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ISSN 2959-409X

The Effect of Kynureninase on Cancer Treatment

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Abstract:

At present, cancer is still one of the most common lethal diseases in human beings. The traditional treatment of cancer has great side effects on human body, and more advanced treatment schemes need to be found to improve the survival rate of cancer. Nowadays, immunotherapy and targeted therapy are very promising directions. In immunotherapy, most of them are immune checkpoint inhibitors, cancer vaccines, cell therapy, etc., and few biological enzymes have been applied in cancer treatment. This paper analyzes the research on kynureninase, and proves that kynurenine accumulation is one of the important factors that lead to the occurrence of tumors. Combined with kynureninase, it can have a significant effect in the treatment of large B16-F10 melanoma, 4T1 breast cancer or CT26 colon cancer tumors. It can provide reference for the future research of immunotherapy, and the source problem of kynureninase has not been solved. Future research can focus on the development and optimization of humanized enzyme.

Keywords: immunotherapy; immunosuppression; kynurenine

I. Introduction

Nowadays, tumor disease is the most common lethal disease in human life, which has brought a huge impact on people's life [1]. Because many tumor diseases are hard to find in the just growing stage of tumor. And people lack of tumor prevention, tumors continue to grow and reproduce, which may develop into malignant tumors, causing mental and economic pressure to people. The body may suffer from tumor disease due to gene mutation, genetic factors, environmental factors, etc. we can reduce the possibility of tumor disease deterioration and improve the probability of successful treatment through regular physical examination, understanding the initial symptoms of tumor, and timely medical treatment. According to relevant statistics in 2022, the relative survival rate of cancer patients in China is only about 40.5% [2]. Although it has improved, it is still low.

Cancer can be cleared by surgery, chemotherapy, radiotherapy and other methods [3]. These schemes are common cancer treatment methods, but the side effects are more serious. Surgical treatment can only be carried out under the condition that the tumor location is convenient for surgery and the volume is large and the diffusion area is small; Chemotherapy and radiotherapy will cause serious damage to the

healthy cells of the body, so breakthroughs in the pathogenesis, treatment plan and early prevention of tumors are still needed to reduce the number of cancer deaths and improve the quality of human life. Nowadays, many advanced treatment methods have been proposed, such as targeted therapy, immunotherapy and so on. Targeted therapy can directly and accurately act on specific molecules of cancer cells, inhibit their growth and reproduction or induce their apoptosis; Immunotherapy acts on cancer cells indirectly by regulating immune cells or regulatory molecules in the immune system. These two treatment schemes have less damage to the body and significant curative effect. Research on tryptophan metabolism is an important part of tumor treatment. Kynurenine pathway is the main pathway of tryptophan metabolism, and its metabolic process affects immune suppression.

Based on immunotherapy, this paper discusses the influence of kynurenine in cancer occurrence and the role of kynureninase in cancer treatment. It can further understand the immune mechanism of kynurenine and the therapeutic effect of kynureninase, and put forward more possibilities for the treatment of cancer.

II. Tumor immunity and metabolic mechanisms

A. Tumor Pathogenesis

In normal cells, the expression of proto oncogenes and tumor suppressor genes is relatively balanced, which jointly affect the growth and metabolism of cells. Proto oncogenes are involved in cell growth, proliferation, differentiation and apoptosis. Its expressed growth factor receptor can receive the external growth signal, transmit it to the cell, and start cell proliferation; Tumor suppressor genes can inhibit the formation of tumors, which can detect the abnormal differentiation and proliferation of cells, induce apoptosis through a variety of mechanisms, so as to maintain the stability of cells. However, when the expression of proto oncogenes and tumor suppressor genes is abnormal, tumor cells will form. Most tumor cells will be cleared by the body's immune system, but there are still a small number of tumor cells that escape immune clearance, or immunosuppression occurs. Because tumor cells activate telomerase and maintain telomere length, it can increase in value indefinitely in the body, consume nutrients and damage human health. The immune system of the body would not work normally due to various factors, resulting in the immune escape of tumor cells. For example, the antigen components on the surface of tumor cells are changed and the immune system cannot recognize the antigen components; Tumor cells will secrete immune factors and inhibit the activity of immune cells; Immune cells express immune checkpoint ligands and inhibit immune cell activation and proliferation. At the same time, the tumor microenvironment will also have adverse effects on the immune system.

