

Cytokine IL-12 Based Cancer Immunotherapy

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Abstract. Immunotherapy is an important method in cancer therapy. Cytokines are signaling molecules produced by the immune system that play a crucial role in regulating immune responses. They can be harnessed in immunotherapy to modulate the immune system's activity to treat various diseases, including cancer and autoimmune disorders. It has been confirmed that Interleukin-12 (IL-12) family cytokines play an important role in tumor immune regulation. Different cytokines in the IL-12 family have different effects and regulatory roles. The IL-12 family of cytokines has shown promise in cancer immunotherapy due to its ability to activate immune responses against tumor cells. This family includes several key cytokines, such as IL-12, Interleukin-23 (IL-23), Interleukin-27 (IL-27), and Interleukin-35 (IL-35), etc., each with distinct roles in modulating the immune system and potential applications in cancer treatment. In this review, I presented the biological functions of several types of cytokines from the IL-12 family and summarized their potential in cancer treatment.

Keywords: Cytokines; IL-12 family cytokines; Cancer immunotherapy.

1. Introduction

The development and spread of cancer are characterized by eight distinct mechanisms, namely persistent proliferation, evasion of growth suppressors, resistance to cell death, replicative immortality, angiogenesis, metastasis, reprogramming metabolism, and evasion of immune destruction [1]. In this process, current immunotherapy methods mainly intervene by applying growth inhibitory factors, destroying the tumor microenvironment, and blocking cancer cell metastasis. Immunotherapy refers to the utilization of substances or techniques aimed at enhancing or restoring the immune system's capacity to prevent and combat diseases. The primary objective of this approach is to achieve immune system equilibrium, leading to the eradication of cancer cells, while mitigating the risk of an uncontrolled autoimmune inflammatory reaction and circumventing the treatment constraints associated with immunotherapy. Based on this comprehension, a range of immunotherapeutic methodologies have been devised, encompassing vaccination, monoclonal antibodies and checkpoint inhibitors therapy, among others. Each treatment seeks to increase immune function and is classified through various mechanisms of action [1]. The notion of tumor growth being susceptible to recognition and regulation by the immune system traces its origins to 1893, when William Coley employed live bacteria as immune stimulants for cancer treatment. However, the level of enthusiasm surrounding cancer immunotherapy has been somewhat restrained, mostly owing to its restricted clinical effectiveness. The poor effectiveness of current treatments can be attributed to the tumor cells' capacity to evade detection and eradication by the immune system. In recent decades, significant advancements have been achieved in comprehending the mechanisms by which cancer cells elude the immune system. Consequently, novel strategies have emerged to impede cancer immune evasion and effectively eradicate malignant cells [2]. The passage is going to talk about Cytokines in the treatment of Cancer, which is also a kind of immunotherapy. Cytokines serve as molecular mediators of both innate and adaptive immunity, facilitating intercellular communication within the immune system through paracrine and autocrine signaling mechanisms [3]. There exist numerous elements that impose limitations on the effectiveness of these immunotherapies. The introduction of cytokines does not exert a targeted influence on the immune response towards a particular tumor. Instead, it necessitates the host to launch an immunological response, even in cases when the host's immune system may be insufficient in combating the tumor. The cytokines, in turn, have the capacity to augment this immune response. Many new strategies are currently being developed to enhance cytokine activity, taking into account the understanding of the regulatory

mechanisms controlling immune responses, which have been validated in animal models. The tumor microenvironment (TME) consists of several cellular components, including endothelial cells, fibroblasts, and immune cells, as well as extracellular elements such as cytokines, growth hormones, hormones, and the extracellular matrix that surrounds tumor cells. Additionally, the TME encompasses blood arteries. Network nourishment TME not only plays a pivotal role in the process of tumor occurrence, progression and metastasis, but it must have an impact on the therapeutic effect [4]. Various types of cells have an impact on the tumor microenvironment through the secretion of cytokines, hence exerting significant influence on processes such as cell proliferation, angiogenesis, immunosuppression, invasion, and metastasis [5]. Hence, the regulation of cytokines serves as a crucial mechanism for controlling the tumor microenvironment. Additionally, it is noteworthy that cytokines belonging to the IL-12 family play a fundamental role in influencing the immunological conditions within tumors.

