Advances in Life Science for Adolescents

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EDITORIAL:

Research Article:

Hypothesis:

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Advances in Life Science for Adolescents

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Supporting Excellence in Youth Science

Voice From Adolescents

Why we found Advances in Life Science for Adolescents?

In recent years, adolescents have much more engagement in scientific and technological innovation in laboratories, universities, and research institutes. Governments around the world are strengthening the cultivation of young people's cutting-edge scientific literacy. However, these undergraduate, high school, and middle school students who enter laboratories are not full-time workers. After some training, they have developed their own innovations and progress in cutting-edge research topics. But their articles may not be published in traditional science journals due to the less data produced in limited working hours. Therefore, the editorial team aims to verify the achievements of adolescent innovation projects through author identity verification, ensuring that these contributions are recorded and promoted.

Why articles from adolescents should be published?

On one hand, adolescents possess highly active thinking patterns, and many creative ideas worthy of further exploration. On the other hand, since they are not subject to performance or graduation assessments, the likelihood of data fabrication in their research is relatively low. Although the data generated from part-time work may be insufficient for a comprehensive elaboration of their ideas, the authenticity of this data is high and deserves to be published and cited. Finally, we also hope to inspire adolescents' enthusiasm for scientific research and encourage more young people worldwide to engage in scientific learning through the publication of this international journal.

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"Advances in Life Science for Adolescents" is the first journal in this series. Based on Sciadolescent Press, we hope to ultimately establish a series of different journals for adolescents, covering natural and social sciences, consisting of multiple journals in life sciences (Advances in Life Science for Adolescents), social sciences (Advances in Social Science for Adolescents), physics (Advances in Physics and Astronomy for Adolescents), and other fields. Currently, we are starting with life sciences, aiming to accumulate experience in journal operation and funding for the press through this journal. We also aim to create innovative peer-review standards and find a suitable evaluation system for adolescents to publish their articles. In the future, we plan to gradually expand our journal system to include other scientific fields.

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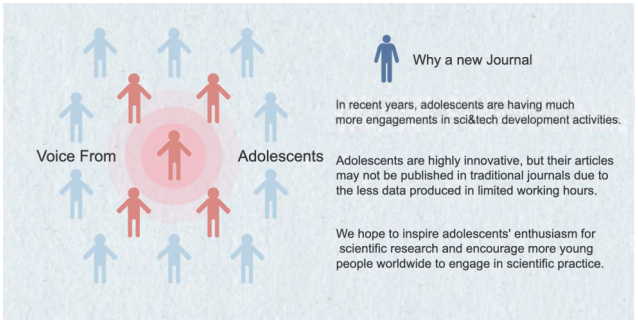

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Advances in Life Science for Adolescents

RESEARCH ARTICLES VOL 1 | Apr 2025 | doi:

Effect of CDAP on myeloid-derived suppressor cells

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Abstract:

The aim of this study is to investigate the influence of Cistanche deserticola polysaccharide (CDAP) on myeloid-derived suppressor cells (MDSCs) within the tumor microenvironment. MTT assays were used to assess the viability of MDSCs, normal fibroblasts (NFs), and cancer associated fibroblasts (CAFs) following exposure to various concentrations of CDAP. Additionally, RT–qPCR was used to measure the expression levels of VEGFA and TNF-γ in treated cells. Our results indicate that CDAP not only facilitates the growth of NFs while suppressing the occupancy of CAFs in the microenvironment but also diminishes the activity of MDSCs. Furthermore, CDAP-treated MDSCs exhibit upregulated TNF-γ expression alongside downregulated VEGFA expression. In conclusion, the traditional Chinese medicine polysaccharide CDAP exhibits a multifaceted capacity to enhance the tumor tissue microenvironment through its influence on various targets and pathways.

Keywords: *Cistanche deserticola* polysaccharide (CDAP); Myeloid-derived suppressor cells (MDSCs); Tumor microenvironment (TME); Cancer-associated fibroblasts (CAFs)

1. Background:

1.1 Tumor Microenvironment and Tumor Immunity

The tumor microenvironment has consistently been a focal point of oncological research. It serves to sustain the vigorous metabolism, proliferation, and differentiation of tumor cells as well as provide a microenvironment unfavorable for immune cell action. Nonneoplastic cells within the tumor microenvironment offer an aberrant nutritional milieu to nourish tumor cells and suppress the activity of immune cells, including NK cells and effector T cells, by secreting inflammatory factors and immunosuppressive agents. Thus, the cells in tumor tissues are essential, as they provide a conducive environment for the growth and proliferation of tumor cells [1].

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According to the cancer immune-editing hypothesis, the interplay between the immune system and tumor cells occurs in three phases: elimination, equilibrium, and escape. The establishment of a stable tumor microenvironment that favors tumor growth marks the transition to the second phase. Research suggests that disrupting such an aberrant environment is beneficial for eliminating tumor cells and inhibiting tumor progression [2].

1.2 The Role of Cancer-Associated Fibroblasts in the Tumor Microenvironment

Cancer-associated fibroblasts (CAFs) are an essential component of tumor tissues, providing mechanical support for tumor cells and shaping the entire tumor microenvironment. In comparison to normal fibroblasts, CAFs are spindle-shaped fibroblasts with a larger volume. CAF activity is regulated by growth factors secreted by tumor cells, and CAFs themselves can secrete growth factors such as fibroblast growth factor (FGF), stromal cell-derived

factor 1 (SDF1), matrix metalloproteinases (MMPs), and insulin-like growth factor 1 (IGF1). These factors promote further tumor growth, angiogenesis, and metastasis. Research has shown that the removal of fibroblasts from tumor tissue disrupts the tumor's immune escape mechanism, leading to a significant reduction in tumor size and cell death [3].

1.3 The Role of Myeloid-Derived Suppressor Cells in the Tumor Microenvironment

Myeloid-derived suppressor cells (MDSCs) are a type of cell widely present in the infiltrated and noninfiltrated tumor microenvironment of cancers such as liver cancer, breast cancer, and ovarian cancer. MDSCs are recruited from the bone marrow by various cytokines, such as VEGF, GM-CSF, M-CSF, IL-6, IL-1β, and β-fibroblast growth factor (β-FGF). [4] MDSCs migrate from the vascular and lymphatic systems to the tumor microenvironment, where they proliferate and secrete cytokines such as TGF-β, which inhibits the immune response mediated by T cells and B cells. Additionally, MDSCs promote tumor proliferation, infiltration, and metastasis and enhance tumor survival and migration. Existing research has shown a correlation between high levels of MDSCs in the tumor microenvironment and poor prognosis and shorter survival in cancer patients. Specific removal of MDSCs from the tumor microenvironment in mice has led to significant reductions in tumor volume, highlighting the importance of MDSCs in tumor survival and proliferation. Targeting MDSCs with drugs such as anti-GT-1 antibodies, anti-GM-CSF antibodies, PGE2/COX-2 inhibitors, CXCR2/4 antagonists, and RA190 has been demonstrated to enhance 'chemotherapy effectiveness to eradicate tumor cells. Notably, immunotherapies that target CXCR4 and IL-10, which are characteristic of MDSCs, have significantly improved the effectiveness of PD-1 monoclonal antibody therapy for ovarian cancer. However, mature targeted therapies with COX-2 inhibitors such as nimesulide, meloxicam, and celecoxib exhibit strong gastrointestinal side effects and kidney toxicity. Therefore, the discovery of effective, natural compounds with low toxicity to suppress MDSCs in tumors is of great significance [5].

1.4 The Bioactivity of Cistanche deserticola Polysaccharide

Traditional Chinese medicine offers descriptions of tumors and cancer that are similar to those in Western medicine, and it has developed a unique system for understanding the etiology, pathogenesis, and treatment of malignant tumors since ancient times. Many diseases are closely related to immune system dysfunction, and tumor development due to weakened immune surveillance is one such association. Most traditional Chinese medicines do not have direct toxic effects to tumor cells, but they can inhibit tumor growth and development by enhancing and improving the immune functions of patients [6].

Chinese herbal polysaccharides are natural extracts of traditional Chinese medicines that are characterized by natural ingredients, minimal toxicity and side effects, and stable and long-lasting therapeutic effects. The primary effective ingredients of Chinese herbal polysaccharides function mainly by increasing host antibody concentrations and boosting immune cell activity for immunomodulation. Empirical evidence has demonstrated that different doses of Astragalus polysaccharide can upregulate the thymus and spleen indices in mice and increase the concentrations of interleukin-2 (IL-2), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) in the serum, enhancing cell-mediated immunity and improving the immune surveillance capacity to inhibit tumor growth and development [7].

Cistanche deserticola (Fig. 1.a) is a yang-tonifying herb, and various historical texts, including Ben Jing and Yao Xing Ben Jing, have highlighted its efficacy in nourishing the viscera and inhibiting cancer development[8] .CDAP mainly containing active ingredients such as fucose, rhamnose, arabinose, et.[9] ,as shown in Fig. 1.a) Researches have proved those ingredients with natural anti-inflammatory, antifatigue effects[10] .We consulted the clinical database of traditional Chinese medicine and found that CDAP has been applied to various tumors, as shown in Fig. 1.c). Through bioinformatics analysis, CDAP has an influence in the breast cancer associated pathways, as shown in Fig. 1.b). However, the mechanism, dosage, and effectiveness of *C. deserticola* polysaccharide (CDAP) in anticancer treatment have not been verified by modern research. By Thus, in this experiment, the effects of CDAP on tumor microenvironment cells are studied from an immunological perspective.

2. Materials and Methods

2.1 Cell Lines: Myeloid-Derived Suppressor Cells (MSC2) and Hepatic Stellate Cells (LX-2)

The cellular resources employed in this investigation were procured from the Cell Repository at the Institute of Biophysics, Chinese Academy of Sciences. These resources encompassed myeloid-derived suppressor cells (MSC2), human hepatic stellate cells (LX-2), and human hepatic fibroblasts associated with breast cancer (ME-iLX-2). The LX-2 cell line, originated from hepatic stellate cells isolated from the livers of healthy adults. ME-iLX-2 cells were methodically generated within our laboratory by inducing hepatic stellate cells (LX-2) with TS/A breast cancer cells.

The cell culture medium was meticulously maintained utilizing high-glucose DMEM (Gibco, USA) supplemented with a 1:10,000 mixture of penicillin and streptomycin (Gibco) and enriched with 10% fetal bovine serum (FBS, Pan Biotech, Germany). Cultivation was carried out in a 37 °C incubator under a 5% CO2 atmosphere. After reaching confluence, the cells were detached with trypsin (Gibco). This was succeeded by a series of washes, centrifugation steps, and subsequent passages.