B. Tumor Microenvironment

Tumor microenvironment refers to the microenvironment surrounding the growth and proliferation of tumor cells. TME provides tumor cells with oxygen, amino acids, glucose and other nutrients to help them achieve rapid proliferation and metabolism. Tumor microenvironments comprise cells like tumor and immune cells, endothelial cells,etc. Additionally, there are non-cellular elements such as the extracellular matrix and biochemical signals like IL-10 and IL-6. These interactions foster tumor growth and proliferation, leading to cancer development and drug resistance [4]. Tumor cells manipulate the immune system by drawing in numerous immunosuppressive cells, including regulatory bone marrow-derived suppressor cells [5] and regulatory T cells. These suppressive cells secrete molecules like interleukin-10, which create a protective shield for the tumor cells. This shield enables them to evade immune surveillance, thus thriving during chemotherapy and targeted therapy and ultimately developing resistance to these treatments. In the tumor microenvironment, both tumor cells and antigen-presenting cells highly express immune checkpoint molecules, notably programmed death receptor 1 ,and its ligand 1 (PD-1/PD-L1 are both immune checkpoint molecules) [6]. These molecules bind to T cell receptors, effectively inhibiting T cell activation and proliferation. Therefore, the tumor cells are able to evade the immune system's monitoring and attack, allowing them to proliferate unchecked.

C. Tryptophan Metabolism

Tryptophan is very important for human and is the essential amino acid which human cannot synthesis by themselves. It is a necessary raw material for protein synthesis. Tryptophan and its metabolites can regulate the immune system and cellular homeostasis can be maintained. According to research, the expression of tryptophan metabolizing enzymes in TME is significantly increased in gastrointestinal cancer, gynecological cancer, hematological malignancies, breast cancer, lung cancer, glioma, melanoma, prostate cancer and pancreatic cancer [6], which can be considered as an important factor to accelerate tumor changes [7]. Tryptophan metabolism can be divided into three pathways: 5-HT pathway, indole pathway and kynurenine pathway. A part of free tryptophan is used for

ISSN 2959-409X

producing protein and serotonin, but more than 95% of free tryptophan will be degraded through the third way -the kynurenine pathway. so the kynurenine pathway is the main metabolic pathway [8].

D. Kynurenine Pathway

In the kynurenine pathway, for Figure 1 Kynurenine pathway, tryptophan is decomposed into n-formylkynurenine by indoleamine 2,3-dioxygenase 1 and tryptophan 2,3-dioxygenase. N-formylkynurenine is degraded to kynurenine by formamidase. Indoleamine 2,3-dioxygenase 2 catalyzes the breakdown of tryptophan into kynurenine, albeit its precise role in cancer therapy remains undefined. Subsequently, kynurenine undergoes a series of transformations, kynureninase converts it into anthranilic acid, kynurenine aminotransferase into kynurenine quinolinic acid, and kynurenine monooxygenase into 3-hydroxykynurenine. This latter compound further decomposes into xanurinoic acid and 3-hydroxyanthranilic acid, eventually leading to the formation of NAD and NADP, key metabolic intermediates.

