risks. Because with long-term administration there will be binding antibodies inside of the patients' serum. Those antibodies can neutralize IFN-β in the serum, and that will affect IFN-β's biological activity and decrease its efficacy. This aggregation of IFN-β is a big problem for IFN-β clinical use especially in effectiveness and safety because it can elicit an immunological response [9].

TNF-α is a soluble multi-effect cytokine with specific antitumor activity, and can directly kill tumor cells. Inside the body, macrophages, natural killer cells and T cells all can secret TNF-α. Even though it is the first clinically used cytokine, it still can not be used as a constitutional therapy. Because it lacks targeting and has severe side effects. First, with low specificity, local high dose TNF-α can also damage normal cells. Second, as a biomarker of aGVHD, the increase of TNF-α means the happening of aGVHD [10]. Therefore, in many cases, using its antagonists could be a good method to reduce toxic and side effects after an organ transplant or blood transfusion [11].

TGF-β is a kind of cytokine that can inhibit the proliferation of immunocompetent cells, differentiation of lymphocytes, production of cytokines, and regulate the cell phenotype. Different from many other cytokines, TGF-β can be secreted from various cells in the body, but most TGF-β is released inactivated. TGF-β can induce protection, apoptosis, and cytostasis, so it can also inhibit tumorigenesis initially. However, TGF-β can also promote tumor later in the presence of oncogenic events and epigenetic perturbations. In some cases, patients with cancer can not benefit from TGF-β therapy because of the potential carcinogenesis promotion. In other aspects, as an immune checkpoint, TGF-β can be blocked by some monoclonal antibodies to release the inhibition of TGF-β on CD8+ T-cells. But if the TGF-β is blocked, it may cause cardiotoxicity [12].

GM-CSF, a monomeric glycoprotein type cytokine, is very common in the blood and usually be produced by macrophages, T cells, mast cells, and even NK cells and fibroblasts. GM-CSF has a significant place in the survival, proliferation, differentiation, maturation, and functional activation of hematopoietic cells, such as monocytes and macrophages. However, when hypoplasia of severe suppression of the marrow occurs in the body, GM-CSF can only produce a limited effect. Several days after haem transplantation, a limited effect may occur in aplastic anaemia and induction therapy for acute myelogenous leukemia. Just like that, toxicities may become more serious than haemato-poietic toxicity and the main effect of CSFs may be also lost as another result. In some cases, GM-CSF can also cause many side effects, for example, fever, vomiting, diarrhea, dyspnoea, exanthema, drowsiness, loss of appetite, and asthenia.

Chemokines are a family of small, soluble, secreted, and structurally similar cytokines. Chemokines play a very important role in inflammation and immunity. And chemokines can also directly influence the proliferation and metastasis of cancer cells. For instance, CCL3 has anti-tumor activity, but it can also be used to induce tumor cell proliferation by attracting immunosuppressive cells to the tumor bed. Therefore, the accurate use of CCL3 could be a great challenge [13].

4. The Improvement of Cytokines Immune Treatment Technology

The disadvantages of cytokines are very clear. In response to these shortcomings, the current main improvement plan is to combine cytokines with other immunotherapy methods, there are already related application examples in clinical practice. Of course, it is useful that new approaches can improve the targeting of cytokines and alter their pharmacokinetics might be useful. There are five obstacles in the clinical translation of next-generation cytokine cancer immunotherapies, including enhancement of pharmacokinetics and pharmacodynamics, improvement of local administration, understanding of context-dependent interactions in the tumor microenvironment (TME), elucidation of the role of genetic polymorphisms, and optimization of combination therapy [14]. According to the latest literature reports, there are three main types of cytokine improvement directions other than some other directions in other fields [15][16].

The First improvement method is named Supercytokine. Scientists introduce mutations at gene levels, to translate improved cytokines. After this is improved, the cytokines can play a different function from the original and avoid the original shortcomings. PEG or antibody sequence can also be connected to the alkali sequence of cytokines through covalent bonds to change the biological characteristics of the translated cytokines. PEG is the most commonly used molecule that can currently change protein or other material's physical and chemical properties with a lot of types. PEGs of different lengths can transform materials into different traits. Antibodies can increase the targeted news of cytokines to improve their specificity. The alkali sequences of multiple cytokines are connected so that the translated cytokine has a stronger effect.