The IL-12 family is distinguished by its exclusive composition of heterodimeric cytokines, which encompass IL-12, IL-23, IL-27, IL-35, and IL-39. The presence of this characteristic endows these cytokines with a distinct array of associations and functional interplays that are absent in other families of cytokines [6]. Despite exhibiting numerous shared structural traits and molecular interactions, cytokines belonging to the IL-12 family have a remarkably wide range of functional effects. The IL-12 family stands out among the extensive array of bioactive cytokines. The exclusive family of heterodimeric cytokines possesses a multitude of unique and distinguishing properties. This particular family exhibits numerous molecular and functional characteristics that present distinct possibilities for both positive and negative feedback regulation. Furthermore, there are still additional traits that remain to be discerned. The phenomenon of chain pair promiscuity is a prevalent characteristic observed among this particular group of heterodimeric cytokines. Also, members of the IL-12 family have shown remarkable results in various combination therapies and have great potential and prospects, which will be discussed below.

2. IL-12 family cytokines

2.1. IL-12

IL-12 is a cytokine composed of two subunits, specifically p40 and p35. It is categorized as a pro-inflammatory cytokine. The synthesis of IL-12 is commonly ascribed to antigen-presenting cells, including dendritic cells and macrophages. The IL-12 cytokines are recognized as proinflammatory or prostimulatory agents that exert a substantial influence on several types of lymphoid cells, such as natural killer (NK) cells and cytotoxic CD8⁺ T cells, including both the innate and adaptive immune responses. Their primary function is to induce the production of IFN- γ , a crucial cytokine with a pivotal role in defending against tumor development, growth, and metastases. In addition, IL-12 has the ability to produce non-IFN- γ -mediated tumor-suppressive effects through the activation of innate natural killer (NK) cells that express the p46-related protein Nkp46, as well as lymphoid tissue inducer cells. Furthermore, the role of IL-12 is crucial in the recruitment of CD8⁺ T and NK cells, which are integral constituents of efficacious immune responses directed towards malignancies. Therefore, IL-12 is of utmost importance in facilitating strong immune responses against tumors and is deemed essential in this regard [7].

Moreover, IL-12 goes beyond its role in activating the anti-tumor immune response and can also directly contribute to immune suppression. One of its notable effects is on regulatory T cells (Tregs). The flexibility of terminally developed Tregs is influenced by IL-12, which induces a transformation of Foxp3⁺ Treg cells into interferon- γ (IFN- γ) producing Foxp3⁺ T cells. Furthermore, the administration of IL-12 results in a decrease in the concentrations of IL-2, a crucial cytokine required for the viability and proliferation of Tregs. Interleukin-12 (IL-12) has the ability to induce the suppression of Treg proliferation in mice through the mediation of IFN- γ . The induction of cell cycle arrest in Tregs and the inhibition of tumor-induced Treg cell proliferation are both consequences of the signaling pathway triggered by IL-12-induced IFN- γ [8].