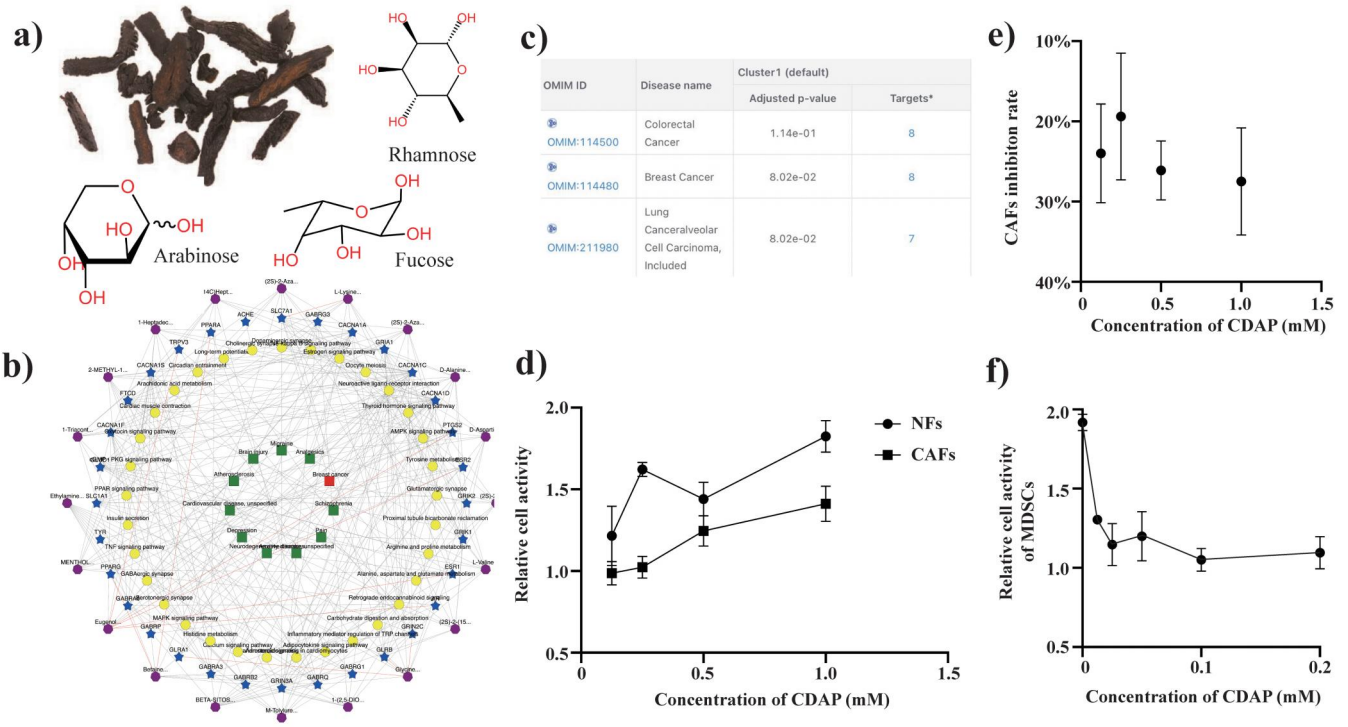

Fig. 1: a) Morphology of C. deserticola. **b)** pharmacological characteristics of Cistanche deserticola. **c)** Predicting targets of CDAP. **d)** Effects of CDAP on fibroblasts. **e)** CDAP suppresses CAF (ME-iLX-2 cell) viability. **f)** CDAP inhibits MDSC viability.

2.2 Extraction and Preparation of C. deserticola Polysaccharide (CDAP)

C. deserticola, a hallmark of traditional Chinese herbal medicine (depicted in Fig. 1.a), was used as the raw material for the extraction of CDAP. The preparation of CDAP was graciously performed by the State Key Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences. Extraction was performed by thermal water-ethanol precipitation, mainly include air-drying the C. deserticola at 40 ◦C and ground it into powder. Then treat the powder in ethanol(60◦C) for 3 h. Diluted the powder with water, refluxed at 90 ◦C, and centrifuged twice (1500× g) to separate the supernatant. Subsequently, 95% ethanol was added. 4◦C overnight and centrifuge (1500× g) twice. Repeat the cycle twice, resulting in 0.15% yield as well as 97.3% purity of polysaccharide. Following this, the resultant extract was subjected to phosphate-buffered saline (PBS) solution and subsequently passed through a 0.22 μm cell filter, culminating in the formulation of a 1 mg/mL stock solution that we denoted as CDAP.

2.3 MTT Cell Viability Assays

MTT experiments were executed with MSC2, ME-iLX-2, and LX-2 cells as the subjects of interest. Given the reduced proliferation of these cells, an augmented cell density was adopted for the initial seeding process. Specimens were judiciously sown within 96-well plates at a seeding density of 5×103 cells per well. Following a 12-hour incubation period at 37 °C under 5% CO2, the supernatant medium was meticulously aspirated. Then, 100 μL of the appropriate concentration of CDAP solution was introduced, and for an additional 24 hours. Following this incubation period, MTT solution was added, and the plates were subjected to an additional 4-hour incubation period at 37 °C. The formed formazan crystals were solubilized by the addition of lysis buffer (comprising 10 g of SDS, 5 mL of isobutyl alcohol, and 0.1 mL of 10 M HCl diluted to a final volume of 100 mL with double distilled water). After undisturbed incubation overnight to ensure complete dissolution, absorbance measurements were taken at a wavelength of 570 nm. The results obtained were normalized by PBS treatment group to determine relative cell viability, thereby offering insights into the cellular proliferation rates.

2.4 RT–qPCR analysis to Determine Relative mRNA Expression

After 72 hours of cocultivation of CDAP and cells in 6-well plates, total RNA was extracted with TRIzol reagent. The extracted RNA underwent reverse transcription into cDNA with the M-MLV reverse transcriptase system. Subsequently, primers targeting TNF-γ, VEGF, and GAPDH were used to perform real-time quantitative PCR (RT–qPCR). The housekeeping gene GAPDH was used as an internal reference. Subsequent data analysis was performed by determining the Ct values of the samples with Rotor Gene 5 software. These data allowed calculation of the relative VEGFA mRNA levels in the specimens through the utilization of the ΔΔCt method.

2.5 WB for protein expression analysis

LX-2, ME-iLX-2, MDSCs cells treated with CDAP for 72 hours were lysed on ice using RIPA lysis buffer (Biyuntian, China), and the collected proteins were quantified

using the BCA assay. Five times loading buffer was added to the remaining protein samples and incubated at 95°C in a metal bath for 10 minutes. Protein separation was performed by electrophoresis on a 12.5% polyacrylamide gel at 150V for 50 minutes. After electrophoresis, proteins were transferred to a PVDF membrane (Millipore, USA) using the wet transfer method at 100V for 70 minutes. After transfer, the membrane was blocked with 3% BSA in PBS-T (containing 0.5‰ Tween-20) for 1 hour and washed five times with PBS-T (five minutes each). The membrane was then incubated overnight at 4°C with the primary antibody. After washing with PBS-T, an HRP-conjugated goat anti-rabbit IgG (Abclonal, USA) was used as the secondary antibody (dilution 1:3000) and incubated at room temperature for 1.5 hours. Following PBS-T washing, the membrane was exposed using a chemiluminescent substrate. b-actin (Abclonal, USA) was used as an internal reference, diluted at 1:6000, and VEGFA (Bioss, China) was diluted at 1:2000.

2.6 Data Analysis and Graphical Representation

Statistical analysis was performed by IBM SPSS version 22. To visually display the findings, GraphPad PRISM 5 software was used. Batman V2.0 was involved for pharmacological research. Furthermore, Adobe Illustrator was used for image annotation.

3. Results

3.1 CDAP inhibits ME-iLX-2 cell growth

Different concentrations of CDAP solution was added to cells in 96-well plates, and relative cell viability was determined using the MTT reagent. The results showed that under the influence of CDAP, the viabilities of both CAFs (ME-iLX-2 cells) and NFs (LX-2 cells) normalized to the control group(PBS treated) increased, indicating that CDAP did not exhibit significant toxicity to fibroblasts, as shown in Fig. 1.d).

Furthermore, CDAP had a greater effect on promoting NF (LX-2 cell) proliferation than on CAF (B-iLX-2 cell) proliferation (22.62%). After normalizing the relative activities of NFs at the same concentration, the calculated CAF relative inhibition rate is shown in Fig. 1e).

3.2 CDAP Suppresses MDSC Growth

Similarly, different concentrations of CDAP solution were added to the culture system with MDSCs (MSC2 cells), and after treatment with the MTT reagent and measuring the light absorbance, it was found that CDAP had a significant, concentration-dependent inhibitory effect on MDSC activity, as shown in Fig. 1.f).

3.3 CDAP Upregulates TNF-γ Expression in MDSCs

The MDSCs (MSC2 cells) that survived after culture with different concentrations of CDAP for 72 hours were

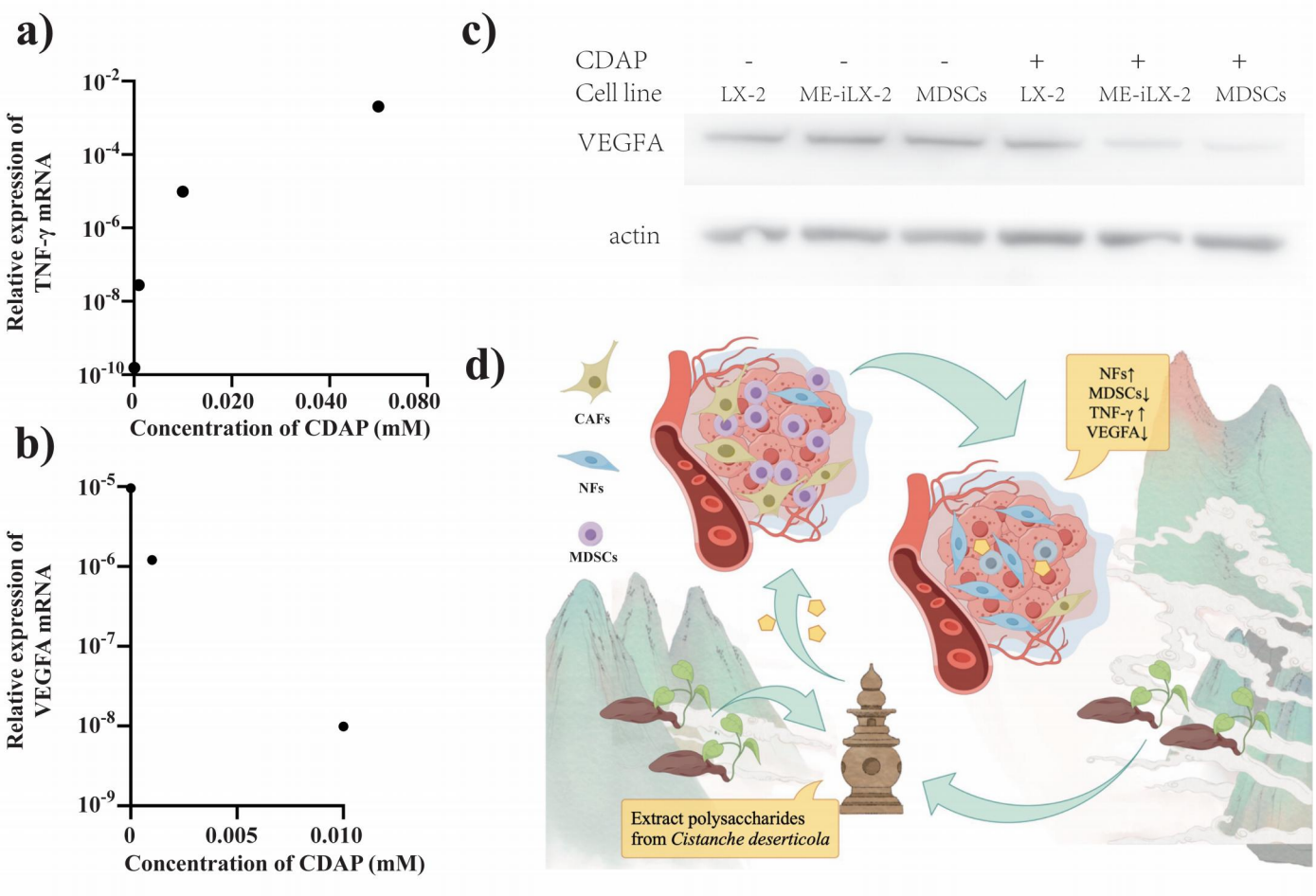

Fig. 1: a) Upregulation of TNF-γ by CDAP in MDSCs. **b)** Downregulation of VEGFA mRNA by CDAP in MDSCs. **c)** Downregulation of protein VEGFA by CDAP in MDSCs. **d)** The ability of CDAP to improve the tumor microenvironment.

used for this experiment. Total RNA was extracted from the samples using TRIzol total RNA extraction reagent, reverse-transcribed, and subjected to qPCR fluorescence analysis, which provided the cycle threshold (Ct) values for the corresponding gene in the samples. After processing, it was found that CDAP significantly upregulated TNF-γ mRNA in MDSCs in a concentration-dependent manner, as shown in Fig. 2a).

