The Mechanism of Action of IL-2 in the Tumor Microenvironment and its Application in Melanoma

Jiayi Chen *

College of Fisheries and Life Science, Shanghai Ocean University, Shanghai, China

* Corresponding Author Email: jychen@stu.sqxy.edu.cn

Abstract. Cancer, the leading cause of death worldwide, causes a large number of deaths each year and this number is expected to increase with the aging of the population. In this context, it has become particularly important to study and understand in depth the role of cytokines in the tumor microenvironment (TME). Cytokines, particularly interleukin 2 (IL-2), are key regulators of the human immune system and play a pivotal role in the TME.IL-2 plays a crucial role in regulating immune cell proliferation, differentiation, and function, thereby influencing the body's immune response to tumors. However, signaling by IL-2 and its receptor (IL-2R) may be dysregulated in TME, and this dysregulation has profound effects on tumor growth and anti-tumor immune responses. Specifically, dysregulated IL-2 signaling may promote tumor growth and spread while suppressing the body's immune response to the tumor. Translated with DeepL.com (free version) This review summarizes the mechanism of IL-2 and its signal pathway in the TME according to the current popular clinical research object. Based on the side effects of IL-2 during clinical application (such as short half-life and toxicity of high doses of IL-2), this article introduces a new strategy for developing IL-2-cytokine immunotherapy in melanoma treatment. Together, this work aims to provide readers with a timely view of the field of engineered IL-2, highlighting a more promising avenue for future research and development.

Keywords: IL-2; tumor microenvironment; melanoma.

1. Introduction

The tumor microenvironment (TME) plays a key role in cancer development and progression, especially in the early stages of tumors. The TME not only provides growth conditions for early-stage cancer cells but also promotes their invasive ability and helps tumors overcome hypoxic and acidic environments by promoting angiogenesis. This complex interaction makes TME a factor to be reckoned with in cancer treatment.

Cancer immunotherapies have gained attention for their targeting and efficacy compared to conventional cancer treatments. Among them, interleukin-2 (IL-2), an important cytokine, plays a crucial role in inhibiting tumor growth. Preclinical studies have confirmed that IL-2 exhibits potent antitumor activity in animal models, which provides a solid theoretical basis for its application in cancer therapy.

Significantly, the U.S. Food and Drug Administration (FDA) has authorized IL-2 as the first cytokine to treat renal cell carcinoma and melanoma. This signifies the acknowledgment of IL-2's significant role in the treatment of cancer. High dosages of IL-2 therapy have shown promise as a cancer immunotherapy by producing long-lasting responses in patients with melanoma and renal cell carcinoma, highlighting IL-2's potential in the fight against cancer [1].

Even yet, IL-2's anticancer effects are strong, and in certain circumstances, its therapeutic effectiveness is still restricted. This is primarily because IL-2 is essential for the survival and growth of regulatory T (Treg) cells [2]. Treg cells have the function of suppressing immune responses, which may somewhat diminish the anti-tumor effect of IL-2. Therefore, researchers are exploring the possibility of pharmacologically re-engineering IL-2 to improve its therapeutic efficacy and minimize side effects.

Engineering cytokines for the treatment of melanoma has become a research hotspot. Modification of IL-2 by genetic engineering means could allow it to better target tumor cells while reducing damage

to normal cells. The development of such engineered cytokines provides new ideas and methods for the treatment of melanoma and other cancers.

The following review provides an overview of the mechanism and obstacles faced by IL-2/IL-2R. This review also categorizes new immunotherapies based on the adverse and limitations of IL-2. The promising results from these studies have led to the exploration of cytokine-based treatments in the clinical setting.

2. The signaling pathway of IL-2

2.1. IL-2

By binding to IL-2R subunits, IL-2 carries out stimulating and regulating actions. This cytokine, which comes from Th and NK cells among other immune cells, is an important one that is necessary for the control of the adaptive immune system. IL-2 is a powerful lymphocyte growth factor that regulates several elements of the immune response through signaling pathways. Because it contributes to T cell homeostasis and enhances immunological responses, this cytokine is essential to the immune system's optimal operation.

The heterotrimeric complex known as IL-2R is composed of three distinct chains: IL-2Rγ (CD25), IL-2Rβ (CD122), and IL-2Rγc (CD132). T and B cells in particular have large distributions of the transmembrane glycoprotein receptor IL-2R on their surface. These chains can result in three different types of receptors: low-affinity (IL-2Rα), medium-affinity (IL-2Rβ and IL-2Rηc), and high-affinity (IL-2Rα, IL-2Rβ, and IL-2Rηc). The majority of immune responses directed against tumor cells are triggered by the IL-2 and IL-2R signaling pathways.