This dual role of IL-12, both in activating anti-tumor immune responses and in suppressing immune inhibition, underscores its complexity and significance in regulating the immune system's response to cancer. According to a study conducted by Agliardi et al., the targeted application of IL-12 has the potential to be a beneficial addition to CAR-T cell therapy for the treatment of glioblastoma multiforme (GBM). In the context of this module, IL-12 serves as an adjuvant. In the present investigation, the investigators utilized a solitary intratumoral administration of recombinant single-chain IL-12 fused to the Fc segment of murine IgG3, denoted as IL-12-Fc, in conjunction with systemic chimeric antigen receptor T-cell (CAR-T) therapy. In the context of a murine model of glioblastoma (GBM), the implementation of this integrated therapeutic strategy led to the total elimination of pre-existing gliomas. The investigation additionally assessed the influence of IL-12 on the viability of EGFRvIII-specific CAR-T cells and the modification of the TME, which had a crucial function in attaining anti-tumor immune response. An essential element of the investigation was to ascertain that the impact of IL-12-Fc was confined to the intracranial tumor site subsequent to its delivery. The significance of this cannot be overstated, as the systemic administration of recombinant IL-12 has been found to elicit substantial deleterious effects in human subjects. The examination of mouse samples obtained on day 4 and day 11 following the injection of IL-12-Fc demonstrated that there were no statistically significant increases in the levels of IL-12 in the overall bloodstream of mice who received IL-12-Fc, in comparison to the control group, during these specific time intervals. However, on the fourth day after the administration of IL-12-Fc [9], there was a little increase in the concentrations of the IL-12-induced cytokines IFN- γ and CXCL9, while there was no statistically significant change in the levels of CXCL10 [10]. The observed effects had a temporary nature, with cytokine levels reverting back to their initial levels by the eleventh day. In addition, the examination of serum concentrations of the pro-inflammatory cytokines IL-6 and GM-CSF revealed no statistically significant disparities across the cohorts receiving different treatments. In general, the results of this study suggest that the administration of IL-12-Fc at the local level is linked to little systemic adverse effects, while simultaneously offering a significant improvement in the management of tumors. Since cytokines used as monotherapy have not consistently demonstrated the efficacy seen in preclinical studies, combining them with other therapies, as demonstrated in this study, may be a more promising approach for cancer treatment. The study conducted by Yanyan Zheng and colleagues involved the development of an adenovirus vaccine that contained the tumor antigen glypican-3 (Glypican-3) and the helper interleukin-12 (IL-12) [10]. This vaccine was administered to subcutaneous tumor models in a series of three vaccinations at 10-day intervals until immunity was established. The results demonstrated that in the group receiving the combined immune therapy (Glypican-3 and IL-12), there was a significant enhancement in the proliferation and multifunctional activity of CD8⁺ T cells compared to the control group. This increase in CD8⁺ T cell activity effectively suppressed tumor growth. The therapeutic effects of the Glypican-3 and interleukin-12 vaccines were primarily attributed to dendritic cell-mediated CD8⁺ T cell-mediated antitumor activity. Furthermore, the study showed promising therapeutic outcomes in a lung metastasis model of hepatocellular carcinoma, suggesting that combined immunotherapy, especially using interleukin-12, has the potential to serve as a therapy for this type of liver cancer. In summary, this research study provides valuable insights and evidence supporting the use of combined immunotherapy treatments like the G3I-12 vaccine for cancer treatment.

2.2. IL-23

IL-23 is a cytokine that consists of two subunits, specifically p40 and p19.[7] It has been found to have a complex impact on cancer, demonstrating both pro-tumorigenic and anti-tumorigenic actions. And it is well acknowledged as a crucial modulator of the Th17 immune response and exerts a substantial impact on modulating the immune response within the framework of cancer. Prior research has established that IL-23 exerts a substantial influence on augmenting the inflammatory response within the TME, hence facilitating tumor formation through a mechanism that is independent of IL-17. An upregulated expression of the IL-23 receptor (IL-23R) on neoplastic cells

has the potential to induce heightened tumor-associated inflammation, hence facilitating the progression and dissemination of cancer. IL-23 exhibits the capacity to initiate and sustain the synthesis of transcription factors that are particular to T-helper 17 (Th17) cells. These transcription factors subsequently have a negative regulatory influence on the immune-suppressive response of Tregs. The IL-23 signaling pathway impedes the maturation of Tregs and maintains the continuous transcription of many cytokines, including interleukin-17 (IL-17), interleukin-21 (IL-21), interleukin-22 (IL-22), and interleukin-23 receptor (IL-23R). Furthermore, it is possible that IL-23 exerts a regulatory influence on the functionality of different subsets of immune cells present in the TME. Significantly, IL-23 elicits distinct downstream signaling pathways in both tumor cells and immune cells, hence exerting either pro-inflammatory or anti-inflammatory effects contingent upon the specific circumstances. Presently, researchers are focused on the development of tailored therapeutics that utilize the potential of IL-23 in order to enhance the efficacy of cancer treatments. Ongoing clinical trials are being conducted to assess the safety and effectiveness of these emerging medicines in individuals diagnosed with cancer. Yung chan et al.'s study about the efficacy of the IL-23 inhibitor Guselkumab in Chinese patients with psoriasis at 20 weeks is a good example to shows the development of it [11]. This initial study examines the experiences of Chinese individuals suffering with psoriasis. The findings demonstrate that guselkumab, a treatment option, effectively and safely produced positive clinical outcomes within a 20-week timeframe. This gives us an idea for inhibitor therapy.

