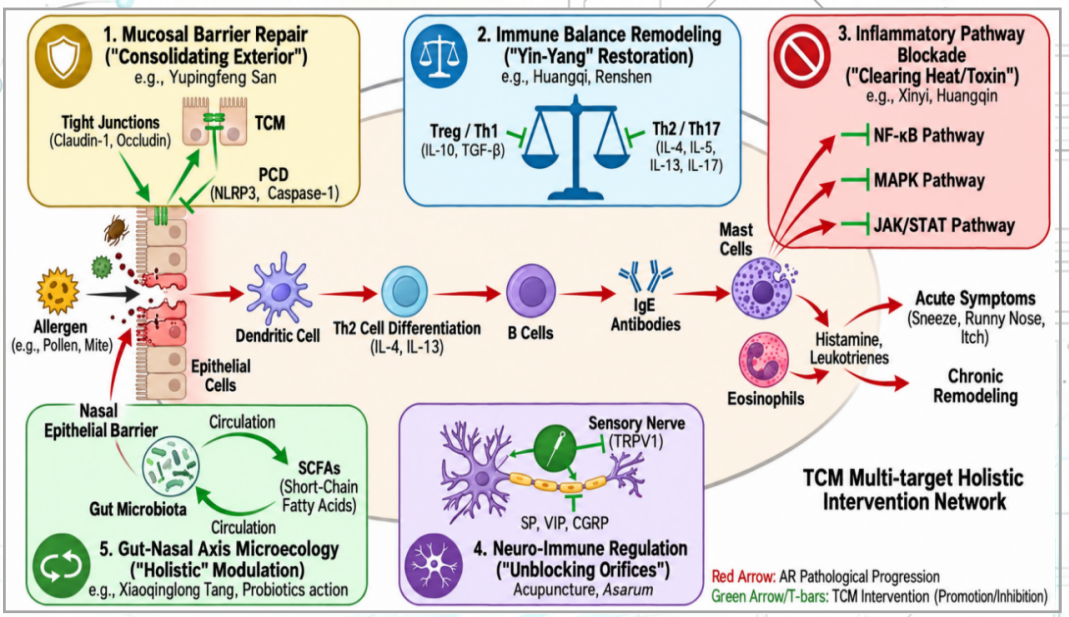
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Mechanisms and Research Progress of Traditional Chinese Medicine in the Treatment of Allergic Rhinitis from the Perspective of Modern Medicine

Ziyang Yu, Zhanfeng Yan



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Mechanisms and Research Progress of Traditional Chinese Medicine in the Treatment of Allergic Rhinitis from the Perspective of Modern Medicine

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Abstract

This article conducts a systematic review of the biological mechanisms and research progress in traditional Chinese medicine (TCM) for treating allergic rhinitis (AR) from a modern medical perspective. Research indicates that TCM exerts systemic therapeutic effects through a multi-target, multi-level network, with core mechanisms focusing on five key areas: first, the regulation of intestinal microecology, reshaping gut microbiota composition and metabolites based on the "gut-nose axis" theory; second, the repair of the mucosal barrier, protecting nasal epithelial cells by modulating programmed cell death pathways such as pyroptosis, autophagy, and ferroptosis; third, the restoration of immune homeostasis, correcting Th1/Th2 and Th17/Treg imbalances and inhibiting IgE-mediated cascade reactions; fourth, the modulation of inflammatory signaling pathways, including TLRs, NF-κB, and MAPK; and fifth, neuro-immune regulation, suppressing neuropeptide release and neurogenic inflammation. Current evidence suggests that TCM offers the advantage of "constitutional regulation and prognosis improvement." Its integration with conventional therapies can significantly lower AR recurrence rates and mitigate drug-related side effects. To address challenges like lack of standardization and mechanistic opacity in TCM research, this review proposes establishing an evidence-based, standardized diagnostic and therapeutic framework. Utilizing whole-genomics, network pharmacology, and artificial intelligence for precise subtyping and personalized treatment is recommended to advance the modernization and scientific development of TCM in AR management.

Keywords: Allergic Rhinitis; Integrated Traditional Chinese and Western Medicine; Mechanism of Action

1. Introduction

1.1. Definition, Epidemiology, and Clinical Burden of Allergic Rhinitis in Modern Medicine

Allergic rhinitis (AR), often known as hay fever, is a chronic inflammatory condition affecting the nasal mucosa that is mainly mediated by immunoglobulin E (IgE) after individuals who are susceptible come into contact with allergens. It is clinically characterized by recurrent episodes of sneezing, rhinorrhea (runny nose), and nasal congestion. In traditional Chinese medicine (TCM), AR falls under the category of the syndromes of Bi Qiu, Qiu Ti, and Qiu Bi.

The global prevalence of AR has increased markedly in recent decades, currently affecting approximately 1.4 billion individuals and establishing it as one of the most common chronic respiratory diseases worldwide. In the United States alone, an estimated 50 million people are affected by AR (Bernstein et al., 2024). The disease imposes a substantial and multifactorial burden. Foremost, it significantly impairs health-related quality of life (HRQoL), leading to sleep disturbances, reduced productivity and absenteeism from work or school, and limited outdoor activity participation in children. Secondly, AR is a major risk factor for several respiratory comorbidities; for example, patients with AR have a several-fold higher risk of developing asthma compared to the general population. Furthermore, AR entails considerable economic costs. Annual work productivity losses attributable to AR in the European Union are estimated at €30–50 billion (Greiner et al., 2011), while direct annual healthcare costs for AR treatment in the United States range from $2 to $5 billion .

As a result, AR has become a major global public health concern characterized by substantial clinical and socioeconomic consequences. Current mainstream management in modern medicine, tailored to disease severity, includes allergen avoidance, pharmacotherapy, immunotherapy, and surgery. Although these interventions provide satisfactory symptomatic control for most patients, AR frequently recurs and is often associated with considerable adverse effects. Recent systematic reviews have indicated that TCM interventions can enhance clinical efficacy, reduce total symptom and sign scores, and exhibit a favorable safety profile with fewer adverse events, underscoring their clinical value . However, due to the theoretical complexity and diverse therapeutic modalities of TCM, while numerous independent studies have explored its mechanisms of action in AR, a recent systematic synthesis of the cumulative evidence is still lacking. Therefore, this study reviews and integrates current studies on the mechanistic underpinnings of TCM in the treatment of AR, with the purpose of offering a distinct and relatively thorough summary of the clarified therapeutic mechanisms.

