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Mechanism of melittin for anti-tumor effects: Research status and future perspectives

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Abstract. Cancer is a primary cause of mortality in humans. Conventional chemotherapy has hazardous side effects and numerous limitations, necessitating the urgent exploration of novel anti-cancer agents. Based on existing literature and data, this article reviews the anti-tumor mechanism, current application status, and future development of bee venom peptides. The research findings demonstrate that bee venom peptide, a natural cell lytic peptide, possesses many anti-tumor mechanisms, including the direct destruction of tumor cells, the induction of tumor cell apoptosis, and immunological modulation. It can disrupt the integrity of the cell membrane and lead to cell death. It can also activate apoptosis signaling pathways through endoplasmic reticulum, mitochondria, and death receptors, promoting apoptosis of tumor cells. Meanwhile, it can reshape the immune microenvironment and stimulate immune responses. The author also discussed issues such as poor stability, low bioavailability, lack of targeting, and potential safety of bee venom peptides. In the future, it is recommended to develop new derivatives of bee venom peptides and optimize combination therapy strategies.

Keywords: melittin, tumor, immunoregulation, combination therapy strategy

1. Introduction

The World Health Organization reports that cancer ranks among the foremost causes of mortality, with over 19 million diagnoses and approximately 10 million deaths attributed to cancer worldwide in 2020. Cancer encompasses a range of disorders resulting from acquired genetic alterations that precipitate unregulated and inappropriate proliferation [1]. Cancer cells can infiltrate adjacent tissues and disseminate to other organs, a phenomenon known as metastasis. Extensive metastasis is a contributing factor to cancer mortality. There are many therapeutic treatments available for cancer, such as chemotherapy, that are not only toxic but also have serious side effects. The systemic toxicity of chemotherapeutic drugs limits their administration. In addition, factors such as poor cell delivery, limited solubility, the inability of drugs to cross the cell barrier, and the lack of overcoming multidrug resistance (MDR) limit the application of chemotherapy drugs [2]. Therefore, it is of vital practical significance to actively explore new anticancer drugs and drug carriers with high efficiency and low toxicity. Melittin (MLT) is a naturally occurring cytolytic peptide that directly induces tumor cell death and possesses diverse immunomodulatory activities. Due to its unique dual mechanism of action, MLT shows minimal resistance and is considered a promising broad-spectrum anti-tumor drug [3]. Based on the existing literature and research results, this paper discusses the research progress, challenges and future prospects of the anti-tumor mechanism of melittin. In-depth study of the anti-tumor mechanism of melittin not only helps to reveal the internal principle of its anticancer activity, but also provides a solid theoretical basis for the development of new anticancer drugs based on melittin and may provide new ideas for the formulation of drug combination strategies.

2. Mechanism of melittin for anti-tumor effects

Melittin constitutes 40-60% of the dry weight of bee venom and is the principal active component responsible for its biological activities. The molecule is a linear, cationic, amphiphilic peptide composed of 26 amino acids, featuring 6 positive charges at physiological pH. In neutral aqueous solution, melittin as a monomer exists in a random curly structure, but with the increase of pH value and ionic strength, melittin can self-crosslink to form an α-helix tetramer structure [4]. Melittin can bind to lipid bilayer membranes, fold into amphiphilic α-helical secondary structures, and destroy permeability barriers [5]. A substantial body of

literature indicates that melittin has several biological actions, including antibacterial, antifungal, antiviral, and antiparasitic properties. In addition to the above properties, melittin has been shown to be an effective cell growth inhibitor [6].

2.1. Killing the tumor cell directly

Melittin can directly interact with the cell membrane, compromise its integrity, and subsequently induce cell death, representing a significant mechanism of its anti-tumor activity. Studies have found that melittin divides and moves laterally in the cell membrane in the form of monomers and then oligomerizes into a ring structure, forming holes, resulting in cell death, and it is found that cells collapse during co-incubation with free melittin and lose their original shape [7]. Jiaojiao Liu's team discovered that following the initial attachment of melittin to the cell membrane, the subsequent accumulation of melittin on the bilayer induced significant variations in the membrane, even extracting certain lipid molecules from the distorted outer lobules of the bilayer. This unique mode of mass removal and the resulting asymmetry in the number of local lipids between the two lobules alters the mechanical state of the membrane, thereby reducing the free energy barrier for apiin insertion. The development of transmembrane pores was markedly enhanced [8]. The permeability of the cell membrane alters, leading to an ionic imbalance within the cell, including potassium ion efflux and calcium ion influx, ultimately ending in cellular swelling, lysis, and death.