3.4 CDAP Downregulates VEGFA in MDSCs

Concurrently, the MDSCs and Me-iLX-2 that survived treatment with CDAP showed a significant decrease in VEGFA mRNA as well as protein expression, indicating that vascular endothelial growth factor A (VEGFA) was downregulated in these cells, but not in normal hepatic cells LX-2. This inhibitory effect was concentration dependent, as shown in Fig. 2b) & Fig. 2c).

4. Discussion

4.1 CDAP May Inhibit Self-Recruitment of MDSCs

Studies suggest that VEGFA is one of the important cytokines involved in the recruitment of MDSCs, and highly expressed VEGFA in MDSCs in the tumor microenvironment can create a positive feedback loop by recruiting more MDSCs and accelerating the deterioration of the tumor microenvironment. The results of this study suggest that CDAP may have a significant effect on breaking this cycle. On the one hand, CDAP can inhibit the proliferation of MDSCs, leading to a reduction in the number of MDSCs in the cell population. On the other hand, the MDSCs that survive treatment with CDAP lose their ability to express VEGFA, further reducing the recruitment effect of the VEGFA secreted outside the tumor tissue and reducing the recruitment of MDSCs.

Moreover, the decrease in the secretion of VEGFA can alleviate excessive angiogenesis in the tumor microenvironment and slow tumor proliferation, differentiation, and migration. It has been well documented that due to the high metabolic demands of tumor cells, tumors highly express VEGFA to increase the rate of angiogenesis, enhance the nutritional density in the local tissue, and promote rapid growth. By inhibiting the secretion of VEGFA by MDSCs, CDAP can somewhat alleviate these phenomena. Furthermore, VEGFA is associated with tumor cell survival and proliferation and acts downstream of VEGFR, regulating the ERK and MAPK pathways to ensure cell survival and promote cell proliferation. CDAP's ability to reduce VEGFA expression in the cells in the tumor microenvironment can slow these processes. Finally, VEGFR is associated with the reorganization of the cytoskeleton, the promotion of cancer cell infiltration and migration and acceleration of tumor development. CDAP may slow these processes by reducing the expression of VEGFA.

4.2 CDAP Suppresses Tumor Growth by Acting on MDSCs

Under the actions of CDAP, the density and activity of myeloid-derived suppressor cells (MDSCs) in the tumor microenvironment significantly decrease, as does the recruitment of new cells from the bone marrow. The above pharmacological effects indicate that the function of the MDSC population decreases along with the concentration of immune inhibitory factors (TGF-β, IL-10, etc.) in the tumor microenvironment, allowing the immune system to no longer be restrained by suppressive cells and increasing the toxicity of immune cells to tumor cells, thus inhibiting tumor growth. Moreover, under the action of CDAP, TNF-γ expression in MDSCs is significantly increased, initiating the tumor cell TRADD protein complex and transmitting signals to caspase 3/7 via caspase 8/10 to initiate tumor cell apoptosis. Therefore, MDSCs that survive treatment with CDAP may not possess their original protumor growth function but may instead exhibit antitumor growth and anticancer functions.

4.3 CDAP Acts on Fibroblasts to Suppress Tumor Development

On the other hand, normal fibroblasts (NFs) in the tumor microenvironment, when under the influence of CDAP, showed increased activity, indicating that CDAP had no significant toxicity to normal cells. This ensures that under the influence of CDAP, normal human tissues can still grow and proliferate normally. However, CDAP displayed a weaker effect on promoting tumor-associated fibroblasts (CAFs), considering the competitive transformation relationship between NFs and CAFs. It can be concluded that CDAP indirectly inhibits the biological activity of CAFs, helping normal fibroblasts (NFs) occupy and improve the tumor microenvironment.

In summary, CDAP not only significantly reduces the population density of MDSCs, causing cells to lose their protumor activity, but also inhibits CAF activity and promotes NF growth. This demonstrates that CDAP, a traditional Chinese medicine polysaccharide, can improve tissue microenvironments as a multitarget, multipath agent.

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HYPOTHESIS VOL 1 | Apr 2025 | doi:

Hypothesis and Evidence: Immune Cell Therapy Might Yield Poor Outcomes in Patients with Diabetes

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Abstract:

Immune cell therapy has rapidly emerged as a novel strategy for cancer treatment, particularly with the development of chimeric antigen receptor (CAR) immunotherapy. However, the clinical efficacy of CAR-T, CAR-NK, and CAR-M therapies remains controversial in patients with metabolic diseases. Diabetes mellitus (DM) is one of the most prevalent metabolic diseases, with studies suggesting that patients with diabetes often develop chronic inflammation and a deteriorated tumor microenvironment (TME), leading to an increased prevalence of tumors. This review summarizes the pathways and molecular mechanisms underlying the deterioration of the tumor immune microenvironment in DM. It highlights the upregulation of reactive oxygen species, fatty acids, HIF-1 & VEGF, O-GlcNAc, TGF-β1, EGF, PGE2, and IGF-1 levels; downregulation of HIPK2 levels; and promotion of a pro-inflammatory bias in microRNA and exosome expression profiles. These changes downregulate immune cell activity through various mechanisms, including signal crosstalk, metabolism, and cellular interactions, thereby influencing the clinical efficacy of CAR-T, CAR-NK, and CAR-M therapies. Additionally, potential drugs targeting these pathways are proposed, offering new insights for improving the TME in patients with diabetes patients and enhancing the efficacy of immune cell therapy.

Keywords: Diabetes Mellitus (DM), Immune therapy, CAR-T therapy, CAR-NK therapy, CAR-M therapy, Tumor microenvironment (TME).

1. Background:

1.1 Immune Cell Therapy as a Novel Strategy in Cancer Treatment

In recent years, immune cell therapy has emerged as a novel strategy in treating cancer and autoimmune diseases, particularly with developing chimeric antigen receptor (CAR) immunotherapy. Anti-tumor immune cell therapies primarily include CAR-T, CAR-NK, CAR-M, NKT, and γδT cell therapies. CAR-T cell therapy involves modifying patients' T cells to target and kill tumor cells by adding receptors that enable T cells to recognize specific tumor antigens. Five CAR-T therapies targeting B-cell markers, such as CD-19 and BCMA, have been approved by the FDA for treating refractory hematologic malignancies [1]. Clinical trials targeting various solid tumor-expressed antigens (e.g., HER2, interleukin-13Rα2 [IL-13Rα2], and GD2) have shown positive outcomes. For example, a clinical study targeting HER2-positive sarcomas included 17 evaluable patients. Four patients experienced stable disease for several months following CAR-T cell therapy, three undergoing tumor removal, and over 90% tumor necrosis observed in one case [2]. However, CAR-T therapy demonstrated less favorable outcomes in the remaining 10 patients.

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Despite significant advancements in CAR-T cell therapy, its efficacy may not always meet "reliable" standards for some patients with metabolic diseases.

CAR-NK cell therapy, which uses the cytotoxicity of natural killer (NK) cells, has shown a lower risk of cytokine release syndrome and neurotoxicity, with broader sourcing and faster preparation than CAR-T cells. Nevertheless, clinical trials have revealed that only 60% of evaluated patients with refractory acute myeloid leukemia achieved complete remission when treated with CAR-NK cells targeting CD33 derived from umbilical cord blood [3]. CAR-M therapy has also garnered attention. Macrophages, as key cells in the immune system, can recognize tumor cells through CAR and modulate the tumor microenvironment (TME) to activate additional immune cells in the anti-tumor response. Although CAR-M therapy shows promising prospects for solid tumor treatment, it is still in its early stages, with only one clinical trial initiated [1]. In summary, immune cell therapy does not guarantee favorable outcomes for all patients, and its lower efficacy remains a subject of scrutiny. The reasons for such variability are not yet fully understood. Observations suggest that some metabolic diseases may downregulate immune cell activity and diminish the effectiveness of cell therapy, particularly in conditions such as obesity and diabetes mellitus (DM) [4, 5]. Therefore, this review discusses the effects of immune cell therapy in patients with diabetes by analyzing the impact of diabetes-induced TME on immune cells.

1.2 Impact of Hyperglycemia on Immune Cell Function

DM is one of the most prevalent metabolic diseases globally, affecting approximately 150 million individuals, with developing countries exhibiting an 11.2% prevalence rate [6]. Recent research suggests that DM is associated with immune dysregulation. Elevated glucose metabolism in immune cells activates Toll-like receptor signaling pathways, leading to increased expression of IL-1β via the NF-κB pathway [7]. Additionally, NOD2 activation releases IL-1β and IL-18, initiating inflammatory responses in patients with diabetes [8]. Evidence indicates hyperglycemic conditions can increase reactive oxygen species (ROS) production, pro-inflammatory mediators, cell apoptosis, and fibrosis [9-11]. Moreover, bone marrow-derived cells (BMDCs) in patients with diabetes tend to express higher levels of tumor necrosis factor-α (TNF-α), contributing to inflammation [12, 13]. These pro-inflammatory signaling pathways support the concept of meta-inflammation, characterized by low-grade chronic inflammation, which is linked to the pathogenesis of diabetic complications [14, 15]. Chronic inflammation is recognized as a major factor in cancer development [16-18]development, establishing a connection between DM and tumor occurrence. Research indicates that cancer cases associated with high body mass index and diabetes are twice as common, with six cancers closely linked to diabetes [19, 20]. Despite the significant impact of diabetes on the immune system, research on the efficacy of cell therapy in patients with diabetes remains limited.

2. DM is Associated with a Deteriorated TME

The TME comprises cellular and non-cellular components embedded in the extracellular matrix (ECM), including cytokines, growth factors, enzymes, proteoglycans, and glycoproteins. These components facilitate proper communication and development of tumor tissue. Deterioration of tumor conditions in patients with diabetes is frequently observed. Hyperglycemia and advanced glycation end-products (AGEs) activate mast cells and increase the expression of pro-inflammatory cytokines, such as TNF-α, which promotes mast cell degranulation and forms an inflammatory microenvironment conducive to tumor development [21]. DM is associated with elevated TNF-α expression in BMDCs in gastric and renal cancers, contributing to inflammation rather than repairing damaged gastric mucosa [13, 22]. These infiltrating immune cells are crucial in reshaping the TME [23, 24]. A key feature of the TME is hypoxia, which induces functional changes in the cells within this environment [25, 26]. Patients with diabetes often experience more severe tumor hypoxia than non-diabetic patients due to their unique metabolic conditions. Low oxygen levels stimulate the proliferation of regulatory T cells via the HIF-1α pathway, inhibiting the differentiation of effector T cells [27-29] and leading to T cell exhaustion and the formation of terminally exhausted T cells [30]. Hypoxia also promotes the recruitment of myeloid-derived suppressor cells (MDSCs) to the tumor transformation site, ultimately promoting tumor growth and suppressing anti-tumor immunity [26].

Evidence supports that remodeling and stiffening of the tumor-associated ECM are key factors behind the highly invasive phenotype of the TME [31-33]. This remodeling supports cancer cell survival, progression, and metastatic invasion [34, 35]. Fibronectin and type I collagen are the most common and abundant fibrous components in cancer-related ECM [36-38]. Their increase, resulting from excessive fibrosis primarily mediated by α-smooth muscle actin-expressing myofibroblasts, contributes to ECM stiffening [39, 40]. Most ECM fibrous proteins exhibit higher levels of AGEs, which are long-lived targets in DM and chronic hyperglycemia [41]. The cross-linking of AGEs with load-bearing proteins leads to ECM stiffening, favoring cancer cell survival and proliferation and promoting interactions between metastatic cancer cells and endothelial cells [42]. Chronic hyperglycemia generates a reservoir of AGEs, which may trigger various receptors for advanced glycation end products (RAGE)-dependent mechanisms, further deteriorating the TME [41]. Recent studies have shown that epithelial-mesenchymal transition is significantly enhanced in a high-glucose environment. In summary, DM can deteriorate the TME and accelerate tumor progression by suppressing anti-tumor immune activity.

3.Influence of DM on immune cell Anti-Tumor Resposes

Following observations that DM exacerbates the TME, we focused on understanding how these microenvironments affect the anti-tumor activity of immune cells.