1.2. Significance of Investigating the Mechanisms of TCM in Treating AR from a Modern Medical Perspective

The methodology of Traditional Chinese Medicine (TCM) differs fundamentally from that of modern medicine. In contrast to the structuralist paradigm of modern pathology, which seeks specific anatomical lesions or pathogens, TCM is guided by functionalism, focusing on the "functional state". For example, the TCM syndrome "Pi Qi Xu" (Spleen Qi Deficiency) does not denote an organic lesion of the anatomical spleen but rather describes a functional state characterized by impaired digestion and absorption, disordered energy metabolism, and immune

dysregulation (Wang et al., 2020). Given these epistemological differences, TCM diagnostic and therapeutic approaches are frequently conceptualized as a “black-box” system from the perspective of modern medicine: its inputs are macroscopic symptom patterns (pulse, tongue appearance, and patient complaints), and its outputs are TCM-specific interventions such as herbal formulas or acupuncture. The intervening pathophysiological mechanisms and active pharmacological constituents, however, have long remained obscure. This “black-box” characterization consequently poses challenges for TCM within the framework of modern medicine, which places a premium on Evidence-Based Medicine (EBM) — the rigorous scientific evaluation of clinical efficacy to determine treatment validity (Cui et al., 2025). Furthermore, in contemporary healthcare, patients increasingly seek not only confirmation of efficacy but also a mechanistic explanation, raising expectations for greater transparency in TCM. Therefore, elucidating the mechanisms of TCM for AR from a modern medical perspective is crucial to addressing these challenges. Such research, by gradually illuminating this "black box", would not only advance TCM itself but also inform the development of novel therapeutic strategies and drugs, thereby facilitating its internationalization and modernization.

1.3. Contemporary Medical Insight into the Key Pathogenic Mechanisms of Allergic Rhinitis

The pathogenesis of allergic rhinitis (AR) involves multiple interrelated systems, including the gut microbiota, nasal epithelium, immune and inflammatory pathways, and neural regulation (Greiner et al., 2011). This complex process is initiated by dysregulation of the “gut-nose axis” (Nunez et al., 2025; Wang et al., 2025). The gut microbiota plays a crucial role in maintaining immune homeostasis (Salem et al., 2018). Dysbiosis can trigger immune dysregulation (Lyu et al., 2022), thereby predisposing individuals to AR.

The nasal mucosal epithelium is central to the AR pathological process (Chang et al., 2025). When the mucosal barrier integrity is compromised, epithelial cells release alarmins (e.g., IL-33, TSLP, IL-25) to orchestrate repair and inflammatory responses (Nur Husna et al., 2021). Allergens like house dust mites facilitate this by either directly damaging the epithelial barrier via proteases (e.g., Der p 1) or activating pattern recognition receptors (e.g., Der p 2). These alarmins serve as upstream regulators that promptly activate type 2 innate lymphoid cells (ILC2s), which in turn secrete IL-5, IL-13, and IL-4. This initiates the type 2 adaptive immune response, promotes IgE class switching, and culminates in a cascading mucosal inflammatory reaction (Bousquet et al., 2020). Epithelial cell death pathways, including apoptosis, pyroptosis, and autophagy, also contribute to AR development, though their precise roles require further elucidation.

These alarmins drive the immune system into a phase of antigen-specific sensitization and response amplification. TSLP promotes dendritic cell-mediated polarization of naïve T cells into pathogenic Th2 (particularly Th2A) cells, while IL-33 directly activates ILC2s. These parallel pathways converge on the massive secretion of IL-4, IL-5, and IL-13 (Niu et al., 2025). IL-4 and IL-13 induce immunoglobulin class switching in B cells to generate antigen-specific IgE, which binds to high-affinity receptors on mast cells and basophils, completing sensitization.

Upon re-exposure, allergen cross-linking of IgE-bound receptors triggers instantaneous mast cell degranulation and the release of histamine, leukotrienes, and prostaglandin D2, triggering the acute onset of clinical symptoms. The intensity and persistence of symptoms are critically modulated by a complex neuroimmune network. Histamine stimulates trigeminal nerve endings, sending impulses to the medullary "sneeze center" to elicit sneezing. Concurrently, activated sensory nerves release substance P and CGRP via an axon reflex, directly acting on mast cells and endothelium to cause vasodilation, plasma exudation (nasal congestion), and further immune cell activation, forming a neurogenic inflammatory positive-feedback loop. Furthermore, parasympathetic reflex activation of the sphenopalatine ganglion releases acetylcholine and VIP, a key autonomic mechanism underlying watery rhinorrhea and refractory nasal obstruction (Seijo-Martinez et al., 2006).

Ultimately, recurrent acute episodes evolve into chronic inflammation and tissue remodeling. Sustained IL-5-mediated eosinophil recruitment primes the nasal mucosa, resulting in a state of heightened sensitivity (the "priming effect") where even minimal stimuli can trigger symptoms. Persistent type 2 inflammatory microenvironment triggers goblet cell hyperplasia, basement membrane thickening, subepithelial fibrosis, and neovascularization in the nasal mucosa. Although these structural changes are typically less severe in AR than in asthma, they form the pathological basis for persistent mucosal hyperreactivity, ultimately rendering AR a chronic, systemic disorder that is difficult to cure (Wang et al., 2025).

1.4. Literature Search Strategy

To ensure the scientific rigor and reliability of this systematic review, a comprehensive literature search was performed across multiple electronic databases, including Google Scholar and the China National Knowledge Infrastructure (CNKI). The search was conducted using a combination of core English keywords: *"Allergic Rhinitis" AND ("Traditional Chinese Medicine" OR "TCM") AND "Mechanism"*, together with their corresponding Chinese terminological equivalents. To guarantee the novelty and timeliness of the included evidence, only recently published literature was retrieved and screened. Studies that were not directly relevant to the research topic or exhibited insufficient scientific quality and rigor were excluded from the review.