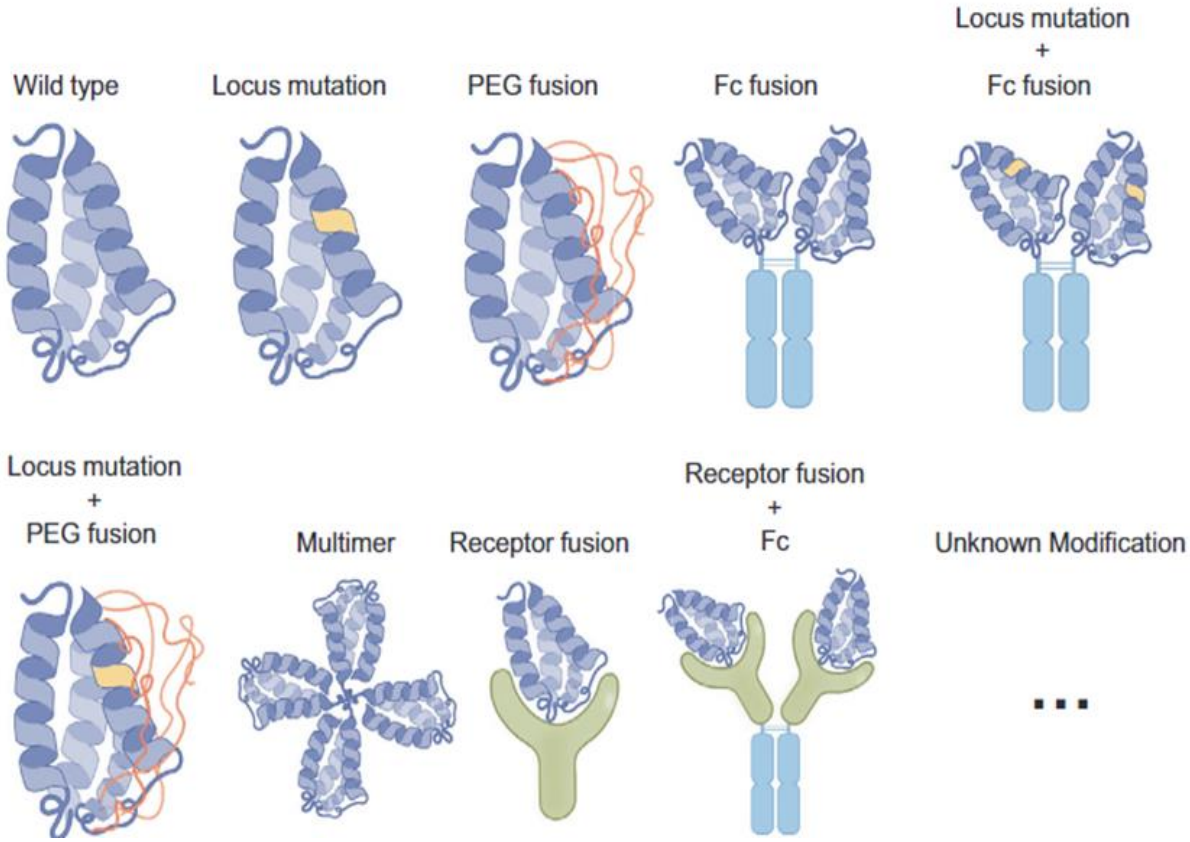


Figure 2. Supercytokine strategy [14].

The second improvement solution is about Antibody-Cytokine Fusion Proteins. Scientists designed connecting different antibodies to the same cytokines to exert the functions of different antibodies and achieve the effect of combined treatment. At present, the combination scheme of immune checkpoint antibodies and cytokine has been a success. Based on this system, there is also a method that connects different types of cytokines to the same antibody, so that the transformed cytokines can play different roles at the same time after being targeted [14].

The plasticity of this cytokine transformation strategy is very strong. Although the function is relatively single, it is better than the first method. To a certain extent, although it can still make up for the lack of function. Generally speaking, the shortcomings of the lack of function still have not been solved well. So this scheme is best used in combination with its cancer treatment plan as a supplementary force for cancer treatment.

The third improvement solution is named Tri-specific Killer Cell Engager Cytokines. It is a plan for improvement based on existing technology, this system is divided into three parts. The engager cytokine protein is connected as a linker to connect the immune cell activation receptor and the tumor antigen, such as NK cells [17]. Immune cell activation receptors can stimulate immune cell proliferation and increase the body's immune response. The system may be used as the main means to improve cytokine functions in the future.

There are also some cytokine improvement methods, such as nanoparticle-localized cytokines, and cytokine-loaded adaptive immune cells. But these directions need higher requirements for researchers and increase their complexities and difficulties, so it is difficult to become the mainstream research direction in the future.

5. Conclusions

Roles of cytokines in immunotherapy become increasingly important. It has already become a relatively mature method in curing disease considering the long history of development from the researches of pus to the discoveries of all the kinds of cytokines, like interferons, interleukins, GM-CSF, and so on. But the drawbacks of this therapy are still significant, including some loss of functions, high-risk of side effects, and lack of efficacy. Due to these defects, cytokines failed to realize their potential value. On the other hand, the bottlenecks of this treatment offer scientists a larger space to bring out improvements and further researches. Many new technologies that aim to make up the defects of cytokines combine cytokines with other therapies or modify the role of cytokines. All in all, cytokine, as a critical modern technology in curing diseases, has its advantages and flaws. Also, the prospect of improvement is substantial and anticipated. Cytokines will be more effective and able to cure more people, as long as more improvements and developments about them are brought out.

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