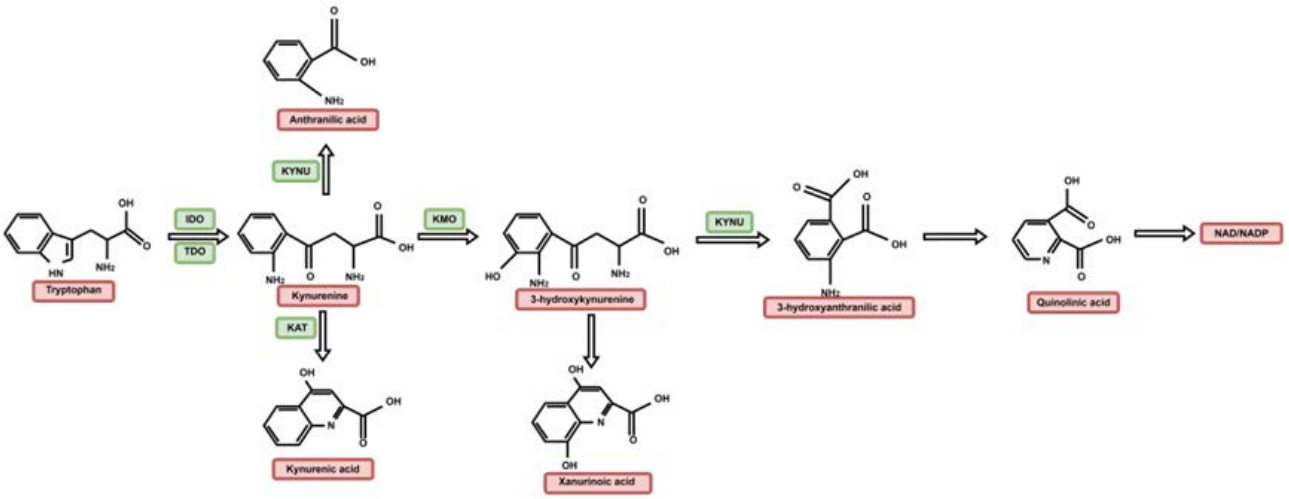

Figure 1. Kynurenine pathway

III. Immunosuppression

A. Expression of IDO 1 and TDO

In TME, tumor cells themselves express IDO 1, and drive other stromal cells and immune cells (antigen-presenting cells, etc.) to express IDO 1. Interferon - γ is a key cytokine that induces Ido 1. It attaches to specific cell surface receptors, initiating signaling cascades. This process phosphorylates transcription factors, enabling them to enter the nucleus and bind to promoters, thereby activating gene transcription. Inflammatory factors can also help the transcription of IDO 1. They are tumor necrosis factor alpha and interleukin-1. The pro-inflammatory cytokine lipopolysaccharide can also promote the gene transcription of IDO 1 and induce the expression of IDO 1. Under steady-state conditions, TDO is expressed by hepatocytes to decompose 80%-90% of tryptophan in food, but in TME, it is expressed by tumor cells. The increased expression of IDO 1 and TDO will lead to a large amount of tryptophan being interpreted as kynurenine, causing tryptophan depletion and kynurenine accumulation.

B. Inhibition of mTOR Pathway

The deficiency of TRP will lead to the activation of glucokinase pathway and further inhibit mTOR pathway [9]. mTOR pathway can determine whether it is involved in protein synthesis or autophagy based on amino acid level. Inhibit autophagy by inhibiting the activity of autophagy related proteins, so that the materials and energy in cells can be preserved and used, thereby regulating cell metabolism; Conversely, inhibition of mTOR pathway induces autophagy in effector T cells. The mTOR pathway mediates the expression of inducible costimulatory molecules on T cells, which interact with B7h to promote the immune infiltration of tumors [10]. ICOS has important influence in the activation, proliferation and regulation of immune response of T cells, so the reduced expression of ICOS will lead to incomplete activation of T cells and thus immunosuppression [11].

C. Eukaryotic Initiation Factor 2 α Phosphorylation

At the same time, when cells detect the decline of TRP content in vivo, they will activate general control repressi-

ble 2 (gcn-2), cause eukaryotic initiation factor 2 α (eIF2 α) phosphorylation, and inhibit the translation initiation of proteins. This will lead to the reduction of protein synthesis related to cell proliferation, thus hindering the normal proliferation of cells. Specifically, they sport the proliferation of effector T cells in the G1 cycle. Meanwhile, gcn-2 can also regulate the activity of some transcription factors, such as activating transcription factor 4 (ATF4), thereby promoting the expression of tryptophan transporters [12]. The expression of tryptophan transporters in tumor cells is much higher than that of effector T cells, so tumor cells will express more tryptophan transporters to take up more tryptophan to meet the growth needs. Effector T cells cannot obtain more tryptophan to meet their needs in the process of activation and proliferation, so that effector T cells are reduced and the body appears immunosuppression.