We aimed to identify the pathways responsible for the diminished efficacy of cellular therapies in diabetes-aggravated tumors. We discovered that DM is associated with the upregulation of ROS, fatty acids, HIF-1α & VEGF, O-linked protein β-N-acetylglucosamine (O-GlcNAc), TGF-β1, EGF, PGE2, and IGF-1 levels in the TME, whereas homeodomain-interacting protein kinase 2 (HIPK2) levels were downregulated. These changes have been shown to inhibit various immune cells. Additionally, microRNA (miRNA) and exosome expression profiles in patients with DM exhibited an immune-suppressive skew. Consequently, these factors limit the efficacy of CAR-T, CAR-NK, and CAR-M therapies, as shown in the figure below.

3.1 High Glucose Inhibits Immune Cells in the TME

High blood glucose promotes glycolysis in tumor cells [43, 44]. Recent studies have indicated elevated glycolysis levels in tumor cells can suppress T-cell function via the TNF-α pathway [45]. In patients with diabetes, chronic high glucose levels may directly inhibit NK cells through NAD metabolism, resulting in reduced NK cell cytotoxicity [46].

Simultaneously, high blood glucose levels induce the upregulation of calpain in the mitochondria, which leads to reduced ATP synthase activity and increased mitochondrial ROS formation[47]. Mitochondrial ROS plays a crucial role in stabilizing hypoxia-induced transcriptional profiles, as it can activate pathways that support tumor angiogenesis [48] and promote myofibroblast deterioration[49]. Additionally, ROS contributes to the generation of diabetes-specific “ox-inflammation”[50]. This “ox-inflammation” includes the downregulation of Bcl2 expression, which leads to T cell apoptosis[51], and the downregulation of TCRζ-CD16ζ-chain expression in NK cells and T cells. This downregulation reduces NF-κB activity, resulting in decreased secretion of interferon-γ, TNF-α, and IL-2, ultimately impairing the efficacy of T-cell[52, 53] and NK cell therapies[54, 55].

In tumor cells, high glucose levels can promote HIF-1α expression under both normoxic and hypoxic condition[47, 48], enhancing the expression of several oncogenes. This leads to increased expression of angiogenic factors such as VEGF and HIF-1 and the inhibitory factor PD-L1 [49]. Furthermore, high glucose levels contribute to macrophage M2 polarization [50], reduce T cell activity and infiltration [51], promote regulatory T cells (Treg) differentiation [52], and reduce cytotoxic T cell activity [53], thereby decreasing the efficacy of T cell therapy. Additionally, high glucose, in synergy with hypoxia, sensitizes macrophages to cytokine stimulation [54] and generates an M2-skewed M1/M2 cytokine spectrum [55]. Hypoxia-induced VEGF activates VEGFR, leading to phosphatidylserine exposure on

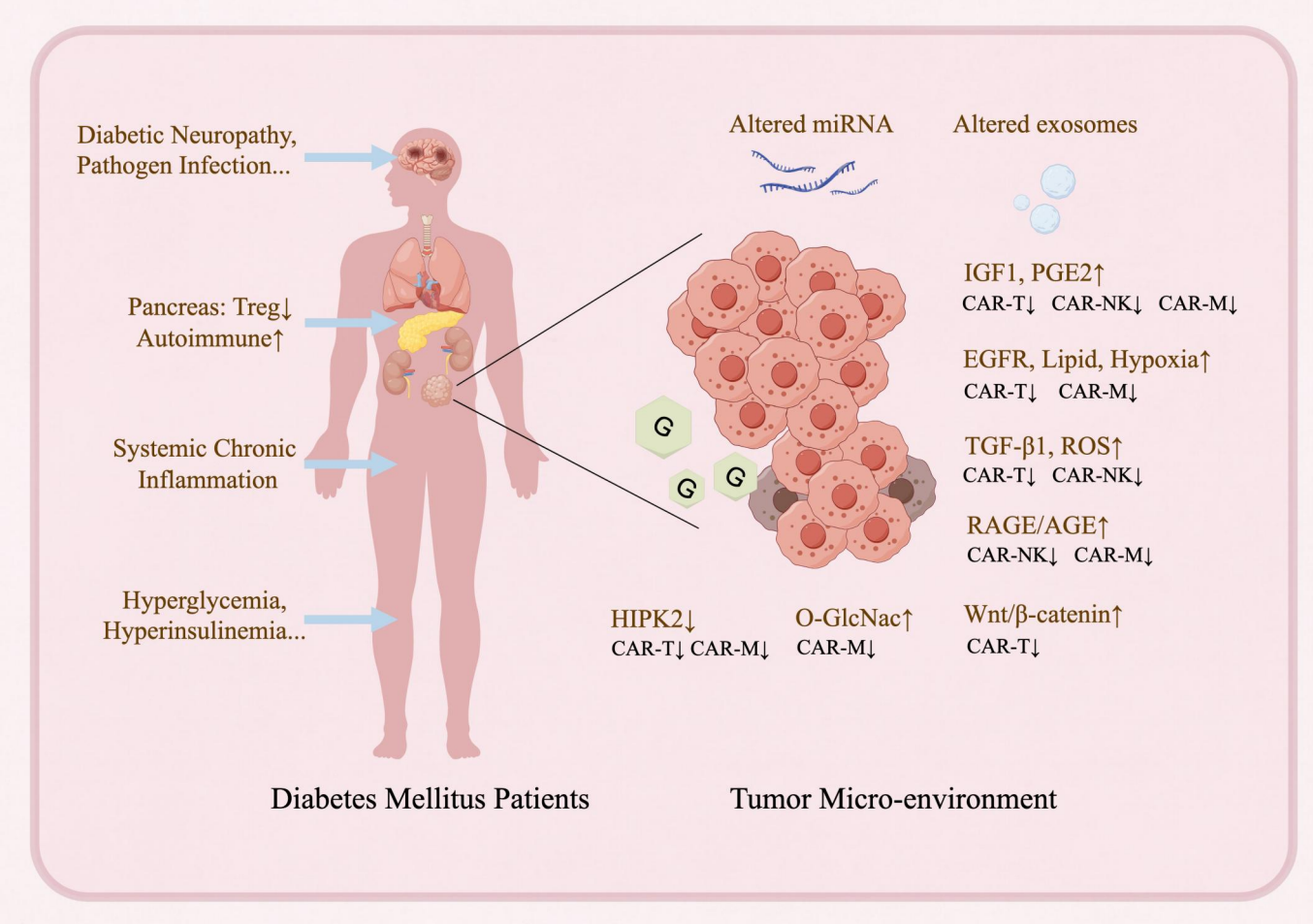

Fig. 1: Influence of DM on Immune cell therapy in Anti-tumor response, G refer to higher Glucose level in the patients with DM.

exosomes and cell membranes, which results in an immune-suppressive microenvironment by recruiting MDSCs, T-regs, and blocking the functions of NK, NKT, CD8+, and CD4+ cells [56].

3.2 High Glucose Upregulates O-GlcNAc Modification

O-GlcNAc modification is a dynamic post-translational modification that influences various proteins involved in cell cycle regulation and is a major contributor to the harmful effects of high blood glucose[66]. This modification includes adding an N-acetylglucosamine molecule to the hydroxyl residue of serine or threonine via O-GlcNAc transferase. In a high-glucose environment, this process leads to the O-GlcNAcylation of oncogenic factors and other cell cycle regulatory factors in tumor cells[67-69]. Recent studies have reported that high blood glucose increases the flux of the hexosamine biosynthesis pathway in tumor-associated macrophages (TAMs), resulting in upregulation of O-GlcNAc, activation of macrophage p56 and IκB, which leads to decreased macrophage activity[70], and induction of M2 polarization[71]. Upregulated O-GlcNAc reprograms active macrophages into TAMs[72-74] and reduces anti-tumor immunity[73].

3.3 Chronic High Glucose Accelerates RAGE/AGE Accumulation

Chronic high glucose is a hallmark of diabetes. It accelerates the formation of AGEs, a heterogeneous group of compounds formed by non-enzymatic reactions between carbohydrates and amino groups of proteins, lipids, and nucleic acids [57, 58]. All cellular components of tumor cells and the tumor stroma express RAGE. Increasing evidence supports the role of the RAGE/AGE axis in promoting tumor growth, with high glucose significantly enhancing RAGE expression. Consequently, the behavior of various cell types in the TME is influenced by diabetes or hyperglycemia. Patients with diabetes exhibit high rates of AGE formation, leading to overexpression of RAGE and overactivation of the RAGE/AGE axis [59]. Activation of this signaling pathway is a significant factor in inflammation-related tumorigenesis through mechanisms such as hypoxia, apoptosis resistance, anti-tumor immunity, angiogenesis, and tumor invasion [60, 61]. Specifically, the RAGE-HMGB1 pathway is believed to decrease the activity of monocytes, macrophages, and NK cells [62, 63]. At the same time, the RAGE-DAMPs/PAMPs axis interacts with the gut microbiota, promoting acute immune reactions in patients undergoing immunotherapy and leading to additional complications [64, 65], which may be more severe in patients with diabetes. Therefore, the upregulation of RAGE/AGE and its downstream pathways threaten the efficacy and safety of innate immune cell therapies for patients with diabetes.

Moreover, activating the RAGE/AGE axis in diabetic tissues and cells significantly increases the expression of COX-2 mRNA and protein and the levels of PGE2 [66]. The COX-2/PGE2-mediated signaling pathway is a key factor in diabetes-related complications such as hypertension and atherosclerosis [67]. This pathway also plays a crucial role in the inflammatory niche of gastric and colorectal cancers [68]. Downstream pathways mediated by PGE2 support tumor growth, metastasis, and neovascularization [69]. Additionally, PGE2 can directly inhibit NK cell anti-tumor activity [70, 71] and suppress T cell activity via dendritic cells, leading to the failure of CAR-T therapy [72], recruitment of MDSCs cells [73] and reduced macrophage activity [74, 75].

3.4 High Glucose Upregulates the Wnt/β-Catenin Pathway

Increasing evidence highlights the significant role of the Wnt/β-catenin pathway in the onset, progression, and metastasis of gastric cancer [76]. High glucose levels can affect the Wnt/β-catenin pathway in cancer cells by continuously enhancing the activity of histone acetyltransferase p300 or suppressing the activity of sirtuin-1, which leads to increased expression of Wnt target genes [77-79]. Consequently, high blood glucose results in elevated levels of β-catenin acetylation, allowing for nuclear accumulation and transcriptional activation of Wnt target genes [80]. This process ultimately leads to decreased T-cell infiltration and limits the efficacy of CAR-T therapy [81].

3.5 High Glucose Promotes TGF-β1 Activity

The TGF-β1 signaling pathway is excessively active in patients with diabetes[100, 101], and its role as a key fibrosis-promoting factor in the progression of diabetic nephropathy is well-documented[101-103]. Extensive research has demonstrated that TGF-β plays a crucial role in gastrointestinal cancer biology, influencing tumor progression; evasion of growth inhibitors; and resistance to apoptosis, angiogenesis, invasion, and metastasis[100, 104, 105]. TGF-β1 can induce T cell dysfunction[106], decrease CD8+ T cell infiltration[107], recruit MDSCs[108], and inhibit NK cell activity[109]. These factors contribute to the potential failure of cell therapies in a high-glucose environment.

3.6 High Glucose Activates the Epidermal Growth Factor Receptor (EGFR) Pathway in the TME

In vivo studies demonstrate that post-translational modifications of the ECM occurring during high blood glucose periods favor the invasion of cancer cells by activating the EGFR signaling pathway [82]. This mechanism is similar to that observed in diabetic nephropathy, a common microvascular complication of diabetes. The interaction between glycated ECM and increased EGFR activity may be a key factor in enhancing cancer cell invasion in patients with DM. Activation of the EGFR signaling pathway promotes cell proliferation, inflammation, and ECM remodeling, all associated with progressive cancer in patients with DM [83]. Notably, several ligands or other biological mediators can activate these signaling pathways, such as ROS[84,85], TGF-β[86], and PKC[87], all upregulated in DM. Studies have shown that upregulation of downstream EGFR

pathways, such as ERK and AKT, leads to upregulation of cell surface CD47, resulting in tumor cell resistance to macrophages and T cells [88-90].