2.3. IL-27

The role of IL-27 in the immune response is multifaceted, since it has been shown to have both stimulatory and suppressive effects.[8] Nevertheless, there is growing evidence supporting its potential as a preventive agent. The synthesis of IL-27 is stimulated when Toll-like receptor (TLR) agonists (namely TLR3, TLR4, or TLR7/8) activate antigen-presenting cells (APCs). The activation of antigen-presenting cells (APCs) in conjunction with the activation of Toll-like receptors (TLRs) results in a synergistic effect that leads to the synthesis of interferon-beta (IFN β) and subsequently induces immunological suppression. IL-27 can also be generated in the resolution phase of an autoimmune response, primarily by antigen-presenting cells (APCs) located in inflamed regions. Several different triggers, such as Tregs, IFN-gamma (IFN γ), and statins, have the ability to induce the production of IL-27, so effectively reducing the initiation of inflammation. While IL-27 does not possess inherent stimulatory qualities, it has the ability to enhance the production of IFN γ from T and natural killer (NK) cells when administered in conjunction with other stimuli. IL-27 elicits the production of IFN γ from various cell types and facilitates the upregulation of STAT1 expression, ultimately resulting in the induction of T-bet expression through signaling pathways, particularly in the presence of IL-12 or IL-2. The cytokine IL-27 has been observed to exert a regulatory influence on cellular proliferation, and the specific outcomes of this modulation are contingent upon the concurrent presence of IL-2. The cytokine IL-27 has the ability to decrease the production of IL-2, hence restricting the proliferation of T cells and natural Tregs (nTregs). Nevertheless, the effects mentioned above are mitigated by IL-2 through the inhibition of WSX1 expression. In addition, it has been observed that IL-27 has the ability to impede the maturation of Th17 cells, while simultaneously promoting the generation of a regulatory population known as IL-10-producing Tr1-like cells. This effect is predominantly mediated by the upregulation of c-Maf. Moreover, the expression of c-Maf caused by IL-27 can promote the formation of T follicular helper (Tfh) cells by increasing the levels of IL-21. The ability of IL-27 to modulate B cell growth and activity enables it to exert an impact on the immune response's efficacy while simultaneously regulating inflammation. Multiple studies have indicated that the intratumoral administration of IL-27, whether paired with immune checkpoint inhibition or adoptive T cell treatment, holds significant potential as a viable approach in the fight against cancer. For example, when Jin-qing liu et al [12]. used lipid nanoparticles for intratumoral delivery of IL-27 mRNA in cancer immunotherapy, IL-27 was activated through the Stat1 and Stat3

pathways. The results showed that IL-27 promoted the Th1/Tc1 response and increased the survival rate of T cells in the tumor microenvironment.

2.4. IL-35

IL-35 is mostly synthesized by regulatory T cell subsets, including nTregs, in both murine and human systems, and it assumes a pivotal function in augmenting their suppressive potential [7]. The inhibitory effects of Tregs through soluble substances can be prevented by genetic deletion or antibody-mediated inhibition of IL-35. The inhibitory effect of IL-35 on T cell proliferation is mediated by the induction of cell cycle arrest specifically at the G1 phase, without concomitant induction of apoptosis or cell death. Furthermore, IL-35 has been observed to elicit the activation of a distinct subset of Tregs referred to as "induced Tregs 35" (iT_h35 cells), in addition to its established involvement in nTregs. The aforementioned cells, characterized by the expression of Foxp3 but the absence of IL-10 or TGF- β , play a significant role in the regulation of inflammation within various locations, such as solid tumors. The immunomodulatory function of IL-35 encompasses a wide range of illness situations, exhibiting its most notable effects in contexts characterized by intense inflammation and robust activation of nTregs. However, within the context of cancer, IL-35 demonstrates a dual function. While it has been observed to facilitate tumor growth in specific cancer models such as melanoma, pancreatic cancer, non-small cell lung cancer (NSCLC), breast cancer, lymphoma, and gastric cancer, it has also demonstrated potential anti-tumor properties, particularly in hepatocellular carcinoma (HCC). The inhibition of IL-35 has demonstrated potential as a therapeutic strategy in the context of cancer treatment, exhibiting the ability to synergistically enhance the efficacy of immunotherapy. The presence of heightened amounts of IL-35 has been linked to the pathogenicity and advancement of cancer, indicating a potential distinctive function in cancer development when generated by immune cells or cancer cells. Additionally, it has been observed that the upregulation of IL-35 in HepG2 carcinoma cells results in the augmentation of major histocompatibility complex class I (MHC I)-specific immune response to tumors, as well as a decrease in cancer cell motility, colony formation, and invasion. These findings suggest that IL-35 has a role in suppressing tumor growth in hepatocellular carcinoma (HCC). In brief, the involvement of IL-35 in cancer is characterized by its diverse impact, exhibiting both tumor-promoting and tumor-suppressing actions across various contexts and disease classifications. The intricate interplay occurring within the tumor microenvironment renders it a captivating subject for additional investigation and prospective therapeutic approaches. Future research efforts should prioritize the exploration of IL-35 as a potential therapeutic target to impede the progression of malignancies. Additionally, it is crucial to assess the physiological impacts of IL-35 on primary tumors through the implementation of human clinical trials [13].