2.2. Signaling pathway

Figure 1 provides a clear depiction of the TME's IL-2 signaling pathway. T and B lymphocyte development and functional maturation are dependent on the signal transducer and activator of transcription (STAT) and Janus kinases (JAK) signaling pathways. As an example, Treg regulates JAK1 and JAK3 differently from effector T (Teff) or conventional T (Tconv). One of the key components that is more active in Treg cells than in Teff or Tconv cells is STAT5. Since Treg cells don't create much IL-2, they use their high affinity IL-2 receptor to block the environment's supply of IL-2, which prevents responder T cells from using it [2]. When this route is activated, it coordinates the nuclear trafficking of certain transcription factors, which controls the expression of many target genes, including IL-2. The result is a positive feedback loop that may maintain itself.5. Furthermore, IL-2R activation initiates two other critical signaling cascades: Mitogen-Activated Protein Kinase (MAPK) and Phosphatidylinositol 3-Kinase (PI3K). Teff or Tconv cells have increased activity in both of these pathways. The PI3K pathway is essential for maintaining cell survival and encouraging lymphocyte proliferation and differentiation, even though the MAPK pathway is critical for controlling activities related to cell proliferation and differentiation.

Moreover, transcription factors and regulators such as ITK, IκB, NFAT, AP-1, C-JUN, and c-Fos are involved in the IL-2/IL-2R signaling pathway. Numerous factors impact the signaling dynamics in Treg, Teff, and Tconv cells. One of the many ways that signals interact in the tumor microenvironment is through the regulation of cytokine signaling by SOCS1. The goal is to provide a deeper knowledge of tumor immunology through the thorough study of the complex IL-2/IL-2R signaling pathway and the clarification of the activities of the related proteins. This may open the door for the creation of novel treatment strategies in the field of cancer immunotherapy.

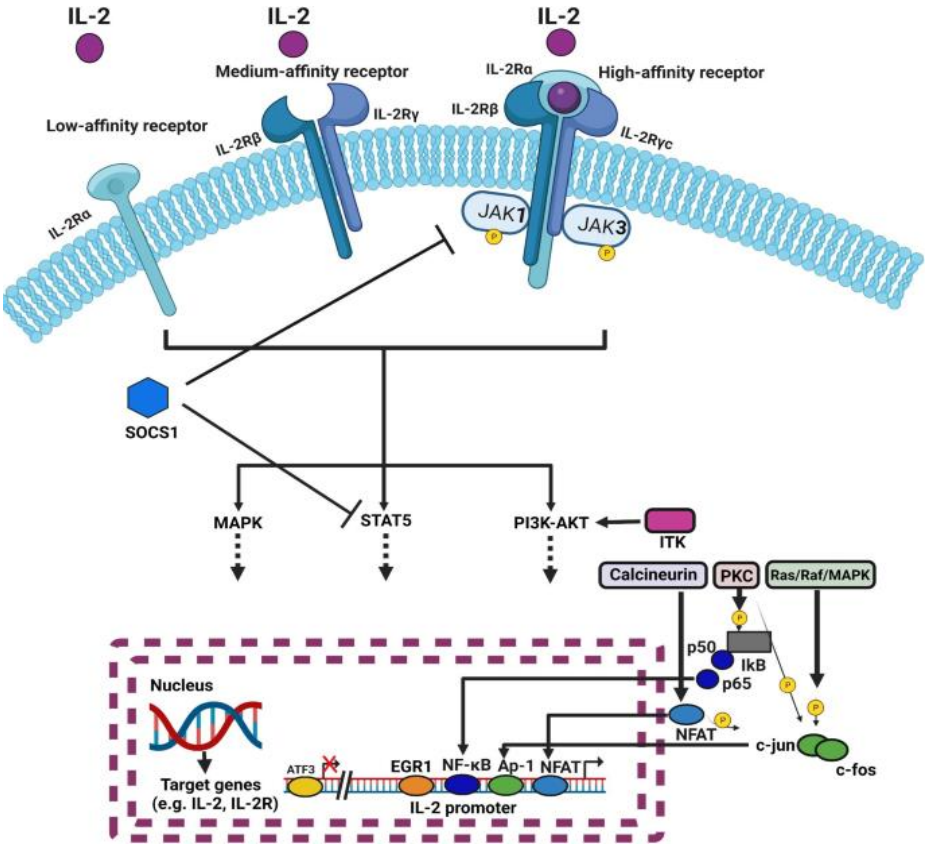


Fig 1. IL-2 and IL-2R pathway within the tumor microenvironment [3].