2. Core Mechanisms of TCM in Treating AR from a Modern Medical Perspective

2.1. Modulation of the Gut Microbiota

The regulation of gut microbiota has become a promising therapeutic approach in the management of AR. Accumulating evidence indicates that the mechanisms of several TCM interventions are closely linked to their regulatory effects on intestinal microecology.

In addition to its previously mentioned roles, *Xiaoqinglong Decoction* has been shown to modulate the "gut-lung axis" (Liu et al., 2026). Using high-throughput sequencing, demonstrated that *Xiaoqinglong Decoction* increased the abundance of intestinal *Lactobacillus*, elevated short-chain fatty acid (SCFA) levels, inhibited histone deacetylase (HDAC) activity, and consequently reduced serum IgE and IL-4 levels in a murine model.

Beyond its mucosal actions, research by showed that *Mahuang Fuzi Xixin Decoction* enriches intestinal SCFA-producing bacteria (e.g., *Butyricicoccus*), modulates the Treg/Th17 balance, and lowers IgE and histamine levels.

Furthermore, ingredients such as *Lycium barbarum* (goji berry) and *Astragalus membranaceus* (huangqi) have been reported to exert prebiotic effects, stimulating the growth of *Bifidobacterium* and *Lactobacillus*, boosting SCFA production, and thereby promoting nasal immune tolerance via the gut-nose axis (Liu et al., 2026).

2.2. Protecting the Mucosal Barrier via Modulation of Programmed Cell Death

Recent research indicates that TCM can mitigate allergic rhinitis by regulating key forms of programmed cell death (PCD)—namely, apoptosis, pyroptosis, autophagy, and ferroptosis .

2.2.1. TCM Monomers

Polygonum cuspidatum (Huzhang) has the effects of promoting diuresis to relieve jaundice, clearing heat and detoxifying, and resolving stasis to relieve pain. Its active component, polydatin, can inhibit pyroptosis. confirmed that polydatin activates the PINK1-Parkin pathway to promote mitophagy.

Vitex negundo var. cannabifolia (Mujingzi) has the effect of resolving phlegm and descending qi. found that vitexin, the main active component of *Vitex negundo var. cannabifolia*, can protect nasal mucosal injury by activating the Sirt1/FoxO1 signaling pathway and inhibiting ferroptosis.

Glycyrrhiza uralensis (Gancao), a commonly used TCM for AR treatment, contains 18β-glycyrrhetinic acid. found that 18β-glycyrrhetinic acid inhibits epithelial cell autophagy.

Psoralea corylifolia (Buguzhi) exerts the therapeutic effects of tonifying the kidney to support yang, securing essence to reduce urination, descending qi for asthma relief, and warming the spleen to alleviate diarrhea. studied its active component, bavachin, and found that it can reduce NLRP3-mediated pyroptosis, promote barrier repair by inhibiting the PI3K/Akt/NF-κB pathway.

Gleditsia sinensis spine (Zaojiaoci) has the effects of promoting the drainage of toxins, antiparasitic effects. found that the extract of *Gleditsia sinensis* spine can inhibit MUC5AC mucin production induced by IL-4/IL-13 through the STAT3/STAT6 pathway, and alleviate mucus hypersecretion to protect the mucosa.

Panax notoginseng (Sanqi) exerts the therapeutic effects of resolving stasis to stop bleeding, as well as reducing swelling to relieve pain. found that its active component, notoginsenoside, can inhibit epithelial cell apoptosis and pyroptosis through AMPK/Drp1/NLRP3 and AMPK/Drp1/Caspase-3 pathways, thereby protecting the mucosa.

Meanwhile, triptolide (from *Tripterygium wilfordii*, Leigongteng) and baicalein (from *Scutellaria baicalensis*, Huangqin) have been confirmed to promote eosinophil apoptosis, thereby inhibiting inflammation and protecting the mucosa.

2.2.2. TCM Formulas

Mahuang Fuzi Xixin Decoction, a famous formula from Zhongjing, consists of *Ephedra sinica*, *Aconitum carmichaelii*, and *Asarum heterotropoides*, with the *effects* of supporting yang and consolidating the exterior. found that it inhibits NLRP3/Caspase-1/GSDMD-N pathway-mediated pyroptosis of nasal epithelial cells. Network pharmacology analysis of Mahuang Fuzi Xixin Decoction has revealed that its core active ingredients, such as naringenin, genkwanin, and deoxyandrographolide, can alleviate allergic rhinitis by modulating the PI3K-AKT and AMPK signaling pathways to suppress inflammatory mediator release (Cao et al., 2025).

Yupingfeng San, a classic TCM formula, contains *Saposhnikovia root*, *Astragalus membranaceus*, and *Atractylodes macrocephala*, exerting the effects of replenishing qi, *consolidating* the exterior, and arresting sweating. found that it inhibits mitochondrial reactive oxygen species (ROS) and the NLRP3 pathway, and downregulates Caspase-1 levels. confirmed that YPF+ (Yupingfeng San-derived *component*) can improve nasal mucosal injury in rats and alleviate AR symptoms such as sneezing, nasal itching, *and* rhinorrhea. Similarly, the specific material basis of Yupingfeng San has been identified through network pharmacology, highlighting active compounds like calycosin, formononetin, and quercetin. These specific ingredients synergistically target the TNF and MAPK signaling pathways to repair the mucosal barrier and restore immune homeostasis (Z. Liu et al., 2022).

Linggui Zhugan Decoction, originating from Jin Kui Yao Lue (Synopsis of the Golden Chamber), is composed of Poria cocos, Cinnamomum cassia, Atractylodes macrocephala, and Glycyrrhiza uralensis, exerting the therapeutic effects of warming yang to disperse fluid retention, invigorating the spleen, and promoting diuresis. demonstrated through an AR rat model that it downregulates the expression of MUC5AC and MUC5B, thereby promoting nasal mucosal tissue repair. For Linggui Zhugan Decoction, network pharmacology studies indicate that active compounds including cinnamaldehyde, glycyrrhizic acid, and atractylenolide can regulate oxidative stress and restore immune balance via multi-target inflammatory mechanisms (Yu et al., 2023).