3.7 High Glucose Downregulates HIPK2

Desmoplasia, or connective tissue hyperplasia, is common in patients with diabetes due to hyperglycemia, which activates the fibrotic pathway by directly stimulating the synthesis of ECM components and triggering the transformation of epithelial and endothelial cells into myofibroblast-like phenotypes. HIPK2 has been identified as a critical negative regulator in this process; its downregulation promotes tumor progression [91][118]. Hyperglycemia leads to the sustained degradation of HIPK2 [92], promoting the differentiation of epithelial and stromal cells into cancer-associated fibroblasts (CAFs). CAFs are known for their immunosuppressive properties, significantly enhancing epithelial-mesenchymal transition in hyperglycemia [93]. HIPK2 also exerts an “anti-inflammatory” effect by inhibiting NF-κB activation and phosphorylating HDAC3 in macrophages [94, 95]. Its downregulation has been reported to inhibit DC cells directly, regulate T helper cell activity [96], inhibit cytotoxic T cells [95], and contribute to tumor immune escape [97]. Consequently, some studies have proposed HIPK2 as a marker for immune escape in breast cancer [98], noting its downregulation in patients with DM.

3.8 High Blood Glucose Concurrent with High Lipid Metabolism TME

High blood glucose promotes glycolysis by inducing the expression of glycolysis-related genes [43, 44], which leads to increased ATP production [99]. This effect exerts a greater inhibitory impact on immune cells than the Warburg effect and is associated with higher lipid concentrations in the TME.

Furthermore, high blood glucose enhances the expression of the carbohydrate-responsive element-binding protein (ChREBP), a known promoter of lipogenesis [100, 101]. In patients with diabetes, lipids in the TME are transported into effector T cells via lipid transporters such as CD36 and Mincle. This leads to oxidative stress in effector T cells, resulting in T cell dysfunction and ferroptosis [102, 103]. However, this high-lipid environment does not similarly affect Tregs [104, 105]. Tregs upregulate the expression of glutathione peroxidase 4, which inhibits ROS accumulation and ferroptosis [105-107]. Therefore, the high-lipid environment induced by diabetes may reduce the efficacy of CAR-T cell therapy. Additionally, the high-lipid microenvironment increases lipid intake and fatty acid oxidation in macrophages due to elevated levels of CD36, leading to ROS production. This promotes M2 polarization of macrophages and prolongs the survival of M2 macrophages while shortening the lifespan of M1 macrophages via the JAK1-STAT6 pathway, potentially impacting the efficacy of CAR-M therapy [108].

3.9 Hyperinsulinemia Directly Inhibits the Immune Response in TME

Hyperinsulinemia is closely associated with type 2 diabetes [109]. Emerging data suggest insulin may be a key regulatory factor in certain human tumors, leading to tumor immune escape in gastric cancer[110], breast cancer[112,115,116], prostate cancer[113,114]. This process enhances tumor proliferation[111] and anti-apoptotic programs [117]. Upon binding, insulin-like growth factor 1 receptor activates the PI3K pathway [118], which results in the downregulation of CD8+ T cell infiltration [119], recruitment of MDSCs [120], TAMs [121], and inhibition of NK cell activity [122, 123].

3.10 High Glucose Induces Changes in miRNA Expression Profiles

miRNAs are small, non-coding RNAs that act as post-transcriptional regulators of gene expression [124]. They are involved in various cellular processes such as cell growth, differentiation, development, and apoptosis across many cancer types [125] including leukemia[126], cervical cancer[127,128] and so on. Hypoglycemia and hyperinsulinemia in patients with DM patients lead to significant changes in miRNA expression profiles, which can possess tumor-promoting characteristics. Tumor suppressor miRNAs, such as miR-497, miR-495p, and miR-203, are downregulated, potentially inhibiting tumor cell proliferation and migration, thereby suppressing immune cell activity in the TME [129, 130]. Additionally, the downregulation of the Let-7 miRNA family is associated with malignancies [131]. Let-7 miRNAs are downregulated under hyperglycemic conditions and insulin resistance [129], leading to the upregulation of PD-L1 and other immune inhibitory factors [132], which may adversely affect immune cell therapy.

3.11 Exosome Skewing Promotes Inflammation in the TME of Patients with Diabetes

Exosomes are submicron-sized extracellular vesicles involved in intercellular and interorgan communication [133]. They participate in the pathogenesis of various diseases, including inflammatory diseases and cancer [134, 135], and can inhibit immune cell activity in cancer [136]. Evidence indicates that chronic hyperglycemia or hyperinsulinemia alters the molecular cargo of exosomes and changes their secretion levels. These alterations induce significant changes in exosome function, enhancing crosstalk between tumor and non-tumor cells [137]. Recent studies have shown that exosomes released by patients with diabetes exhibit pro-inflammatory cargo skewed characteristics, which reduce the activity of relevant immune cells in the microenvironment. This pro-inflammatory shift may contribute to the reduced efficacy of cell therapy in patients with diabetes [137].

4.Potential Adjuvants for Immune Therapy in Patients with DM

The targets mentioned above may contribute to the poor prognosis observed in patients with diabetes undergoing immune cell therapy. Based on these findings, we have identified several drugs that could be combined to improve the precision of immune therapy for patients with diabetes. Notably, these targets and pathways are derived from literature and primarily offer ideas and directions for future research. Additional molecules and clinical mechanisms remain to be explored.

To address glucose and ROS upregulation in the TME of patients with diabetes, nanoenzymes such as copper nanoparticle-based POD-mimicking nanozymes have gained attention for their effectiveness in clearing ROS from tissues [138]. These nanozymes can significantly reduce ROS levels in the TME, enhancing CAR-T efficacy [139]. Additionally, silicon dioxide nanoparticle-encapsulated CaO2 and MnO2-based nanozymes (combined iron-manganese-based nanozymes) are believed to help downregulate tumor hypoxia, M2 macrophage polarization, and Treg activity, while activating T cells [156]. For CAR-NK cell therapy, IL-15 co-treatment may mitigate ROS-induced damage to NK cells, thereby improving CAR-NK therapy efficacy in patients with diabetes [140]. Furthermore, drugs targeting SLC1A5, SLC3A2, and SLC7A5 (e.g., IGN523 [141], JPH203 [141], V-9302 [157]) have shown significant potential in strengthening anti-tumor immunity in the TME [158].

In cases of hyperlipidemia induced by hyperglycemia in the TME, recent studies indicate that the ChREBP inhibitor SBI-993 can effectively reduce lipid content and enhance T cell activity, improving the efficacy of PD-L1 monoclonal antibody therapy [142]. GLUT inhibitors such as BAY-876 and fasentin can also achieve similar effects [143]).

For severe hypoxia and highly activated HIF pathways in the TME of patients with diabetes, studies have shown that the hypoxia-activated agent TH-302 can significantly enhance the efficacy of anti-PD-L1 and anti-CTLA4 therapies [144]. Additionally, HIF-1α inhibitors can serve as adjuvants to improve immune cell therapy efficacy [144, 145, 159].

Regarding the highly activated RAGE/AGE pathway in the TME of patients with diabetes, 4'-methoxyresveratrol can inhibit the inflammatory microenvironment induced by RAGE/AGE activation, potentially serving as an effective adjuvant to enhance immune therapy efficacy in patients with diabetes [146, 160, 161]. However, its clinical safety, dosage, and other aspects require further validation.

For the downregulated HIPK2, anti-tumor drugs such as cisplatin, adriamycin, and roscovitine have been reported to activate the HIPK2/p53Ser46 apoptotic signaling pathway and upregulate HIPK2 concentration in the TME, which could be beneficial for treating patients with diabetes with immune cell therapy [147].

In the case of upregulated O-GlcNAc in patients with diabetes, studies suggest that OGT and OSMI-1 can alleviate this process [148, 162]. However, whether these adjuvants can enhance immune therapy efficacy remains to be further investigated.

For the upregulation of the Wnt signaling pathway in patients with diabetes, the highly specific Wnt pathway inhibitor ICG-001 has effectively enhanced T cell infiltration and cytotoxicity[149,150], thus improving immune therapy efficacy [81, 163].

Regarding the upregulation of the TGF-β signaling pathway, various mature TGF-β inhibitors may enhance immune therapy efficacy in patients with diabetes [164]. Additionally, compounds such as Cu(sal)phen [151] and ST80 [152] targeting TGF-β could be potential adjuvants [153].

Tab1. DM may deteriorate the TME and inhibit immune therapies

pathways	DM patients	Innate immune therapy			potential adjuvants	Ref.
		CAR-T	CAR-NK	CAR-M		
Glucose & ROS	↑	↓	↓		Nanoenzymes, IL-15, JPH203, IGN523	[138] [139] [140] [141]
Fatty acid	↑	↓	↓	↓	SBI-993, BAY-876, fasentin	[142] [143]
HIF1&VEGF	↑	↓		↓	TH-302, HIF1a inhibitors	[144] [145]
RAGE/AGE	↑		↓	↓	4'-Methoxyresveratrol	[146]
HIPK2	↓	↓		↓	cisplatin, roscovitin, adriamycin	[147]
O-GlcNAc	↑			↓	OGT, OSMI-1	[148]
Wnt/β-catenin	↑	↓			ICG-001	[149] [150]
TGF-β1	↑	↓	↓		Cu(sal)phen, ST80	[151] [152]
EGFR	↑	↓		↓	DRD1	[153]
PGE2	↑	↓	↓	↓	DP1, DP2, EP4	[154]
IGF1	↑	↓	↓	↓	CT102	[155]
miRNA	alterned					
Exosomes	alterned					

doi:

expression of EGFR associated with diabetes, DRD1 can inhibit its activity, improving the efficacy of cell therapy [153].

In response to high PGE2 concentrations in the TME induced by hyperglycemia, various anti-inflammatory drugs, including aspirin and COX2-specific inhibitors, are commonly used. Additionally, analogs of PGE2, PGF2α, and PGI2, TP antagonists, and antagonists of DP1, DP2, and EP4 are undergoing clinical evaluations for various indications [154, 165].

Finally, for upregulated IGF in patients with diabetes complicated by hyperinsulinemia, studies have shown that CT102 can serve as an adjuvant to promote tumor therapy by reducing the expression of inhibitory signals such as PD-L1 on tumor cell membranes and enhancing the prognosis of CAR cell therapy [155].

5. Conclusion and Future Directions

High glucose levels in patients with DM not only promote chronic inflammation, increasing cancer prevalence but also exacerbate immunosuppression within the TME. This results in the upregulation of various factors, including ROS, HIF-1 & VEGF, O-GlcNAc, TGF-β1, EGF, PGE2, IGF1, and fatty acid levels, alongside the downregulation of HIPK2 levels. Additionally, high glucose promotes a pro-inflammatory bias in miRNA and exosome expression profiles, potentially leading to the failure of immune cell therapy.

The targets and drugs discussed have shown potential to enhance the efficacy of immune cell therapy in patients with DM by mitigating the anti-immune effects of the high-glucose TME. However, due to the limited data available on CAR cell therapy, specific adjuvants targeting metabolic disorders have not yet been developed despite their significant potential to improve the efficacy of immunocytes. A successful treatment might yield poor outcomes simply due to the patient's underlying metabolic disease.

The efficacy of CAR therapy has improved significantly since its inception by Steven A. Rosenburg in the 1990s. The next step in advancing CAR therapy may involve the development of adjuvants tailored for the precise treatment of patients with specific metabolic disorders. Given that over 11% of the population develops DM, there is a pressing need for further research to identify effective and safe adjuvants for immune cell therapy. Such advancements are crucial for creating successful tumor treatments in heterogeneous patient populations.