D. Aryl Hydrocarbon Receptor Ligands

Kynurenine and its downstream metabolites in tryptophan metabolism are aryl hydrocarbon receptor (AHR) ligands, which activate the transcription of AHR. They are 3 '- hydroxy kynurenine (3'oh-kyn) and 3' - hydroxy anthranilic acid (3'oh-aa). AHR can promote the expression of immunosuppressive interleukin-10 (IL-10) and the reprogramming of helper T cell 17 (Th17) into regulatory T (Treg) cells, making T cells decrease and Treg cells increase. Tumor cells use AHR ligand signaling pathway to promote their own proliferation, invasion and immune escape.

IV. Kynureninase:

A. Enzyme Selection

At present, clinical research mainly focuses on the inhibition of IDO, TDO and dual inhibition to improve the body's immunosuppression. Its side effects include the occurrence and aggravation of autoimmune diseases, affecting the metabolism of neurotransmitters and so on, which are more harmful to the body. From a therapeutic perspective, enzyme therapy can provide several important advantages over small molecule ido1 inhibitors currently under clinical evaluation, including the ability to treat not only ido1 upregulated cancers, but also TDO expressing tumors [13]. At present, Kmo, Kat and kynureninase can decompose kynurenine, but only kynureninase has the highest activity and the product has the least impact on human body. It has biochemical and pharmacological properties [14]. Kynureninase will degrade kynurenine into immune inert, non-toxic, and easily cleared metabolites: anthranilic acid and L-alanine [15].

B. Modification of kynurenine with polyethylene glycol (PEG):

Because the enzyme modified by PEG has the ability to resist protease hydrolysis; Reduce the immunogenicity of the enzyme and reduce the possibility of being clearly recognized by the immune system; Increase the molecular weight of the enzyme and reduce the filtration and clearance ability of the kidney. Then by adding PEG modification, the retention time of kynureninase in the body is extended so that KYN can be completely eliminated, so as to improve the body immunity and inhibit tumor growth.

V. Application value evaluation

Todd A. Triplett and colleagues conducted research demonstrating that combining PEG kynureninase with approved checkpoint inhibitors or cancer vaccines yields substantial therapeutic benefits in treating large tumors, including B16-F10 melanoma, 4T1 breast cancer, and CT26 colon cancer [13].

A. Blend with Immune Checkpoint Inhibitors

α PD-1 and α CTLA-4 were blend into PEG kynureninase to treat B16-F10 melanoma model and 4T1 breast cancer model. α PD-1/ α CTLA-4 can block the pathway of PD-1/PD-L1 or CTLA-4, relieve the inhibitory signals on the surface of T cells, and restore their effector functions. In the B16-F10 melanoma model, only 10% of mice showed complete tumor remission with single agent α PD-1; While the combined use can improve the complete remission rate to 45%, and 100% of the surviving mice can generate immune memory and reject tumors when they are inoculated with tumor cells again after 2 months. In the 4T1 breast cancer model, single agent α CTLA-4 only transiently inhibited tumor growth and had no survival benefit; However, the median survival was prolonged by 45% when the combination was used, and the tumors in 8% of mice completely regressed and produced immune memory. KYN depletion can enhance the sensitivity of T cells to PD-1 blockade and avoid the resistance of IDO pathway to checkpoint inhibitors. Both experiments can prove that compared with single drug therapy, the combination therapy has a significant therapeutic effect on B16-F10 melanoma and 4T1 breast cancer.

B. Blend with Cancer Vaccines

Relevant studies have shown that the combination of impact vaccine and PEG kynureninase can treat CT26 colon cancer model. Impact vaccine can express gp96 Ig fusion protein, present tumor antigen through heat shock protein, activate cross presentation of dendritic cells (DCS), and induce potent cd8+ T cell responses. The use of single

ISSN 2959-409X

agent impact vaccine only prolonged the median survival by about 10-15 days, and the tumor free survival rate was <20%; However, 60% of the mice achieved complete tumor regression in the combined use, and no tumor growth occurred when the surviving mice were inoculated with CT26 cells again. KYN depletion can reverse Ido mediated inhibition of DC function (such as defects in antigen uptake and cytokine secretion), making the vaccine more effective in initiating initial T cell responses. It is also proved that the effect of cancer vaccine combined with PEG kynureninase is much greater than that of single drug treatment.