2.5. IL-39

IL-39 is a recently discovered constituent of the interleukin 12 family, characterized by its composition of heterodimeric cytokines comprising subunits p19 and EBi3 [14]. Although initial research has predominantly concentrated on examining the impact of this substance on cancer cell lines, there is a scarcity of literature regarding its involvement in animal models or human subjects. At this juncture, it is premature to categorize IL-39 conclusively as either proinflammatory or regulatory. Nevertheless, recent studies have indicated a probable association between IL-39 and the development of lupus erythematosus in mice, so suggesting its potential significance in the context of inflammatory conditions, specifically systemic lupus erythematosus [15]. Moreover, it has been postulated that IL-39 may confer a safeguarding influence on human keratinocytes, perhaps fostering the process of wound healing through the inhibition of the inflammatory response. However, it is crucial to acknowledge that the involvement of IL-39 in human physiology is mostly uncharted territory and necessitates additional research. Several investigations, like those conducted by Ecoeur et al., suggest that IL-39 may possess immunomodulatory properties predominantly in murine models.

However, the precise role and importance of IL-39 in humans have yet to be comprehensively investigated and comprehended.

3. Conclusion

The IL-12 family of cytokines plays a pivotal role in regulating cancer immunity. They exert their influence on the immune system by modulating the activity of other cytokines and immune cells. The members of the IL-12 family display unique properties within different signaling pathways. They serve not only as markers for assessing immune responses but also as adjuvants to enhance the tumor microenvironment or improve the efficacy of single-drug treatments. The clinical studies of IL-12 have demonstrated its potential in diverse cancer models, highlighting the need for more optimization in terms of dosage, administration method, and combination therapy approaches.

IL-23's role in mediating Th17 responses and its impact on other immune cytokines and cells warrant investigation, as does its potential involvement in both anti-inflammatory and pro-inflammatory pathways. IL-27, despite its lack of intrinsic stimulation, can effectively regulate signaling pathways when combined with other stimuli, showcasing its remarkable adaptability. For IL-35 and IL-39, two newer members of the IL-12 family, they primarily exhibit pro-tumorigenic functions in mouse experiments but also have signaling roles in gene expression. Combination therapies are still viable options for both.

In conclusion, members of the IL-12 family hold great promise in the field of cancer immunotherapy. While individual immunotherapy approaches to block or promote their activation can influence tumor immunity, the low controllability, dosage considerations, and potential risks make them challenging to use independently. More commonly, they are employed as adjuvants in combination with other drugs, complementing each other's effects. Currently, indicators for measuring IL-12 family members are frequently used in experiments to monitor tumor growth, and their future use in drug treatments is anticipated.