Xinhuan Decoction is one of the classic TCM formulas, with *Magnolia biondii* and *Astragalus membranaceus* as the main herbs, and it has the effects of tonifying qi and unblocking the nasal orifices. confirmed that *Xinhuan Decoction* can downregulate the expression of Cyt-c, Caspase-3, and PARP in mucosal tissue, thereby alleviating mucosal lesions.

Xiaoqinglong Decoction, derived from the classic TCM work *Shang Han Lun* (Treatise on Febrile Diseases), consists of *Paeonia lactiflora*, *Cinnamomum cassia*, *Ephedra sinica*, *Zingiber officinale* (dried), *Asarum heterotropoides*, *Glycyrrhiza uralensis*, *Pinellia ternata*, and *Schisandra chinensis*. found that *Xiaoqinglong Decoction* can regulate the NLRP3/Caspase-1/GSDMD-N pathway, reduce the proliferation of nasal mucosal goblet cells, mast cell infiltration, and collagen fiber hyperplasia, and downregulate the expression of IL-4, IL-5, and IL-13 in serum and nasal mucosa. Furthermore, recent network pharmacology and molecular docking studies have elucidated the material basis of Xiaoqinglong Decoction. Core active ingredients such as ephedrine, quercetin, and paeoniflorin exert synergistic effects by directly

intervening in the TLR4/NF-κB signaling pathway to reduce allergic inflammation (Yang et al., 2025).

Yiqi Jiemin Decoction is a clinical empirical formula for treating allergic rhinitis with lung-spleen qi deficiency syndrome, developed by multiple experts in the Department of Otorhinolaryngology, the First Clinical Medical College of Beijing University of Chinese Medicine, through clinical practice (Dong, 2024). It is modified from *Xiaoqinglong Decoction* and *Yupingfeng San*, including herbs such as *Astragalus membranaceus*, stir-fried *Atractylodes macrocephala* with bran, *Saposhnikovia root*, honey-fried *Ephedra sinica*, *Cinnamomum cassia*, white *Paeonia lactiflora*, *Zingiber officinale* (dried), *Schisandra chinensis*, *Asarum heterotropoides*, *Magnolia biondii*, *Bupleurum chinense*, *Cicada slough*, and honey-fried *Glycyrrhiza uralensis*. Clinical practice has confirmed its significant efficacy (Yan et al., 2017). Yan et al. (2018) found that *Yiqi Jiemin Decoction* can effectively reduce VAS, TNSS, RQLQ, and nasal sign scores, reduce nasal secretions, and play a role in protecting the mucosa.

2.2.3. External Therapy

Traditional Chinese medicine (TCM) catgut embedding therapy exerts a favorable therapeutic effect on AR. Relevant studies have demonstrated that catgut embedding at the Yingxiang (LI20) acupoint can upregulate the expression of tight junction proteins Claudin-1 and Occludin, thereby enhancing the nasal mucosal barrier. Furthermore, this therapy can decrease the level of Osteopontin (OPN) in lung tissue and retard airway remodeling.

2.3. Regulation of Immune Homeostasis

The core mechanism of some TCM monomers, TCM formulas, and TCM external therapies lies in correcting immune dysregulation in AR patients, which is mainly manifested by inhibiting Th2 and Th17 responses, enhancing Th1 and Treg functions, and directly blocking the production and action of IgE.

2.3.1. Single Chinese Herbs and Their Active Components

Lonicera japonica (Jinyinhua), according to TCM theory, exerts the therapeutic effects of clearing heat to detoxify, as well as dispersing wind-heat. Lonicera japonica inhibits the occurrence of allergic reactions by reducing specific IgE levels and suppressing histamine release from mast cells. Its polysaccharides can inhibit splenic Th17 differentiation and decrease IL-17 levels (Liu et al., 2026). Meanwhile, Jian et al. (2017) observed that mice in the *Lonicera japonica* treatment group exhibited significantly reduced nose rubbing, sneezing, and rhinorrhea compared with the model group. Moreover, the eosinophil count in nasal mucosal pathological sections was markedly reduced, the serum concentrations of IL-4, IL-17, and sIgE were significantly decreased, and the expression of IL-17 mRNA and protein in tissues was suppressed, while the serum levels of IL-2 and IFN-γ were elevated. These results suggest that Lonicera japonica can modulate the expression of inflammatory factors in sensitized mice and exert a regulatory effect on immune function.

Astragalus membranaceus (Huangqi) has the effects of tonifying qi and elevating yang, benefiting the defensive qi and consolidating the exterior, promoting diuresis to reduce swelling,

generating fluid to quench thirst, activating stagnation to unblock obstruction, removing toxins and draining pus, and astringing sores to promote granulation. *Astragalus membranaceus* can regulate Th17/Treg cells and related inflammatory factors. found that astragaloside IV can effectively alleviate symptoms of allergic rhinitis in mice, reduce nasal mucosal tissue damage, decrease Th17 cells, and increase Treg cells in peripheral blood; this component can also significantly regulate the levels of IL-10, IL-6, IL-17, IgE, and TGF-β1 in the serum of allergic rhinitis mice, as well as effectively downregulate the expression levels of IL-17 and TGF-β mRNA in the nasal mucosal tissue of allergic rhinitis mice. Moreover, astragalus polysaccharide contained in *Astragalus membranaceus* also has a good curative effect on reducing the inflammatory response of allergic rhinitis, which may be because astragalus polysaccharide can act on the NF-κB-mediated Treg/Th17 imbalance, thereby effectively reducing the release of inflammatory factors (He et al., 2022).

Scutellaria baicalensis (Huangqin), in line with TCM theory, exerts the therapeutic effects of clearing heat to dry dampness, purging fire to detoxify, and calming the fetus. Baicalein can regulate the balance between regulatory T cells and helper Th17 cells, and inhibit serum histamine and IgE levels (Liu et al., 2026). Yang et al. (2022) found that baicalein treatment significantly reduced the number of sneezes and nose rubbings in AR rats, decreased serum histamine and IgE levels, and alleviated sinus mucosal pathological damage and leukocyte infiltration.

Panax ginseng (Hongshen) has the effects of greatly tonifying primordial qi, restoring pulse and relieving collapse, and benefiting qi to control blood. As one of its active components, ginsenoside Rd can inhibit immune cell activation. Kim et al. (2019) found that ginsenoside Rd can inhibit the expression of IL-4, IL-5, and IL-13 in the colon of OVA-sensitized mice, reduce the frequency of rubbing/scratching, and alleviate nasal mucosal inflammation.