3. The mechanism of IL-2

IL-2 can stimulate the infiltration of cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells to bolster anti-tumor immune responses. When the body is in steady-state rest, some antigens generate a significant amount of IL-2, which directly activates CD4+ and CD8+ T cells to respond. Afterward, this cytokine is used by CD25+Teff and Treg cells. Specifically, it helps CD4+ T cells proliferate and differentiate into T helper cells 1 and T helper cells 2, and it supports the development of CD8+ T cells into CTLs. Moreover, it has been demonstrated that IL-2 benefits the B cell lineage by encouraging plasma cell differentiation and aiding in the growth of Treg cells that express FOXP3, which may inhibit the anti-tumor immunity response [2]. This presents a complex and contradictory picture. In the context of cancer immunotherapy, this leads to a complicated and nuanced scenario that poses a contradictory difficulty. More IL-2 (HD IL-2) is needed to start signaling through the mediate-affinity IL-2R expressed on memory CD8+ T cells and NK cells (Jared E Lopes et al., 2020). One of the many impacts of dysregulated IL-2/IL-2R signaling is the stimulation of Treg cells, which can inhibit anti-tumor responses.5. Low levels of IL-2, or LD IL-2, on the other hand, trigger signaling through the highly specialized IL-2R expressed on Treg cells.7. It has also been demonstrated that IL-2 can promote the growth and survival of certain tumor cells. Studies have demonstrated the involvement of IL-2R in angiogenesis, or the development of new blood vessels, in malignancies. [3]. the mechanism of action of HD IL-2 is dependent on immune-mediated pathways rather than direct anticancer effects. This is the rationale behind the interest that many researchers have in the connection between IL-2 and the tumor microenvironment.

One important factor in T-cell regulation, IL-2 and IL-2R signaling, may be therapeutically useful in rebalancing the TME. TME is an intricate, dynamic ecosystem made up of immune cells, tumor cells, and blood vessels. By releasing signals, encouraging angiogenesis, and triggering immunological tolerance, tumors can have an impact on the microenvironment [4]. T cell function is a crucial factor in determining the formation and progression of tumors inside the TME. Tumors can

release growth factors, chemokines, and cytokines that lead to the accumulation and development of Treg cells in the TME. The cytokine IL-2, which is produced by activated T-cells, is essential for effector T-cell survival, proliferation, and functionality [5]. In particular, IL-2 signaling can promote Teff cell growth and activity while inhibiting Treg cell growth and activity. Furthermore, this signal can induce Treg cells to become Teff cells, which changes the TME from a pro-tumor to an anti-tumor state.

In conclusion, it has been shown that HD IL-2 stimulates immune cells by raising the quantity of cytotoxic lymphocytes. On the other hand, LD IL-2 could encourage the development of a regulatory milieu, which might aid in the growth of tumors. The complex relationship between the IL-2 and IL-2R signaling pathways extends beyond standard immune regulation since strong evidence suggests that they play a role in the TME.

4. The application of IL-2 in melanoma

A patient with metastatic melanoma was completely cured of all malignant illnesses and disease-free for over 29 years after a clinical study using systemic HD IL-2, just 8 years after IL-2 was discovered [5]. The worst skin cancer is still melanoma, which is predicted to cause 7650 fatalities and 99,780 new cases in the United States by 2022. 10 The immune system is intrinsically connected to the treatment of cutaneous melanoma, the deadliest kind of skin cancer [6]. Following some patients' long-lasting, full responses in cases of metastatic melanoma and metastatic renal cell cancer, HD IL-2 was licensed in the 1990s. Roughly 10% of patients with melanoma and kidney carcinoma have demonstrated persistent responses to efficacious immunotherapy [7]

Due to its extreme toxicity, immunotherapy has shown poor effectiveness, and IL-2 monotherapy has little therapeutic value. HD IL-2 therapy has been shown by researchers to have a high incidence rate of severe cardiotoxicity, neurotoxicity, and treatment-related mortality [4]. Three primary constraints have been identified in the therapeutic use of IL-2. For instance, a major obstacle to the substance's effectiveness is its very short (15-minute) in vivo half-life. Furthermore, this chemical may cause significant toxicity at therapeutic levels, which might have some very negative consequences. Lastly, IL-2 may promote the selective proliferation of Treg cells that result in antitumor immunity, opening up a possible treatment pathway for this drug [8].