2.2. Inducing apoptosis of tumor cells

Apoptosis is a physiological process of selective cell clearance [9]. It is of great significance for maintaining normal physiological function and cell homeostasis. In addition to directly killing tumor cells, melittin can also play an anti-tumor role by inducing tumor cell apoptosis. In the process of tumorigenesis and development, tumor cells are often able to evade the normal apoptotic regulatory mechanisms, thus achieving infinite proliferation [10]. Melittin can activate the apoptosis signaling pathway in tumor cells through various pathways such as endoplasmic reticulum and mitochondrial apoptosis and promote the apoptosis of tumor cells, thus inhibiting the growth and spread of tumor[11-13].

2.2.1. Endoplasmic reticulum pathway

The endoplasmic reticulum (ER) is a crucial organelle in the cell, responsible for the synthesis, folding, and modification of proteins, in addition to the storage of calcium ions. Endoplasmic reticulum stress denotes the impairment of the endoplasmic reticulum caused by various factors, leading to the buildup of unfolded or misfolded proteins within the endoplasmic reticulum, which triggers a cascade of stress responses. Melittin can induce endoplasmic reticulum stress to induce tumor cell apoptosis by affecting the endoplasmic reticulum. Xuan Li's team discovered that melittin stimulates the endoplasmic reticulum (ER) stress-CHOP (C/EBP homologous protein) apoptotic pathway, which is closely linked to elevated intracellular ROS levels. 4-Phenylbutyric acid, an endoplasmic reticulum stress inhibitor, has been utilized to validate that the apoptotic signal of endoplasmic reticulum stress-CHOP constitutes a molecular mechanism for melitin-induced A549 cell death [14].

2.2.2. Mitochondria apoptosis pathway

Mitochondria are pivotal in the mechanism of apoptosis. Melittin can directly impair mitochondrial function and trigger cell death, as well as alter mitochondrial membrane permeability, resulting in the release of apoptosis factors such as Cytochrome C into the cytoplasm, so activating the apoptosis signaling cascade.

Edward Gasanoff's team found that melittin can directly affect mitochondrial oxygen consumption and respiratory control index (RCI) and confirmed that the decline of mitochondrial biological energy caused by it is the main cause of cell death [15]. On the other hand, the increase of intracellular calcium ion concentration caused by melittin may also indirectly affect mitochondrial function. High concentrations of calcium ions will accumulate in mitochondria, resulting in mitochondrial calcium overload, and eventually cancer cell apoptosis [16].

In addition to directly affecting mitochondrial function, melittin can also affect apoptosis-related pathways. Xuan Li's team's research indicates that melittin alters mitochondrial membrane potential (MMP) by directly affecting the mitochondria of human lung adenocarcinoma cells (A549), instigating a mitochondrial ROS surge and activating the mitochondria-associated apoptosis pathway Bax/Bcl-2 [17]. The anti-apoptotic protein Bcl-2 can prevent the rise in mitochondrial membrane permeability, block the release of Cytochrome C, and hence impede cell apoptosis. The pro-apoptotic protein Bax can enhance mitochondrial membrane permeability and expedite apoptosis [9].

2.2.3. Death receptor pathway

Death receptors are part of the tumor necrosis factor receptor (TNFR) superfamily, including Fas (CD95), TNFR1, DR4 (TRAIL-R1), and DR5 (TRAIL-R2). After binding to the corresponding ligand on the cell surface, these death receptors can activate the

apoptosis signaling pathway in the cell, leading to apoptosis [18]. Melittin can interact with the death receptors on the surface of tumor cells to activate the apoptosis signaling pathway mediated by death receptors, thereby inducing apoptosis of tumor cells.