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Research Progress on the Role of PKM2 in the Cell Cycle of Tumor Cells and Immune Cells

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Abstract:

This article comprehensively discusses the important role of pyruvate kinase M2 (PKM2) in cell cycle regulation, immune cell function, and cancer treatment. As a multifunctional protein, PKM2 plays a key role in cell metabolism, signal transduction, and transcriptional regulation, affecting biological processes such as cell proliferation, differentiation, and polarization. We conduct an in-depth analysis of the mechanisms by which PKM2 influences cell cycle checkpoints, Th17 cell differentiation, and macrophage polarization, and explore its potential value in cancer therapy. Finally, we look forward to future research directions to more fully reveal the biological functions and clinical application prospects of PKM2.

Keywords: PKM2, Cell Cycle, Tumor immunity, Cell metabolism

1. Background:

1.1 PKM2

Pyruvate kinase (PK) is one of the key enzymes in the glycolytic pathway, catalyzing the final step of glycolysis where phosphoenolpyruvate is dephosphorylated to pyruvate. There are four isoforms of pyruvate kinase: PKL, PKR, PKM1, and PKM2. PKL is expressed in the liver, PKR in red blood cells, PKM1 is highly expressed in normal tissues, and PKM2 is also expressed to some extent in normal tissues. In normal cells, M2-type pyruvate kinase (PKM2) exists in three forms: monomer, dimer, and tetramer. Tetrameric PKM2 has higher pyruvate kinase activity than dimeric PKM2 and primarily functions as a metabolic enzyme, while dimeric and monomeric PKM2 often enter the nucleus to regulate the expression of related genes. [1, 2]

1.2 Cell Cycle

Cell devision is a crucial process in cell proliferation, beginning at the end of the previous cell division and ending at the start of the next division. The eukaryotic cell cycle can be divided into interphase (including G0/G1, S, and G2 phases) and the mitotic phase (M phase). Cells that are not in the cell cycle or have temporarily exited the cycle are referred to as G0 phase cells. The G1 phase prepares necessary materials for DNA synthesis in the S phase, such as DNA polymerase and related regulatory proteins. The S phase is primarily dedicated to DNA synthesis. The G2 phase involves protein and RNA synthesis in preparation for mitosis. [3] The M phase is the mitotic phase, during which cells undergo mitosis. Additionally, the cell cycle has checkpoint mechanisms to ensure its smooth progression. The G1/S checkpoint controls the transition from G1 to S phase by monitoring cell size, extracellular environment, DNA integrity, and other factors. The G2/M checkpoint ensures genomic integrity and stability before cells enter the M phase. The spindle assembly checkpoint (SAC) monitors the connection between spindle microtubules and chromosomal kinetochores. Any issues detected at these checkpoints may cause cells to enter the G0 phase or undergo apoptosis. [4, 5]

Beyond checkpoints, the cell cycle is regulated by various proteins. Two key regulatory proteins are cyclins and cyclin-dependent kinases (CDKs). Cyclins

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and CDKs have highly specific binding relationships. When they bind, cyclins activate CDKs, enabling CDKs to phosphorylate and exert their effects. The periodic formation and degradation of cyclin-CDK complexes ensure the orderly progression of the cell cycle. Additionally, cyclins are regulated by kinase inhibitors and proteins encoded by certain oncogenes or tumor suppressor genes (e.g., p53). [6]

1.3 Characteristics of PKM2 and the Cell Cycle in Cancer Cells

Currently, PKM2 upregulation has been detected in almost all known tumor cells. [7, 8] Moreover, when PKM1 replaces PKM2 expression, tumor cell growth and the Warburg effect are suppressed. [9-11] This demonstrates that PKM2, rather than PKM1, plays a critical role in tumor cells. In cancer cells, PKM2 participates in metabolic reprogramming and enters the nucleus to influence gene expression, thereby promoting tumor development. Meanwhile, cancer cells exhibit characteristics such as unlimited proliferation, prolonged stagnation, gene mutations, dysregulated control, apoptosis inhibition, and abnormal damage repair, all of which are associated with PKM2.

1.4 Immune Cells

The human immune system can be divided into the innate immune system and the adaptive immune system. At the cellular level, innate immune-related cells include phagocytes, neutrophils, macrophages, natural killer cells, mast cells, basophils, dendritic cells, and eosinophils. Adaptive immune-related cells include T cells (cell-mediated immunity) and B cells (humoral immunity). [12]

2. Impact of PKM2 on the Cell Cycle

2.1 Tumor cells

2.1.1 Metabolic Aspects

As mentioned earlier, the structural characteristics of PKM2 enable it to possess both metabolic and non-metabolic functions, each of which can influence the cell cycle. The unlimited proliferation of cancer cells means they require vast amounts of energy and materials to meet their biosynthetic needs. Experiments have shown that the pyruvate kinase activity of PKM2 plays an important role in coordinating glycolysis and deoxynucleotide synthesis. [13] The regulation of PKM2 activity by tyrosine kinase signaling is crucial for metabolic changes during tumor growth and proliferation. [9, 14] Oxidation at the C358 site of PKM2 reduces its enzymatic activity and increases the flux of the oxidative pentose phosphate pathway (PPP), helping to regulate the redox state in cells. [15] Under serine-limiting conditions,[16,17] reduced PKM2 activity can also promote serine biosynthesis and proliferation. Downregulation of pyruvate kinase activity is important for nucleotide biosynthesis in non-transformed cells. [18] Additionally, studies suggest that the ability to regulate pyruvate kinase activity according to cell state may have varying importance for cell proliferation in different cancers. [13] Furthermore, PKM2 can be activated by fructose-1,6-bisphosphate (FBP) and serine and inhibited by high concentrations of ATP and alanine. [19] PKM2 localizes to the nucleus, where it recruits HIF-1α to hypoxia response elements (HREs) and brings in p300 to acetylate H3K9, thereby promoting the transactivation of genes encoding glucose transporters and glycolytic enzymes in cancer cells. [20] These factors collectively provide the metabolic foundation for the rapid repetition of the tumor cell cycle.

2.1.2 Non-Metabolic Aspects

PKM2 also affects the cell cycle of tumor cells through various non-metabolic functions. First, dimeric and monomeric PKM2 can enter the nucleus to regulate the transcription of specific genes, thereby influencing cell cycle progression. After epidermal growth factor (EGF) receptor activation, PKM2 directly binds to histone H3 and phosphorylates the T11 site of histone H3, participating in EGF-induced Cyclin D1 and c-Myc expression, tumor cell proliferation, cell cycle progression, and tumorigenesis. [21] Studies have shown that in human glioblastoma, epidermal growth factor receptor (EGFR) activation induces PKM2 (but not PKM1) and promotes its translocation to the nucleus. At the K433 site, PKM2 binds to the c-Src-phosphorylated Y333 site of β-catenin, forming a complex that recruits to the CCND1 promoter binding element. This leads to the decoupling of histone deacetylase 3 (HDAC3) from the promoter binding element, promoting histone H3 acetylation and Cyclin D1 expression. PKM2-dependent β-catenin activation contributes to EGFR-promoted tumor cell proliferation and tumor development. [22]

At the same time, PKM2 can also bind to proteins to exert its effects. Experiments have shown that PKM2 can bind to the Cdk1-Cyclin B complex, which is critical for the G2-M phase, and in turn promote the activation of Cdk1-Cyclin B, driving cells into mitosis. [23] Studies indicate that PKM2 (but not PKM1) binds to the spindle checkpoint protein Bub3 during mitosis and phosphorylates Bub3 at the Y207 site. This phosphorylation is essential for the recruitment of the Bub3-Bub1 complex to kinetochores and its interaction with Blinkin (also known as KNL1, Spc7, Spc105, AF15q14, D40, and CASC5). This process is crucial for proper kinetochore-microtubule attachment, mitotic checkpoint function, accurate chromosome segregation, cell survival and proliferation, and EGFR-induced tumorigenesis. [24] [25] Aurora B phosphorylates PKM2 at the T45 site but does not phosphorylate PKM1. This phosphorylation is necessary for PKM2 to localize to the myosin light chain 2 (MLC2) region of the contractile ring in mitotic cells and interact with it. PKM2 phosphorylates MLC2 at the Y118 site, enabling the binding of Rho-associated coiled-coil kinase 2 (ROCK2) to MLC2 and its phosphorylation at the S15 site. PKM2-regulated MLC2 phosphorylation is significantly enhanced by EGF stimulation of EGFR

vIII, K-Ras G12V, and B-Raf V600E mutations, playing a key role in cytokinesis, cell proliferation, and tumor development. [26] [27] [28] PKM2 phosphorylates STAT3 at the Y705 site, activating downstream gene expression. [29] [30] 5-Aminoimidazole-4-carboxamide ribonucleotide (SAICAR), a metabolic intermediate in purine nucleotide biosynthesis, can directly activate the pyruvate kinase activity of PKM2. The PKM2-SAICAR complex can phosphorylate over 100 human proteins, including Erk1/2. Activated ERK/MAPK signaling increases PKM2 nuclear localization and promotes cell proliferation. [31] The p53 protein phosphorylates PKM2 at the Tyr 105 site via mTOR signaling. [32] PKM2 can be acetylated at the K433 site by the p300 acetyltransferase. Acetylation interferes with fructose-1,6-bisphosphate (FBP) binding, preventing PKM2 activation, promoting its nuclear accumulation, and enhancing its protein kinase activity, thereby driving cell proliferation and tumorigenesis. [33] Additionally, recent studies suggest that PKM2 membrane localization may be related to intercellular communication, particularly trogocytosis in immune cells, and has become a new research focus.

2.2 Immune cells

Meanwhile, the role of PKM2 in immune cells also contributes significantly to cancer research. Studies have found that checkpoint kinase 2 (Chk2) phosphorylates PKM2 at the T95 and T195 sites, promoting glycolysis and M1 macrophage polarization. [34] PKM2 is also a key mediator of Th17 cell differentiation and autoimmune inflammation. During in vitro Th17 cell differentiation experiments and the development of experimental autoimmune encephalomyelitis (EAE) models, PKM2 is highly expressed. When PKM2 is deleted in T cells, Th17 cell-mediated inflammation and demyelination are reduced, Th17 cell differentiation is inhibited, and EAE symptoms improve. Under normal conditions, PKM2 can translocate to the nucleus and interact with STAT3, enhancing its activity and promoting Th17 cell differentiation. [35] It is reasonable to speculate that the interaction between nuclear PKM2 and STAT3 in Th17 cells may also promote cell cycle progression and proliferation. PKM2 has been shown to be a key determinant of metabolic reprogramming in macrophages stimulated by lipopolysaccharide (LPS) via HIF-1α. After LPS activation, PKM2 dimers stabilize HIF-1α, regulating the expression of HIF-1α target genes such as Il1b and genes encoding glycolytic machinery, thereby playing a significant role in M1 macrophage differentiation and function. [36-37]

3. Potential Applications in Cancer Therapy

Based on the above research, we can explore PKM2 as a target for cancer therapy. First, PKM2 may serve as a potential prognostic biomarker. In lung cancer, compared to adjacent normal tissues, PKM2 expression is elevated in tumor tissues. Prognostic analysis indicates that high PKM2 expression is associated with poorer outcomes in lung adenocarcinoma (LUAD) patients. PKM2 also shows strong correlations with B cells and CD4+ T cells in LUAD, as well as with B cells, CD8+ T cells, CD4+ T cells, and macrophages in lung squamous cell carcinoma (LUSC). Moreover, PKM2 expression exhibits significant negative correlations with immune cell marker expression in LUAD and LUSC. [38] PKM2 can also serve as a biomarker for gastrointestinal cancers. [39] Additionally, researchers have designed cancer-related drugs targeting PKM2. For example, the herbal extract Shikonin can inhibit PKM2 activity by binding to it. [40] Other PKM2 inhibitors include PKM2-IN-1 and TEPP-46. [41-44]

On the other hand, research on PKM2's role in immune cells provides a foundation for cancer therapy. Immune checkpoint blockade (ICB), an immunotherapy approach including PD-1/PD-L1 inhibitors and CTLA-4 inhibitors, primarily blocks immune checkpoint proteins to activate the immune system, enabling it to attack tumor cells. However, studies show that ICB may promote hyperprogressive disease (HPD). Researchers found that CD8+ T cell-derived IFNγ targets FGF2, selectively inhibiting PKM2 and reducing NAD+ production, thereby increasing β-catenin activity in tumor cells and promoting cancer progression and tumorigenesis. [45]

4. Conclusion

As a multifunctional protein, PKM2 plays a vital role in cell cycle regulation, immune cell function, and cancer development. Its complex metabolic and signaling networks influence cell fate and disease progression. By studying PKM2's mechanisms in different physiological and pathological states, we can better understand its impact on cell biology and disease development, providing new insights for designing cancer treatments and immune disease therapies. With advancing technology, we are confident that further revelations about PKM2 will lead to breakthroughs in disease treatment.