Numerous studies have suggested that a variety of redesigned IL-2 designs provide a promising alternative for targeted trafficking into TME. The method of producing antibody clones (such as the mimobody, NARA1) to mimic IL-2Rα binding and hence block IL-2Rα binding and Treg activation in vivo is illustrated in Figure 2. It has recently been found that administering IL-2 coupled to certain anti-IL-2 monoclonal antibodies greatly boosts the biological activity of IL-2 in vivo, either by promoting the growth of MP CD8+ T cells and NK cells or by encouraging the formation of CD25+ CD4+ Tregs. These CD25-directed complexes, called IL-2/mAbCD25 complexes, preferentially increased CD25+ CD4+ Treg cells [9]. Boyman and Garcia demonstrate that IL-2 superkine treatment reduced tumor burden more favorably than WT IL-2 in a syngeneic B16F10 melanoma model, and IL-2/anti-IL-2 mAb complexes exerted very effective tumor control. Because of Super-2's structural difference in IL-2, cytotoxic CD8+ T cells, and NK cells can be activated for anti-tumor immune responses less toxically. It is possible to reevaluate Super-2 for IL-2 therapeutic purposes [10]. These CD25-directed complexes, called IL-2/mAbCD25 complexes, preferentially increased CD25+ CD4+ Treg cells [9]. Boyman and Garcia demonstrate that IL-2 superkine treatment reduced tumor burden more favorably than WT IL-2 in a syngeneic B16F10 melanoma model, and IL-2/anti-IL-2 mAb complexes exerted very effective tumor control. Because of Super-2's structural difference in IL-2, cytotoxic CD8+ T cells and NK cells can be activated for anti-tumor immune responses less toxically. It is possible to reevaluate Super-2 for IL-2 therapeutic purposes [11].

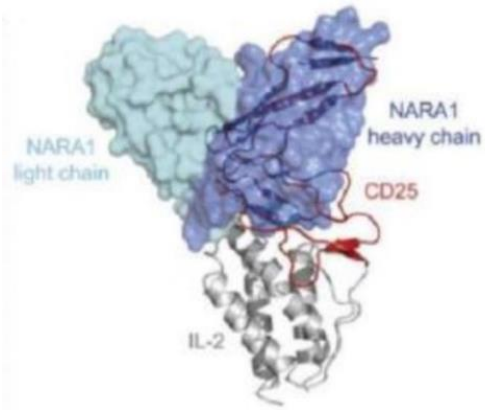


Fig 2. NARA-1 binding with IL-2 concerning immunosuppressive CD25 binding [5].

NKTR-21, also known as bempegaldesleukin, showed promise as a stand-alone treatment when combined with anti-CTLA-4 antibody [12]. It also provided long-lasting immunity that was resistant to tumor rechallenge. By conjugating polyethylene glycol (PEG) to IL-2 at the interface where IL-2/IL-2Rα attaches, the cytokine-polymer conjugates in question allow immune-cell selectivity (Figure 3).1. This material is a prodrug of conjugated IL-2, which targets immune cells preferentially by altering ligand/receptor interactions. The IL-2 core is linked to six releasable PEG chains. When administered in vivo, the PEG chains progressively release to create active IL2 conjugates [12]. Immunotherapy may reduce peripheral toxicity, extend the time that a medication is in circulation, and increase the peripheral blood and TME's affinity for CD8 + T cells. Deborah and colleagues found that in a mouse model of melanoma tumor, NKTR-214 was well tolerated in non-human primates. As a result, the NKTR-214 therapy has improved, and there has been a notable rise in the overall number of NK cells, CD4+ T cells, and CD8+ T cells. Thus, bempegaldesleukin has been shown to effectively stimulate immune clearance and memory against recurrence in melanoma, as well as to increase the robustness and antitumor immunity in preclinical cancer models.

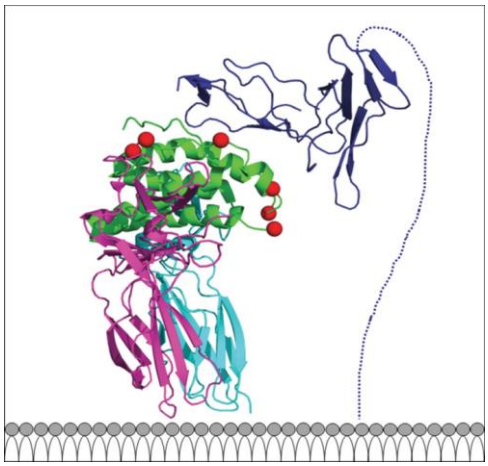


Fig 3. Mechanism of action of NKTR-21 [12].