Miran Jo's team discovered that melittin suppresses cell proliferation by increasing the expression of death receptor (DR) in human ovarian cancer cells SKOV3 and PA-1. In SKOV3 and PA-1 cells treated with melittin, the expressions of pro-apoptotic proteins caspase-3, caspase-8, and Bax, downstream of the death receptor, were concurrently elevated, whereas the expression of Bcl-2 was suppressed. Melittin is determined to cause apoptosis in ovarian cancer cells by upregulating the expression of DR3, DR4, and DR6 [19].

In addition, when melittin is combined with other chemotherapy drugs or cytokines, it may synergistically enhance the activation of the death receptor pathway, improve the apoptosis rate of tumor cells, and provide new ideas and strategies for the combination therapy of tumors.

2.3. Immunoregulatory activity

Upon the presentation of antigens by antigen-presenting cells (APCs) in tumor-draining cells, lymph nodes (LNs) are immunological organs that generate an adaptive immune response to combat malignancies. It is a promising approach to improve the efficacy of cancer immunotherapy to deliver nanovaccines to lymph nodes (LNs) that contain tumor antigens and adjuvants via targeted delivery. Melittin, a cationic host defense peptide, demonstrates a variety of immunomodulatory effects. However, due to their blood solubility and their narrow safe dose range, they are prevented from exerting their maximum immunomodulatory effect in LNs. Xiang Yu's team successfully synthesized an ultra-small (10-20 nm) hummingtin-lipid nanoparticle (named alpha-hummingtin-NP) and demonstrated that alpha-hummingtin-NP effectively shielded the positive charge of hummingtin-NP within the phospholipid monolays, thereby reducing cytotoxicity to red blood cells (RBCS). They assessed the capacity of alpha-melitin-NPS to target lymph nodes and modify the immunological microenvironment. The data indicated that alpha-melitin-NPS was swiftly and effectively administered into the lymph nodes, subsequently activating antigen-presenting cells, including macrophages and dendritic cells. Furthermore, they confirmed the vaccination efficacy of alpha-melitsine-NPS in a bilateral flank B16F10 tumor model. Consequently, α-melitin-NPS can facilitate the release of whole tumor antigens and activate antigen-presenting cells, making it a promising whole-cell nanovaccine for targeting lymph node metastasis in tumor immunotherapy [20].

3. Research status

Melittin, the principal peptide constituent of bee venom, is a potent cytolytic anticancer peptide with anti-tumor efficacy. Nonetheless, the actual utilization of melittin in malignancies has been impeded by its pronounced non-specific hemolytic activity and intrinsic instability. A delivery mechanism is employed to rectify the deficiencies of cellulin and ensure its secure administration. A range of melittin vectors has evolved. A recent study demonstrated that encapsulated melittin retains its immunogenic properties and can function as an adjuvant to elicit a potent antibody immunological response against the delivery vector. In vivo studies demonstrate that DMM is safer than free peptides and exhibits superior efficacy in inhibiting tumor growth. They jointly suggest the inaugural application of micellar D-melittin in oncological treatment.

4. Challenges and prospects

4.1. Challenges

4.1.1. Stability and bioavailability issues

As a bioactive peptide, melittin faces the problem of poor stability and low bioavailability in the vivo environment, which seriously limits its potential for clinical application. Melittin is easily degraded by many factors in vivo, such as hydrolysis of protease, change of acid-base environment and interaction with other substances in vivo. Its short half-life in the blood, usually only a few minutes to ten minutes, means that most melittin may have been degraded and inactivated before reaching the tumor site, unable to fully exert its anti-tumor effects. The hydrophilicity and hydrophobicity of melittin also limit its solubility and distribution in vivo, which further reduces its bioavailability. In order to solve the problem of the stability and bioavailability of melittin, researchers have carried out a lot of exploration, but there are still many technical difficulties. Therefore, the development of high efficiency, stability and high bioavailability of melittin preparations is still a problem to be solved.