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The Etiological Correlation of Seven Common Types of Alopecia

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Abstract:

In recent years, the population suffering from alopecia in China has been increasing, which severely affects the daily life and mental health of individuals, making it one of the diseases that cannot be ignored. The occurrence of alopecia is associated with various factors, including genetic predisposition, induction by other diseases or medications, and environmental influences. Current research on the specific etiology of alopecia is not comprehensive, which hinders the diagnosis and treatment of various types of alopecia and the inhibition of hair loss from its roots or progression. This article summarizes several types of alopecia with high incidence rates, such as androgenetic alopecia, alopecia areata, frontal fibrosing alopecia, and seborrheic dermatitis, aiming to elucidate the specific causes of different types of alopecia and to organize the relationships between various forms of alopecia. This work lays a solid foundation for the diagnosis and treatment of alopecia by considering potential common etiologies and proposing three biological indicators for triggering alopecia: hormones, microorganisms, and the "common pathway" – immune dysregulation.

Keywords: Alopecia, Etiology, Follicular Immunity.

1. Background:

Alopecia is a degenerative hair follicle disorder caused by various factors, characterized by localized or generalized hair loss. It can be classified into scarring and non-scarring types and is associated with genetic factors, lifestyle habits, psychological stress levels, medication use, environmental pollution, gender, and other factors, exhibiting significant individual variability. As human civilization progresses into the modern era, alopecia has become increasingly prevalent among younger populations and affects a broader demographic [1]. Meanwhile, treatment technologies for alopecia are rapidly advancing, with progress in exosome therapy [2], hair transplantation [3], and novel drugs targeting new pathways [4, 5]. However, for the development of treatment methods, etiological research is crucial. This article aims to analyze the causes of seven common types of alopecia (androgenetic alopecia, frontal fibrosing alopecia, telogen effluvium, lichen planopilaris, central centrifugal cicatricial alopecia, alopecia areata, and folliculitis decalvans) to explore similarities in their pathogenic pathways and identify potential common targets. This will provide new insights for developing innovative therapies and enhancing the universality of existing treatments.

2. Etiological Analysis of Seven Common Types of Alopecia

2.1 Androgenetic Alopecia (AGA)

Androgenetic alopecia (AGA) is a non-scarring form of hair loss characterized by progressive reduction of hair follicles or the appearance of non-functional or dead follicles in a specific pattern on the scalp. Hormonal factors, genetics, micronutrient deficiencies, microinflammation, and stress are all implicated in its development [6]. AGA is the most common type of alopecia, accounting for approximately 90% of all

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hair loss cases. Typical symptoms include thinning hair starting from the frontal and temporal regions, receding hairline, and gradual extension to the vertex. This condition often has a familial predisposition, with significant gender differences—21.3% in males and 6% in females. AGA also exhibits racial disparities, with higher prevalence among Caucasians than among Asians and Africans. The incidence of AGA is age-dependent, beginning at puberty and increasing with age. It is non-contagious and often accompanied by excessive sebum production [7]. The causes of AGA include the following:

2.1.1 Genetic Factors

Susceptibility to AGA is primarily determined by genetics and is considered a polygenic disorder. Androgens are the most critical regulators of human hair growth, with varying effects in different body regions due to differences in gene expression responses to androgens. In the scalp follicles of susceptible males, androgens inhibit hair growth [8]. The *StuI* polymorphism is linearly correlated with AR activity and is associated with AGA. Variants in the *EDA2R* gene also increase susceptibility to AGA [9]. Studies have identified genetic susceptibility loci for AGA on chromosome 7p21.1, 3q26 (*HDAC9*), 20p11 (*PAX1/FOXA2*), and the X chromosome (androgen receptor/*EDAR2*).

2.1.2 Androgen Receptor (AR) and 5α-Reductase Factors

Research indicates that circulating androgen levels in AGA patients are normal, but increased AR expression makes scalp follicles highly sensitive to these hormones. 5α-reductase has two isoforms (type I and II), which catalyze the conversion of testosterone (T) to dihydrotestosterone (DHT). DHT has a fivefold higher affinity for AR than T, and both isoforms play significant roles in androgen metabolism [10].

2.1.3 Cellular Senescence Factors

Hair follicle stem cells (HFSCs) and dermal papilla cells (DPCs) play dominant roles in follicular morphogenesis and cyclic hair growth [11, 12]. DHT, the most potent androgen in hair growth, acts on DPCs, which mediate signaling by secreting growth factors and extracellular matrix components [13, 14]. DHT induces DPCs, leading to progressive follicular miniaturization, shortening the anagen phase and prolonging the telogen phase, ultimately resulting in baldness. Senescent HFSCs exhibit impaired ability to enter the hair growth phase, Wnt-inhibited hair growth signaling, and DNA damage-triggered senescence, leading to follicular miniaturization. Changes in HFSC subpopulations have also been observed in AGA [15].

2.1.4 Other Disease Factors

Risk factors for coronary artery disease (CAD), such as low HDL, high LDL, very low-density lipoprotein, triglycerides, serum lipoprotein-a, and serum homocysteine, increase with the severity of AGA. The risk of CAD in AGA patients rises with the grade of AGA [16]. Conditions like benign prostatic hyperplasia, prostate cancer, and CAD are more common in AGA patients than in non-bald individuals [17].

2.1.5 Emotional Factors

The onset of AGA also depends on psychological and emotional factors. Compared to the general population, psychiatric disorders are more prevalent among individuals with hair loss.

2.2 Frontal Fibrosing Alopecia (FFA)

Frontal fibrosing alopecia (FFA) is a band-like scarring alopecia in the frontal-temporal scalp, classified as a special form of lichen planopilaris. It primarily affects postmenopausal women, though its incidence in men may be underestimated due to potential overlap with androgenetic alopecia [18]. Dermoscopy of the eyebrows reveals "road sign" patterns, hairs growing in different directions, and the presence of black, yellow, and gray dots, diffuse erythema, and follicular unit loss [19]. Histological examination shows abundant perifollicular and infundibular lymphocytic infiltration, hyperkeratosis of the infundibulum and follicular ostia, and apoptosis and vacuolar degeneration of basal keratinocytes [18]. The etiology remains unclear, with potential links to sex hormones, genetic predisposition, autoimmunity, environmental factors, defects in lipid metabolism, and neurogenic inflammatory responses [20]. Hormonal changes during pregnancy, breastfeeding, hysterectomy, or hormone/raloxifene therapy, as well as exposure to sunscreen ingredients, moisturizers, or sunlight, may also play a role [18].

2.2.1 Pathogenesis

Immune cells and cytokine-mediated inflammatory responses appear to play a significant role in FFA [21]. Increased infiltration of CD8+ cytotoxic T cells and dendritic cells around damaged follicles (particularly near the bulge region) exacerbates inflammation, leading to the collapse of immune privilege (IP) and damage to epithelial hair follicle stem cells (eHFSCs) [22]. This results in progressive fibrosis of the entire follicular unit, causing scarring alopecia [23].

2.2.2 Genetic Factors

Research has focused on the role of human leukocyte antigen (HLA) in FFA development. The HLA-B*07:02 mutation appears to promote an autoinflammatory response against eHFSCs, significantly increasing the risk of FFA [24]. A study of FFA patients identified that 83.8% carried the rs9258883 polymorphism in HLA-B*07:02, with most lacking the protective rs1800440 polymorphism in *CYP1B1* (75.2%) [25]. Alterations in genetic pathways such as PPAR-γ and mTOR are also associated with FFA. These pathways influence lipid metabolism, sebocyte differentiation, and immune responses, potentially affecting follicular

health through inflammation and fibrosis induction. Reduced PPAR-γ activity leads to fibrosis and inflammatory cell infiltration, while mTOR signaling interacts with PPAR-γ to regulate lipid homeostasis and inflammatory processes [23]. Specifically, decreased peroxisome and cholesterol production may cause the accumulation of pro-inflammatory lipids in hair follicles, leading to inflammatory cell infiltration in the bulge region [26].

2.2.3 Environmental Factors

Exposure to light, particularly ultraviolet radiation, has been hypothesized to contribute to FFA [27]. Light may influence the synthesis of certain compounds, such as 6-formylindolo[3,2-b]carbazole (FICZ), which can have pro- or anti-inflammatory effects depending on their concentration. Retrospective studies using surveys and clinical cases have also suggested a link between FFA and the use of sunscreen and other facial cosmetics in both male and female patients [28, 29].

2.2.4 Hormonal Factors

Changes in hormone levels, particularly during menopause, may influence the onset and progression of FFA [23]. Reduced levels of DHEA and androgens can induce fibrosis in FFA [21]. Based on its frequent comorbidity with androgenetic alopecia and clinical improvement with anti-androgen therapy, some propose a hormone-dependent etiology for FFA [30].

2.3 Telogen Effluvium (TE)

Telogen effluvium (TE) is a scalp disorder characterized by diffuse, non-scarring hair loss [31], which is a reactive process triggered by metabolic stress, hormonal changes, or medications. Studies indicate that TE has no genetic cause [32].

2.3.1 Pathogenesis

TE is triggered when physiological stress causes a large number of hairs in the anagen phase of the hair cycle to abruptly enter the telogen phase. During this stage, telogen hair growth ceases for 1 to 6 months (average 3 months). When affected hairs re-enter the anagen phase, the telogen hairs are extruded from the follicles, resulting in noticeable hair loss. At the molecular level, etiological factors may disrupt the delicate balance of growth factors, neuroendocrine signals, and cytokines involved in follicular homeostasis. Such disturbances can lead to premature induction or prolongation of the catagen phase, accelerating the transition of hairs into the telogen phase. Relevant studies suggest that inflammatory mediators, oxidative stress, and changes in the follicular niche microenvironment contribute to the persistence of TE.

2.3.2 Inflammatory Response Factors

Surveys indicate that the proportion of TE patients among all hair loss types increased after the COVID-19 pandemic. In addition to direct damage to hair follicles by various viruses, pro-inflammatory cytokines can also harm follicles, and the coagulation cascade accompanied by microthrombi (blocking follicular blood supply) may form [33].

Viral infections trigger the intense release of pro-inflammatory cytokines. Viruses induce a robust antiviral response, particularly through interferons, which are TE-inducing molecules [34]. High levels of IL-6 act on hair follicles, leading to the collapse of immune privilege and inducing the catagen phase, causing localized inflammation. Other molecules elevated in COVID-19 include matrix metalloproteinases 1 and 3 and IL-1b, which may inhibit hair follicle growth [35]. Simultaneously, during high fever, cytokines initiate apoptosis of follicular keratinocytes, pushing them into the catagen phase and subsequently the telogen phase. Additionally, malnutrition, severe illness, and chronic wasting diseases can disrupt the hair growth cycle, causing premature entry into the telogen phase and immediate anagen release [36].

2.4 Lichen Planopilaris (LPP)

Lichen planopilaris (LPP) is a scarring alopecia that predominantly affects middle-aged women, with an estimated incidence of 1% to 7%. Patients present with hair thinning, which may be accompanied by scalp itching or tenderness [37]. It is characterized by a chronic and destructive inflammatory process [38]. LPP is now classified as a primary lymphocytic disorder based on lymphocyte, neutrophil, or mixed infiltrates [39] and is often irreversible [40].