VI. Conclusion

Based on immunotherapy, this paper introduces the kynurenine pathway in detail, the feasibility of using PEG kynureninase to decompose kynurenine, and the experimental effect of this scheme. The significance of this study is to prove that the accumulation of kynurenine is one of the important factors leading to tumorigenesis. Combined with kynureninase, it can have a significant effect in the treatment of certain tumor diseases, which puts forward a new direction for cancer immunotherapy. However, this paper does not analyze the source of kynureninase, which can be from mammals, bacteria, plants, etc. if kynureninase can be cultured from human body, its modification and design can greatly reduce immunogenicity and human rejection. In the future, exploring kynureninase sources can accelerate its clinical application and transformation.

References

[1] Crosby D, Bhatia S, Brindle K M, *et al.*, "Early detection of cancer," *Science*, vol. 375, no. 6586, p. eaay9040, 2022.

[2] Xia C, Dong X, Li H, *et al.*, "Cancer statistics in China and United States, 2022: Profiles, trends, and determinants," *Chinese Medical Journal*, vol. 135, no. 5, pp. 584–590, 2022.

[3] Yan Z, Yao S, Wang Z, *et al.*, "Treatment of extranodal NK/T-cell lymphoma: From past to future," *Frontiers in Immunology*, vol. 14, 2023.

[4] Belli C, Trapani D, Viale G, *et al.*, "Targeting the microenvironment in solid tumors," *Cancer Treatment Reviews*, vol. 65, 2018.

[5] Feng X, Meng M, Li H, *et al.*, "T-cell dysfunction in natural killer/T-cell lymphoma," *Oncoimmunology*, vol. 12, no. 1, p. 11, 2023.

[6] Ala M, "The footprint of kynurenine pathway in every cancer: A new target for chemotherapy," *European Journal of Pharmacology*, p. 173921, 2021.

[7] Puccetti P, Francesca, *et al.*, "Accumulation of an endogenous tryptophan-derived metabolite in colorectal and breast cancers," *PLoS One*, 2015.

[8] Yi S, Yang M, Duan K, "Immune-mediated metabolic kynurenine pathways are involved in the postoperative cognitive dysfunction after cardiopulmonary bypass," *The Thoracic and Cardiovascular Surgeon*, vol. 63, no. 07, pp. 618–623, 2015.

[9] Metz R, Rust S, Duhadaway J B, *et al.*, "IDO inhibits a tryptophan sufficiency signal that stimulates mTOR: A novel IDO effector pathway targeted by D-1-methyl-tryptophan," *Oncoimmunology*, vol. 1, no. 9, pp. 1460–1468, 2012.

[10] Lu Z, Zhang C, Zhang J, *et al.*, "The kynurenine pathway and indole pathway in tryptophan metabolism influence tumor progression," *Cancer Medicine*, vol. 14, no. 6, 2025.

[11] Xie D L, Wu J, Lou Y L, *et al.*, "Tumor suppressor TSC1 is critical for T-cell anergy," *Proceedings of the National Academy of Sciences*, 2012.

[12] Timosenko E, Ghadbane H, Silk J D, *et al.*, "Nutritional stress induced by tryptophan-degrading enzymes results in ATF4-dependent reprogramming of the amino acid transporter profile in tumor cells," *Cancer Research*, vol. 76, no. 21, 2016.

[13] Triplett T A, Garrison K C, Nicholas M, *et al.*, "Reversal of indoleamine 2,3-dioxygenase–mediated cancer immune suppression by systemic kynurenine depletion with a therapeutic enzyme," *Nature Biotechnology*, 2018.

[14] Cervenka I, Agudelo L Z, Ruas J L, "Kynurenines: Tryptophan's metabolites in exercise, inflammation, and mental health," *Science*, vol. 357, no. 6349, p. eaaf9794, 2017.

[15] Cory M, Vladimir L, Lena B, *et al.*, "Three-dimensional structure of kynureninase from Pseudomonas fluorescens," *Biochemistry*, vol. 43, no. 5, p. 1193, 2004.