References

- [1] Maura Abbott, Yelena Ustoyev, Cancer and the Immune System: The History and Background of Immunotherapy, Seminars in Oncology Nursing, Volume 35, Issue 5, 2019, 150923, ISSN 0749-2081
- [2] Yang Y. Cancer immunotherapy: harnessing the immune system to battle cancer. J Clin Invest. 2015 Sep;125(9):3335-7.
- [3] Conlon KC, Miljkovic MD, Waldmann TA. Cytokines in the Treatment of Cancer. J Interferon Cytokine Res. 2019 Jan;39(1):6-21. doi: 10.1089/jir.2018.0019. Epub 2018 Jun 11.
- [4] Wu T, Dai Y. Tumor microenvironment and therapeutic response. Cancer Lett. 2017 Feb 28; 387:61-68.
- [5] Li L, Yu R, Cai T, Chen Z, Lan M, Zou T, Wang B, Wang Q, Zhao Y, Cai Y. Effects of immune cells and cytokines on inflammation and immunosuppression in the tumor microenvironment. Int Immunopharmacol. 2020 Nov; 88:106939.
- [6] Teng, Michele W.L., et al. "IL-12 and IL-23 cytokines: from discovery to targeted therapies for immune-mediated inflammatory diseases." Nature Medicine, vol. 21, no. 7, July 2015, pp. 719+. Gale Academic OneFile
- [7] Mirlekar B, Pylayeva-Gupta Y. IL-12 Family Cytokines in Cancer and Immunotherapy. Cancers (Basel). 2021 Jan 6;13(2):167.
- [8] Vignali, Dario A.A., and Vijay K. Kuchroo. "IL-12 family cytokines: immunological playmakers." Nature Immunology, vol. 13, no. 8, Aug. 2012, pp. 722+. Gale Academic OneFile
- [9] Agliardi G, Liuzzi AR, Hotblack A, De Feo D, Núñez N, Stowe CL, Friebe E, Nannini F, Rindlisbacher L, Roberts TA, Ramasawmy R, Williams IP, Siow BM, Lythgoe MF, Kalber TL, Quezada SA, Pule MA, Tugues S, Straathof K, Becher B. Intratumoral IL-12 delivery empowers CAR-T cell immunotherapy in a pre-clinical model of glioblastoma. Nat Commun. 2021 Jan 19;12(1):444.

- [10] Zheng Y, Lu Z, Ding J, Jiang N, Wang J, Yang J, Song J, Chen H, Fang L, Li H, Tian H, Wang G, Zheng J, Chai D. Therapeutic Adenovirus Vaccine Combined Immunization with IL-12 Induces Potent CD8⁺ T Cell Anti-Tumor Immunity in Hepatocellular Carcinoma. *Cancers (Basel)*. 2022 Sep 17;14(18):4512.
- [11] Chan Y, Tong BSB, Ngan PY, Au CS. Effectiveness of IL-23 Inhibitor Guselkumab in Real-World Chinese Patients with Psoriasis During a 20-Week Period. *Psoriasis (Auckl)*. 2021 Jun 1; 11:53-58.
- [12] Jin-Qing Liu, Chengxiang Zhang, Xinfu Zhang, Jingyue Yan, Chunxi Zeng, Fatemeh Talebian, Kimberly Lynch, Weiyu Zhao, Xucheng Hou, Shi Du, Diana D. Kang, Binbin Deng, David W. McComb, Xue-Feng Bai, Yizhou Dong, Intratumoral delivery of IL-12 and IL-27 mRNA using lipid nanoparticles for cancer immunotherapy, *Journal of Controlled Release*, Volume 345, 2022, Pages 306-313
- [13] Liu K, Huang A, Nie J, Tan J, Xing S, Qu Y, Jiang K. IL-35 Regulates the Function of Immune Cells in Tumor Microenvironment. *Front Immunol*. 2021 May 21; 12:683332.
- [14] Gabriela Fonseca-Camarillo, Janette Furuzawa-Carballeda, Jesus K. Yamamoto-Furusho, Su1528: UP-REGULATION OF THE IL-39 (IL-23P19/EBI3) EXPRESSION IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE, *Gastroenterology*, Volume 162, Issue 7, Supplement, 2022, Page S-615, ISSN 0016-5085
- [15] Ecoeur F, Weiss J, Schleeger S, Guntermann C. Lack of evidence for expression and function of IL-39 in human immune cells. *PLoS One*. 2020 Dec 1;15(12): e0242329.