Ephedra sinica (Mahuang) has the effects of inducing diaphoresis to relieve exterior syndrome, dispersing cold to unblock stagnation, dispersing the lung to relieve asthma, and promoting diuresis to reduce swelling. Its main active components include ephedra polysaccharide, which has the effect of regulating the balance of helper T cells. found that ephedra polysaccharide promotes the secretion of miR-146a-5p, increases IFN-γ and decreases IL-4 in AR rats through Smad3/GATA-3 interaction, and promotes the pathological repair of nasal mucosa(Liu et al., 2026).

Amomum villosum (Sharen) can resolve dampness to stimulate appetite, warm the middle to stop diarrhea, and regulate qi to prevent abortion; it has the effects of regulating Th1/Th2 balance and inhibiting mast cell degranulation. confirmed through experiments that its internal volatile oil components can reduce the frequency of nose rubbing and sneezing in AR mice, inhibit NF-κB phosphorylation, and alleviate inflammation in nasal and lung tissues.

Magnolia biondii (Xinyi) can disperse wind-cold, unblock nasal orifices, and has antihistamine, anti-allergic, and immune homeostasis-maintaining effects. The volatile oil components in *Magnolia biondii* can inhibit inflammation, reduce the release of inflammatory mediators such as IL-1 and tumor necrosis factor (TNF) in the downstream signaling pathway of TLRs, inhibit the

number and activity of eosinophils and mast cells, and suppress T cell activation and mast cell aggregation, thereby improving AR symptoms in patients (Choi et al., 2021). Meanwhile, a study (Hu et al., 2021) found that its volatile oil components can reverse the Th1/Th2 imbalance in the peripheral blood of AR guinea pigs, decrease the levels of IL-4 and IFN-γ, and maintain immune dynamic balance.

Salvia miltiorrhiza (Danshen), according to TCM theory, exerts the therapeutic effects of promoting blood circulation to eliminate blood stasis, unblocking meridians to alleviate pain, clearing the heart to calm the mind, and cooling blood to resolve carbuncles. Its tanshinone IIA component can inhibit Th2 cytokines. It can also reduce histamine release from mast cells by inhibiting the NF-κB pathway, thereby alleviating AR reactions.

Asarum heterotropoides (Xixin), in line with TCM theory, exerts the therapeutic effects of relieving exterior cold, dispelling wind to ease pain, unblocking orifices, and warming the lung to disperse fluid retention. The internal volatile oil components of this herb can regulate the distribution of T lymphocyte subsets, reduce the ratio of T helper cells Th1/Th2, and restrain the production of endorphins and interferons. Experiments have shown that it can regulate the distribution of T lymphocyte subsets, decrease the Th1/Th2 ratio, and inhibit the formation of specific IgE.

2.3.2. TCM Formulas

Chuanxiong Chatiao San, derived from *Taiping Huimin Heji Ju Fang* (Formulas of the Peaceful Benevolent Dispensary), is a classic TCM formula with the effect of dispersing wind to relieve pain, consisting of *Mentha haplocalyx*, *Ligusticum chuanxiong*, *Schizonepeta tenuifolia*, *Asarum heterotropoides*, *Saposhnikovia root*, *Angelica dahurica*, *Notopterygium incisum*, and *Glycyrrhiza uralensis*. *Chuanxiong Chatiao San* can reduce the level of serum ovalbumin-specific IgE (OVA-sIgE) and the expression of related inflammatory factors. Mao et al. (2021) confirmed that this formula significantly increases the mRNA expression of T-bet and STAT1, decreases the expression of GATA3, and reverses Th1/Th2 drift by regulating the JAK/STAT pathway. In the case of Chuanxiong Chatiao San, network pharmacology has identified ligustilide, senkyunolide I, and ferulic acid as the primary bioactive components. These ingredients exert anti-inflammatory effects by directly acting on neuro-immune regulation and modulating vascular permeability in the nasal mucosa (Wang et al., 2022).

Gancao Ganjiang Decoction, derived from the classic TCM work *Shang Han Lun* (Treatise on Febrile Diseases), consists of *Glycyrrhiza uralensis* and *Zingiber officinale* (dried), with the effects of transforming yang through acridity and sweetness, and warming the middle to tonify qi. It has the function of regulating cytokine imbalance. found that this formula can increase serum IFN-γ and decrease IL-4 in AR rats, reverse the deviation of Th cell differentiation, and repair the pharyngeal mucosal structure. Furthermore, the material basis of Gancao Ganjiang Decoction has been linked to key compounds such as liquiritin, glycyrrhetinic acid, and 6-gingerol. Network pharmacology demonstrates that these ingredients synergistically inhibit the NF-κB signaling pathway and reduce oxidative stress, thereby ameliorating mucosal inflammation (Lee et al., 2021).

Huangqi Guizhi Wuwu Decoction, derived from the classic TCM work*Shang Han Lun* (Treatise on Febrile Diseases), consists of *Astragalus membranaceus*, *Cinnamomum cassia*, fresh *Zingiber officinale*, *Ziziphus jujuba*, and *Paeonia lactiflora*, which can tonify qi and deficiency, warm the meridians to unblock collaterals, and regulate nutritive and defensive qi. Studies have proved that it has a certain immunomodulatory effect. Chen et al. (2020) found that modified *Huangqi Guizhi Wuwu Decoction* can effectively regulate the immune balance of Th1/Th2 and Th17/Treg cells in the body.

Modified *Wuwei Shigao Decoction* is developed by Liu by adding *Magnolia biondii* to *Wuwei Shigao Decoction* from *Sisheng Xinyuan* (Four Sages' Heart Source), including *Schisandra chinensis*, gypsum, *Prunus armeniaca*, *Pinellia ternata*, *Scrophularia ningpoensis*, *Poria cocos*, *Platycodon grandiflorus*, and fresh*Zingiber officinale*; it can disperse with acridity and warmth, unblock orifices with aroma, dispel wind-cold pathogens externally, and ascend and diffuse lung-stomach clear qi internally. Liu et al. (2018) found that this formula can improve the symptoms of AR mice, inhibit the levels of serum IgE, TNF-α, and IL-1β, and increase serum IFN-γ and nasal mucosal T-bet expression.