2.4.1 Immune-Inflammatory Response Factors

Cell-mediated immunity can drive the clinical manifestations of LPP. The immune response primarily involves the bulge region, a continuous part of the outer root sheath rich in stem cells. The participation of T lymphocytes (CD4 and CD8) is activated by an increase in Langerhans cells in the dermis and epidermis. Reports indicate that Th17 cells (a subset of CD4+ T helper cells) also play a crucial role in promoting immune-inflammatory responses in autoimmune diseases [41].

2.4.2 Microbial Population Factors

One study demonstrated an imbalance in the scalp microbiota of LPP patients, which plays a role in its pathophysiology. Another study found that compared to healthy controls, the LPP group had increased abundance of Cyanobacteria and Euryarchaeota phyla, fewer Firmicutes, and higher microbial diversity [38].

2.4.3 Environmental Factors

Due to global climate change, particularly air pollution, certain members of the Cyanobacteria phylum have proliferated in the atmosphere. This is associated with higher cyanobacterial richness in LPP patients, and metabolites produced by this phylum have negative effects on human health [42]. Pollutants such as particulate matter and heavy metals may

accumulate on hair [43], inducing oxidative stress by increasing reactive oxygen species (ROS) production [44] and contributing to clinical conditions related to hair loss, including LPP [45].

2.5 Central Centrifugal Cicatricial Alopecia (CCCA)

Central centrifugal cicatricial alopecia (CCCA) is a scarring alopecia characterized by permanent patches of hair loss that begin at the vertex or crown of the scalp and gradually spread outward in a centrifugal pattern [46]. CCCA shows significant racial and gender predispositions: it most commonly affects individuals with tightly coiled or kinked hair, and 2.7% of African American women are affected by CCCA [47].

2.5.1 Genetic Factors

A positive correlation has been found between mutations in type III peptidylarginine deiminase and CCCA. This enzyme specifically acts on the deamination of proteins within the hair shaft. Loss of this function increases hair fragility, leading to hair loss [48].

2.5.2 Inflammatory Response Factors

Various inflammatory cytokines extracted from the scalps of women with CCCA indicate it is an inflammatory disease. Multiple studies have confirmed a positive correlation between a history of fungal infections and the development of CCCA. One study showed that STAT3 is activated in perifollicular lymphocytes of CCCA patients. STAT3 activation is significant for increasing Th17 cells, which secrete pro-inflammatory cytokines that may play a role in the progression of this disease [49].

2.5.3 Other Disease Factors

The incidence of CCCA is higher in women with a history of depression and anxiety [50]. Additionally, CCCA is associated with type 2 diabetes and uterine leiomyomas. Some studies found that CCCA patients have at least a fivefold increased risk of developing leiomyomas, particularly among women of American descent [48].

2.6 Etiology of Alopecia Areata (AA)

Alopecia areata (AA) is a common chronic tissue-specific autoimmune disease characterized by non-scarring hair loss with preserved hair follicles. The clinical presentation ranges from small patchy hair loss to diffuse or complete alopecia, potentially affecting the entire body surface. Biopsies reveal lymphocytic infiltration in the hair bulb or lower half of hair follicles during the anagen phase. Approximately 2% of the global population experiences AA at some point in their lives [51]. Depending on severity, it may lead to psychiatric disorders like depression and anxiety, as well as psychosocial issues [52]. Surveys indicate that while the global disease burden of AA improved between 1990-2019, it remains relatively high overall [53]. AA tends to be milder in elderly patients but more severe in children, often progressing to alopecia totalis/universalis [54]. The exact pathogenesis remains unclear, though current research provides evidence implicating genetic, immunological, and psychological factors.

2.6.1 Genetic Factors

Recent genome-wide association studies have linked AA to Treg cells, CTLA-4, IL-2/IL-21, CD25 (IL-2RA), ULBP6 (UL16-binding proteins), and NK cell receptor NKG2D. Polymorphisms in AIRE-207 (autoimmune regulator-207) and TNF/LTA (lymphotoxin-alpha) genes are also frequently observed in AA patients [54]. Genetic epidemiological studies show that 8.4%-25.0% of AA patients have a positive family history, with first-degree relatives having higher susceptibility. Compared to the general population, if either parent is affected, the first twin is more likely to develop AA regardless of other family members' status [55].

2.6.2 Immune Dysfunction

AA is recognized as a common type of immune-mediated hair loss where autoimmune attack on hair follicles causes non-scarring alopecia. The pathogenesis is attributed to collapse of hair follicle immune privilege (HF-IP) [56]. As an immune-privileged (IP) site, HF-IP breakdown is considered prerequisite for AA development, while its restoration may enable spontaneous or long-term remission. Therefore, accelerating HF-IP reconstruction represents a promising therapeutic approach. Local immunosuppressive molecules like TGF-β1, IL-10, α-MSH, IDO, and VIP may help maintain this immunoinhibitory microenvironment [57].

Additionally, human leukocyte antigens (HLA) significantly influence autoimmune diseases. AA patients exhibit markedly increased expression of HLA-A, HLA-B, and HLA-C - rarely seen in healthy individuals. Clinical observations also show AA patients frequently present with other autoimmune diseases [58], further supporting AA's autoimmune nature.

2.6.3 Mental Health Factors

Multiple studies demonstrate close relationships between AA onset and psychological stress/mental health status. While anxiety/depression may not play primary roles in AA pathogenesis among limited patient cohorts [56], exacerbated life stress can trigger disease onset/worsening. Complex interactions among insecure attachment, alexithymia, and poor social functioning may increase AA risk. The observed high alexithymia traits and avoidant attachment in AA patients reflect emotional regulation deficits. Though mental health is important, AA occurrence in infants/-neonates necessitates consideration of alternative factors.

2.6.4 Smoking Factor

As a classic inflammatory dermatosis, AA associates

with environmental stimuli including smoking. While the precise smoking-related mechanism remains unclear, cigarette smoke: Elevates proinflammatory cytokines (IL-17, IFN-γ) while reducing anti-inflammatory cytokines ,Activates Th17-mediated skin inflammation, Increases Th2 cytokine IL-13, exacerbating Th2-dominant immune responses [56,58] Thus, AA patients should strictly avoid smoking.

2.7 Folliculitis Decalvans (FD)

Folliculitis decalvans (FD) is a chronic, recurrent pustular folliculitis of the scalp, typically involving the central scalp with crusted plaques and centrifugal progression of follicular pustules [59]. Histopathology reveals this rare primary neutrophilic scarring alopecia [60]. Staphylococcus aureus is frequently detected in affected areas and considered pathogenic [61]. No definitive cure exists; treatment aims at stabilization with antibiotics/immunosuppressants [61]. Characteristic features include: Tufted hairs (multiple shafts per follicular opening), Perifollicular pustules/scaling, Late-stage follicular destruction [62] Deep fungal scalp infections may mimic FD (and vice versa), particularly in African Americans. Light microscopy and fungal culture are diagnostic essentials [63].

2.7.1 Staphylococcus aureus Infection

S. aureus colonizes 80% of FD patients' skin - the only bacterium consistently present in >2/3 cases [61]. During neutrophilic phases, follicular structural changes/immune dysfunction enable pathogenic S. aureus colonization, triggering neutrophilic inflammation that damages follicles [61]. S. aureus toxins complex with MHC proteins to stimulate T cells while evading immune detection. FD may result from abnormal host responses to post-infection toxin release [63].

2.7.2 Synergistic Effects of Polymicrobial Communities

Follicular biopsies from FD patients reveal the most prevalent bacterial genera include Staphylococcus, Cutibacterium, and Bacteroides. Staphylococcus accounts for 25.9% of the follicular microbiome in FD-affected biopsies, compared to only 6.6% in healthy follicles. Pathological follicles in FD patients exhibit distinct bacterial microbiota profiles versus healthy controls, suggesting this unique microbial signature may contribute to FD pathogenesis [63].

2.7.3 Propionibacterium acnes

Bacterial biofilms were observed in all patients and two of three control groups. These biofilms exclusively consisted of morphologically similar bacilli in both cohorts. While these bacilli were tentatively identified as Propionibacterium acnes, further validation is required. This study demonstrates the existence of bacterial biofilms in the infundibular-subinfundibular regions of human scalp follicles, detectable in both FD patients and controls, indicating their commensal existence with potential pathogenic transformation in FD.

2.7.4 Immunological Factors

As S. aureus may not be the sole pathogen, bacterial infection might only initiate rather than sustain the entire pathogenic process. Once immune-inflammatory cells are activated, follicular destruction persists even after pathogen clearance [64]. FD patients' intrinsic follicular, epidermal, or immune system alterations may predispose to localized microbial colonization (e.g., S. aureus) and subsequent folliculitis. However, whether specific bacteria are primary pathogens remains debatable.

During neutrophilic phases, hypothesized structural/immune dysfunction enables pathological S. aureus colonization, triggering neutrophilic inflammation that damages follicles. Chronic overstimulation by aberrant follicular microbiota may dysregulate monocyte cytokine production due to immune exhaustion. This impaired immunity complicates bacterial clearance, exacerbating disease progression.

3. Summary and Perspectives

Etiological analysis of these seven alopecias reveals that all ultimately converge on scalp/follicular immune imbalance. Whether through P. acnes/S. aureus-induced hyperimmunity or cellular immune dysregulation in AA due to genetic/lifestyle factors, the final common pathway involves immune attacks on follicles and arrested hair growth.

Thus, alopecias can be categorized into three types by etiology:

1. Androgen-induced immune activation (AGA, FFA): Minimal microbial involvement, primarily hormonal imbalance-driven immune dysregulation with significant genetic influence on hormone-related enzymes/receptors.
2. Microbe-induced immune activation (FD, LPP, TE): Chronic inflammation/immune stress from microbial dysbiosis/viral infection, where ROS pathway activation and IL-family cytokine dysregulation play pivotal roles.
3. Stress-associated multifactorial immune activation (CCCA, AA): Complex etiology with minimal microbial triggers, involving diverse cell types.

All pathways culminate in the "final common pathway" - immune imbalance disrupting follicular immune privilege.

For drug development, beyond disease-specific targets, universal therapeutic strategies should focus on: Androgen pathway modulation, Microbial homeostasis, Immune regulation targets. Emerging approaches like exosome-mediated immunomodulation and repurposing cancer immunotherapy targets (e.g., immune checkpoint molecules) may inspire localized scalp immunotherapies. With advancing research, clearer etiological mechanisms and shared pathways will undoubtedly emerge, guiding the development of broadly effective anti-alopecia therapies.

Table 1. Etiological Analysis of Several Diseases Leading to Hair Loss

	Immune Factors	Psychological Factors	Microbial Infection	Genetic Factors	Disease-Associated Factors	Possible Classification
Androgenic Alopecia (AGA)	+	+		+++	Coronary artery disease, benign prostatic hyperplasia, prostate cancer, psychological disorders (correlation)	I
Frontal Fibrosing Alopecia (FFA)	++			+	AGA, hormonal changes during menopause (direct factors)	I
Telogen Effluvium (TE)	+++		+++		High fever, malnutrition, wasting diseases, pro-inflammatory cytokines (direct factors)	II
Lichen Planopilaris (LPP)	+++			+++	Environmental pollution, heavy metal particles (correlation)	II
Central Centrifugal Cicatricial Alopecia (CCCA)	++	+	++	++	Leiomyoma, anxiety disorders, etc. (correlation)	III
Alopecia Areata (AA)	+++	+	++	+	Psychological disorders, poor living habits (correlation)	III
Folliculitis Decalvans (FD)	+++			+++	Bacterial, fungal infections (direct factors)	II

Note:"+" indicates the presence or involvement of the factor, "+++" indicates the highest level of involvement or strong direct correlation. The "Possible Classification" column categorizes the diseases based on the complexity and severity of their etiology.

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