The science of cancer immunotherapy is always changing, and new studies have shown encouraging findings on the use of IL-2 as a possible cancer treatment. These intriguing structures comprise cytokines that are mutant or designer, cytokine-polymer conjugates, and antibody-cytokine complexes and fusions. To improve IL-2's anti-tumor efficacy, antibody-cytokine complexes and fusions have been created that target certain cancer cells. As a result, IL-2 is a fantastic prospect for the creation of cutting-edge cancer immunotherapies.

5. Conclusion

The survival and proliferation of Treg in TME are significantly influenced by the IL-2 and IL-2R signaling pathways. Because Treg cells help the immune system reduce the immunological response,

controlling IL-2/IL-2R signaling may be able to manage Treg cell survival and suppression, which would maximize the benefits of tumor immunotherapy. In meanwhile, one of the key defense mechanisms of the immune system against cancers is the ability of CD4+ and CD8+ T cells to identify tumor antigens or self-antigens found in healthy persons. In this mechanism, the balance between effector T cells and Treg cells inside the TME is determined in large part by IL-2R-mediated IL-2 signaling.

Significant advancements in tailored cytokine immunotherapy for malignancies like melanoma have been made possible by the field's fast growth. Among them, a few cutting-edge engineered IL-2 treatments, such as NKTR-214 and IL-2-NARA1, have demonstrated the capacity to encourage CD8+ T-cell proliferation, which contributes to the inhibition of tumor development, enhancement of patient survival, and other positive therapeutic benefits. Inducing efficient immune elimination and immunological memory in melanoma is made possible by these medicines, which offer a powerful tool against cancer reactivation. These developments not only provide melanoma patients fresh hope for treatment, but they also open the door for the creation of a new class of cytokine-engineered medicines in the future.

Despite the remarkable progress of these novel therapies, there are still many challenges and uncharted territories to be explored. For example, how to further optimize the safety and efficacy of these therapies, how to apply them to other types of cancer treatments, and how to combine them with other treatments to achieve better therapeutic effects are all important directions for future research. In conclusion, through in-depth research and continuous exploration, it is believed that researchers can make more breakthroughs and progress on the road of cancer immunotherapy, bringing hope and the possibility of recovery to more cancer patients.

References

[1] Skrombolas D., et al. 2 Challenges and developing solutions for increasing the benefits of IL-2 treatment in tumor therapy. Expert Review of Clinical Immunology, 2014, 10(2): 207–217.

[2] Tanaka A., & Sakaguchi S. 3 Regulatory T cells in cancer immunotherapy. Cell Research, 2017, 27(1): 109–118.

[3] Jared E Lopes, et al. 7 ALKS 4230: A novel engineered IL-2 fusion protein with an improved cellular selectivity profile for cancer immunotherapy. Journal for ImmunoTherapy of Cancer, 2020, 8(1): e000673.

[4] Li Z., et al. 8 Anti-VEGFR2-interferon-α2 regulates the tumor microenvironment and exhibits potent antitumor efficacy against colorectal cancer. OncoImmunology, 2017: 6(3), e1290038.

[5] Uricoli B., et al. 9 Engineered Cytokines for Cancer and Autoimmune Disease Immunotherapy. Advanced Healthcare Materials, 2021, 10(15): 2002214.

[6] Kumar P., et al. 10 the cure from within? A review of the microbiome and diet in melanoma. Cancer and Metastasis Reviews, 2020, 41(2): 261–280.

[7] Boyman O., & Sprent, J. 6 The role of interleukin-2 during homeostasis and activation of the immune system. Nature Reviews Immunology, 2012, 12(3): 180–190.

[8] Silver A.B., et al. 13 an engineered immunocytokine with collagen affinity improves the tumor bioavailability, tolerability, and therapeutic efficacy of IL-2. Cell Reports Medicine, 2023, 4(11): 101289.

[9] Létourneau S., et al. 4 IL-2/anti-IL-2 antibody complexes show strong biological activity by avoiding interaction with IL-2 receptor alpha subunit CD25. Proceedings of the National Academy of Sciences of the United States of America, 2010, 107(5), 2171–2176.

[10] Levin A.M., et al. 15 exploiting a natural conformational switch to engineer an interleukin-2 “superkine”. Nature, 2012, 484(7395): 529–533.

[11] Arenas-Ramirez N., et al. 16 Improved cancer immunotherapy by a CD25-embody conferring selectivity to human interleukin-2. Science Translational Medicine, 2016, 8(367): 367ra166-367ra166.

[12] Charych D. H., et al. 11 NKTR-214, an Engineered Cytokine with Biased IL2 Receptor Binding, Increased Tumor Exposure, and Marked Efficacy in Mouse Tumor Models. Clinical Cancer Research, 2016, 22(3): 680–690.