Sanfeng Tongqiao Dropping Pills is a Chinese patent medicine used for the treatment of AR, exerting the therapeutic effects of clearing heat, dispelling wind, dispersing cold, and unblocking nasal orifices. It is clinically applied to alleviate nasal congestion, runny nose and other related symptoms caused by AR. It has the effect of inhibiting type II immune response. Through network pharmacology and experiments, Sun et al. (2023) confirmed that it acts on the T cell receptor and HIF-1α pathway, reduces the levels of IL-4 and IL-13, and stabilizes the mast cell membrane.

Lu'e Biyan Formula is an empirical formula by Professor Zhang Shunan from China-Japan Friendship Hospital, consisting of deer antler glue, *Centipeda minima*, *Stellaria dichotoma*, *Prunus mume*, *Saposhnikovia root*, *Tribulus terrestris*, *Xanthium sibiricum*, and *Glycyrrhiza uralensis*, with the effect of warming yang and unblocking nasal orifices. The research teams led by Jia et al. (2022) and Yan Jiaxin found that *Lu'e Biyan Formula* restores the balance of Th1/Th2 and Th17/Treg by inhibiting the Notch1-Jagged1 signaling axis (Yan et al., 2024).

Yiqi Wenyang Formula, consisting of *Astragalus membranaceus*, *Codonopsis pilosula*, *Zingiber officinale* (dried), *Cinnamomum cassia*, *Ephedra sinica*, *Schisandra chinensis*, *Pheretima aspergillum*, *Magnolia biondii*, and *Glycyrrhiza uralensis*, is an empirical formula by Professor Yan Daonan, a renowned TCM doctor in Jiangsu Province, with the effects of tonifying qi, warming yang, and dispersing the lung to unblock nasal orifices. Shi et al. (2014) found that this formula promotes the increase of Treg cells in the body, increases the levels of IL-10 and TGF-β1, and inhibits IL-4 and IL-13 produced by Th2, thereby alleviating AR symptoms.

In addition, some self-designed formulas treat AR by regulating immune mechanisms(Gu et al., 2025). *Wenfei Yiqi Formula* improves AR symptoms and promotes the recovery of nasal function by regulating the balance of Th1/Th2 and Th17/Treg cells (Jiang et al., 2023). *Yiqi Gufei Qufeng Formula* reduces the recurrence of AR by improving immune function and alleviating inflammatory response (Fan et al., 2023). *Buqi Sanhan Formula* exerts its therapeutic effect on

AR by reducing the levels of serum total IgE, specific IgE, and IL-17A (Li et al., 2022). *Majie Formula* inhibits the expression of IgE by B cells through the IL-4 and IL-13 signaling pathways, thereby exerting a therapeutic effect on AR (Wu et al., 2020).

2.3.3. External Therapy

Studies (Kim et al., 2022; Li et al., 2024; Li et al.; Zhang et al., 2022; Zhu et al., 2024) have reported that the mechanisms of some TCM external therapies in the treatment of AR are related to immune regulation. Acupuncture (body acupuncture) can effectively improve the symptoms of allergic rhinitis (AR), reduce eosinophil count, and serum levels of IL-4, IL-5, IgE, and sIgE. Heavy moxibustion at acupoints can improve nasal obstruction in AR patients by reducing serum IL-33 and EOS levels. Zhang et al. found that thunder-fire moxibustion significantly reduces IgE and IL-10 levels. Ear acupoint pressing has the effect of activating the "periphery-central-target organ" regulatory mode. Its mechanism involves inhibiting the differentiation of Th1 to Th2 and reducing IgE synthesis through the stimulation of vagus nerve fibers in the cavum conchae (tVNS).

2.4. Regulation of Inflammatory Signaling Pathways

Certain TCM monomers, TCM formulas, and TCM external therapies can intervene in signaling pathways including TLRs, NF-κB, MAPK, and JAK/STAT to treat allergic rhinitis.

2.4.1. TCM Monomers

Tripterygium wilfordii (Leigongteng), in TCM theory, has the effects of dispelling wind and dampness, promoting blood circulation to unblock collaterals, reducing swelling and relieving pain, and killing insects and detoxifying. *Tripterygium wilfordii* possesses immunosuppressive and anti-inflammatory effects. Chen et al. (2020) confirmed that triptolide, an active component in *Tripterygium wilfordii*, reduces serum total IgE and histamine levels through the TLR4/NF-κB pathway, downregulates IL-4 expression in the nasal mucosa of AR rats, decreases TGF-β1 secretion by EOS, and regulates Th1/Th2 balance.

Lycium barbarum (Gouqi), in TCM theory, has the effects of nourishing the liver and kidney, and benefiting essence to improve eyesight. Its main active component, Lycium barbarum polysaccharide (Xie et al., 2024), exerts an immunomodulatory effect (Xiao et al., 2022). Wang et al. (2024) found that it corrects Th1/Th2 imbalance and alleviates nasal mucosal inflammation by inhibiting the expression of proteins related to the TLR/NF-κB signaling pathway.

Saposhnikovia root (Fangfeng) has the effects of dispelling wind to relieve exterior syndrome, overcoming dampness to relieve pain, and relieving spasm. Among them, saposhnikovia polysaccharide is its main active component. Ren et al. (2023) found that saposhnikovia polysaccharide can inhibit the NF-κB/STAT3 signaling pathway, promote AQP5 expression, and improve the pathological morphology of AR rats. In addition, He et al. (2024) found that saposhnikovia polysaccharide can significantly promote macrophages to release NO, TNF-α, and IL-6, increasing the density of immune cells and the number of macrophages.

Rheum palmatum (Dahuang) , according to TCM theory, exerts the therapeutic effects of purging intestines to relieve accumulation, clearing heat to purge fire, cooling blood to detoxify,

stopping bleeding, removing blood stasis to unblock meridians, and promoting diuresis to alleviate jaundice. Studies have found that its extracted component, emodin, can downregulate miR-375 and promote KLF5 expression, thereby inhibiting the inflammatory response in AR mice.