4.1.2. Poor target specificity and high incidence of side effects

At present, the distribution of melittin in the body lacks specificity, and it is difficult to accurately act on tumor tissues, which not only reduces its killing effect on tumor cells but also may produce toxic side effects on normal tissues. Due to the complex microenvironment of tumor tissues, there are many physiological barriers, such as the vascular endothelial barrier and extracellular matrix, which makes it difficult for melittin to penetrate these barriers effectively and accumulate in the tumor site. The

indiscriminate distribution of melittin inside the body may result in its binding to receptors on normal cells, leading to adverse toxic reactions such as hemolysis and allergies, hence constraining the dosage and safety of its therapeutic use. In order to improve the targeting of melittin, researchers have tried a variety of strategies, such as combining melittin with tumor-specific antibodies, ligands or targeted nanocoliths to construct targeted drug delivery systems. In addition, the heterogeneity and individual differences of tumor tissues also increase the difficulty of achieving precise targeted therapy. How to design personalized targeted therapy according to different tumor types and individual patient conditions is one of the key issues to be solved in the current research on melittin targeted therapy.

4.2. Future research direction and prospect

4.2.1. Development of novel melittin derivatives

By modifying and modifying the structure of melittin, it is expected to design and develop melittin derivatives with higher activity, lower toxicity and stronger targeting, so as to improve its clinical application value. The structural alteration of melittin can enhance its physical and chemical characteristics, as well as its biological activity, by including certain functional groups or altering the amino acid sequence via chemical synthesis. The development of nanotechnology also provides new ideas and methods for the research and development of melittin derivatives. Encapsulation of melittin in nanoparticles, such as liposomes, polymer nanoparticles, gold nanoparticles, etc., can effectively protect melittin from degradation by enzymes in vivo and improve its stability and bioavailability. At the same time, the surface of the nanoparticle can modify various targeting molecules, such as tumor-specific antibodies, folic acid, polypeptides, etc., so that it can accurately deliver melittin to the tumor site, enhance the targeting, and reduce the side effects on normal tissues. Moreover, the nanoparticles facilitate the sustained release of the drug, extend the duration of melittin's action within the body, and enhance its anti-tumor efficacy.

4.2.2. Optimization of combination treatment strategies

Combination therapy is one of the important means to improve cancer treatment effect and overcome tumor drug resistance, and the combined application strategy of melittin and other therapeutic methods will be further optimized in the future [21]. On the one hand, continue to explore the combined application of melittin with traditional chemotherapy drugs, radiotherapy, targeted therapy drugs and immunotherapy drugs, through in-depth study of the synergistic mechanism between different drugs, to determine the best drug combination, dose ratio and administration sequence, in order to achieve the maximum synergistic effect, while reducing the side effects. On the other hand, it pays attention to individuation and precision of combination therapy. The response to combination therapy varies due to changes in tumor kind, stage, hereditary traits, and physical circumstances. Therefore, future research will be devoted to the development of individual combination therapy based on biomarkers, by detecting patients' specific biomarkers, such as tumor gene mutation, protein expression level, immune cell infiltration, and so on, to tailor the most suitable melittin combination therapy for patients, achieve precision medicine, and improve the effectiveness and safety of treatment.

5. Conclusion

In summary, melittin, the most active constituent of bee venom, exhibits multiple anti-tumor mechanisms, such as the direct eradication of tumor cells, the activation of tumor cell death, and immunomodulatory effects. It has demonstrated considerable anti-tumor efficacy in both in vitro and in vivo studies, offering new potential pharmaceuticals and therapeutic approaches for cancer treatment. However, the current research and application of melittin still face many challenges, such as stability and bioavailability problems, insufficient targeting and safety considerations in clinical application, which limit its further clinical transformation and application. Nevertheless, with the development of biotechnology, nanotechnology, drug synthesis technology and in-depth understanding of the biological mechanism of cancer, the future research of melittin has broad prospects. By developing novel melittin derivatives, optimizing combination therapy strategies, and conducting more clinical trials, it is expected to overcome current challenges and fully leverage the anti-tumor potential of melittin to bring more effective treatment options and better clinical outcomes to cancer patients.

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