Both *Paeonia lactiflora* (Chishao, red peony root) and *Paeonia lactiflora* (Baishao, white peony root) are rich in paeoniflorin. Paeoniflorin has anti-inflammatory and immunomodulatory effects. Studies have confirmed that paeoniflorin regulates cellular immune balance by increasing IFN-γ levels and decreasing the levels of IL-33, ST2, TGF-β1, ICAM-1, IL-4, and IgE (Wang et al., 2016). Meanwhile, He et al. (2024) found that paeoniflorin can inhibit oxidative stress closely related to immune response, regulate cellular autophagy, and maintain Th1/Th2 balance.

2.4.2. TCM Formulas

Cang'er Wendan Decoction consists of *Poria cocos* (Poria), *Xanthium sibiricum* (Xanthii Fructus), processed *Pinellia ternata* (Qingbanxia, prepared Pinellia tuber), bamboo shavings processed with ginger (Jiangzhuru, ginger-processed bamboo shavings), *Angelica dahurica* (Baizhi), *Citrus reticulata* peel (Chenpi, dried tangerine peel), stir-fried *Aurantii Fructus* with bran (Fuchaoshis hi, bran-stir-fried Aurantii Fructus), *Mentha haplocalyx* (Bohe, peppermint), *Magnolia biondii* (Xinyi, Biond Magnolia Flower), and raw *Glycyrrhiza uralensis* (Shenggancao, raw Liquorice). Li et al., 2025) found that *Cang'er Wendan Decoction* controls allergic rhinitis by inhibiting the TLR4/MyD88/NF-κB signaling pathway, reducing the levels of IgE and IL-6, and increasing IL-10 levels.

Huanglian Jiedu Decoction, derived from *Zhouhou Beiji Fang* (Emergency Prescriptions Worth a Thousand Gold), is a classic TCM formula composed of *Coptis chinensis* (Huanglian, Coptis Root), *Scutellaria baicalensis* (Huangqin, Baical Skullcap Root), *Phellodendron chinense* (Huangbai, Amur Corktree Bark), and *Gardenia jasminoides* (Zhizi, Cape Jasmine Fruit), which can clear fire from the triple jiao. Liu et al. (2021) found that *Huanglian Jiedu Decoction* reduces the proportion of nasal mucosal goblet cells by inhibiting the TLR4/NF-κB signaling pathway.

Biyuan Tongqiao Granules is a commonly used Chinese patent medicine with the effects of dispersing wind-heat and diffusing the lung to unblock nasal orifices. Chai et al. (2025) showed that this granule can target PTGS2, MAPK, and HIF-1 signals by inhibiting the TLR4/NF-κB signaling pathway, regulate Th cell balance, and reduce the levels of IL-4 and TNF-α.

Jingfang Granules is a commonly used Chinese patent medicine with the effects of inducing diaphoresis to relieve exterior syndrome and dispersing wind to eliminate dampness. found that *Jingfang Granules* can regulate the endoplasmic reticulum stress signaling pathway (GADD, ATF4, p-eIF2α), inhibit metabolic and inflammatory pathways, thereby controlling allergic rhinitis.

Bimin Formula consists of *Astragalus membranaceus* (Huangqi), *Atractylodes macrocephala* (Baizhu), *Saposhnikovia root* (Fangfeng), *Cinnamomum cassia* (Guizhi), *Paeonia lactiflora* (Baishao, white peony root), *Cicada slough* (Chanyi), *Pheretima aspergillum* (Dilong), *Angelica dahurica* (Baizhi), *Asarum heterotropoides* (Xixin), *Siegesbeckia orientalis* (Xixiancao), *Cynanchum paniculatum* (Xuchangqing),*Terminalia chebula* (Hezi), and raw *Glycyrrhiza*

uralensis (Shenggancao). It exerts anti-inflammatory and anti-allergic effects and improves AR symptoms by regulating the transmembrane protein 16A (TMEM16A)/MUC5AC/NF-κB/Th17/IL-17A signaling pathway and related factors (Shi et al., 2024; Xu et al., 2024).

2.4.3. External Therapy

Studies have reported that acupoint application and acupoint injection can regulate inflammatory pathways(Xin & Ming, 2025). Jin et al. (2018) found that acupoint application significantly reduces nasal mucosal inflammatory cell infiltration by inhibiting the TLR4/NF-κB pathway. Meanwhile, acupoint injection can downregulate the expression of histamine receptors H1R and H4R; Zhou confirmed that it corrects Th1/Th2 imbalance through the TLR4/AP-1 pathway. In addition, it can also inhibit p38 MAPK phosphorylation and reduce MUC5AC secretion.

2.5. Neuro-Immune Regulation

The main mechanism of some TCM monomers and TCM external therapies in the treatment of AR lies in the intervention of neuropeptide release and neuroreceptors, which are introduced as follows.

2.5.1. TCM Monomers

Ephedra sinica (Mahuang), an exterior-releasing herb, has the effects of inducing diaphoresis to relieve exterior syndrome, dispersing the lung to relieve asthma, promoting diuresis to reduce swelling, and dispersing cold to unblock stagnation. Ephedrine is one of its main active components. Ling et al. (2022) found that it regulates the Th2-type immune response in AR rats through the TSLP/OX40L pathway. Meanwhile, in addition to the functions mentioned earlier, modern pharmacological studies have found that the volatile oil of *Magnolia biondii* (Xinyi) can counteract neurogenic vasodilation (Liang et al., 2011), constrict nasal mucosal blood vessels, inhibit the release of IL-1 and TNF-α downstream of TLRs, and alleviate neurogenic edema.

2.5.2. External Therapy

Acupuncture has a significant therapeutic effect on AR through the regulation of the nervous system (Xiao & Xu, 2025). Intranasal acupuncture (at Neiyingxiang/EX-HN9 and nasal concha) has the effect of rapidly improving the allergic state of the nasal mucosa. Studies confirmed that it reduces TRPV1 activity and blocks the release of neuropeptides through the "axon reflex". Meanwhile, acupuncture at the nasal concha increases NPY levels, effectively constricting blood vessels and relieving nasal obstruction. Studies found that after acupuncture, the serum IgE level of patients and the contents of SP and VIP in nasal secretions decreased significantly. Meanwhile, Tian found that acupuncture at the sphenopalatine ganglion (Xinwu point) has a direct effect on regulating the neurofunction of the nasal mucosa. It inhibits the TLR4/MyD88/NF-κB pathway and effectively balances the Th1/Th2 and Treg/Th17 systems. Catgut embedding therapy has long-acting physical stimulation and neuroregulatory effects; studies have found that it inhibits the conduction of C-type sensory fibers and reduces the release of SP, CGRP, and VIP through the "Yingxiang (LI20)-trigeminal nerve-sphenopalatine ganglion" pathway.

3. Summary and Outlook

3.1. Mechanism Review: Biological Basis of Systematic Intervention

Research on the mechanisms of TCM in treating allergic rhinitis has transcended the superficial level of symptom alleviation and probed into the in-depth logic of the body's regulatory network. From the perspective of modern systems biology, the TCM theory focusing on syndrome differentiation and treatment has been specifically deconstructed into the synergistic effects of multiple dimensions, including the regulation of the microecological axis, the repair of cellular barriers, the regulation of immune homeostasis, the blocking of inflammatory signals, and neuro-immune regulation.

These five mechanisms do not exist in isolation but exhibit a highly coupled network relationship. For example, the remodeling of intestinal microecology can distally regulate the function of Treg cells through the "gut-nose axis", thereby promoting the recovery of immune homeostasis; the improvement of the immune environment can reduce the attack of inflammatory mediators on the nasal mucosal epithelium, creating conditions for the repair of the physical barrier. This multi-target, multi-level dynamic regulatory mechanism effectively corrects the abnormal biological network state of AR patients, establishing a solid biological basis for TCM in the treatment of AR. However, despite significant progress in the exploration of micro-mechanisms, TCM still faces multiple challenges from theoretical cognition to clinical evaluation paradigms when attempting to integrate into the mainstream modern medical system dominated by reductionism.

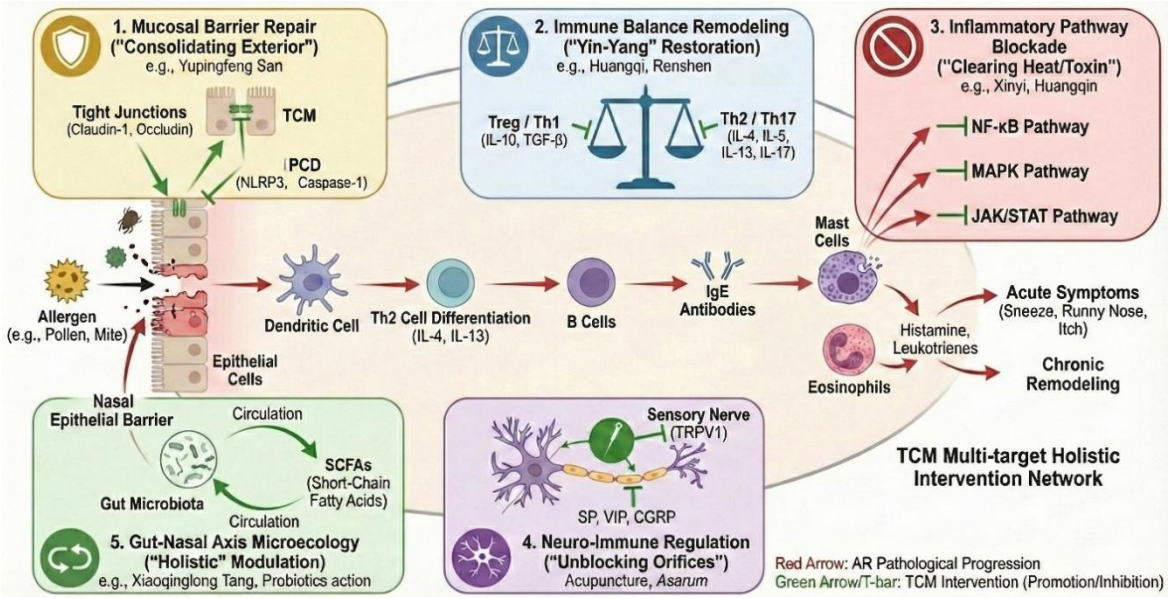


Figure 1. Panoramic View of Allergic Rhinitis Pathogenesis and TCM Regulatory Targets

3.2. Strategic Synergy and Clinical Value of Integrated Traditional Chinese and Western Medicine (TCM-WM) with Complementary Advantages

The integrated TCM-WM treatment of allergic rhinitis is not a simple superposition of two medical systems, but an in-depth integration and strategic synergy based on their respective

advantages, aiming to solve clinical bottlenecks that are difficult to overcome with a single therapy. Modern conventional Western medicine treatment, based on the ARIA guidelines, takes second-generation antihistamines, nasal glucocorticoids, and leukotriene receptor antagonists as the cornerstone. With its strong anti-inflammatory and anti-allergic effects, it can quickly control acute symptoms such as nasal obstruction and rhinorrhea within hours, serving as a vanguard in the acute attack stage of AR. However, Western medicine treatment often faces the dilemma of recurrence after drug withdrawal; long-term use of hormones may lead to nasal mucosal atrophy, drug-induced rhinitis, and corticosteroid resistance, and some patients cannot tolerate the somnolent side effects of antihistamines. In contrast, relying on the holistic concept and syndrome differentiation and treatment, TCM has unique advantages in regulating immune imbalance, improving allergic constitution, and preventing recurrence. This complementary strategy of "treating acute symptoms urgently and addressing the root cause in remission" constitutes the core of the clinical value of integrated TCM-WM.

Evidence-based medicine strongly supports the superiority of this synergistic effect. Large-scale Meta-analysis (Zhu & Zhang, 2022) has shown that TCM decoction combined with conventional Western medicine in the treatment of AR is not only superior to the single Western medicine group in short-term symptom control, but also significantly improves the long-term effective rate (RR=1.79), indicating that combined treatment can effectively break the vicious cycle of the inflammatory cascade reaction. Especially in terms of controlling the recurrence rate, the combination of exterior-consolidating preparations such as *Yupingfeng San* and loratadine can significantly reduce the six-month recurrence rate of AR from 41.7% in the single Western medicine group to 23.1% (Tong & Yan, 2025), with no serious adverse reactions, achieving the clinical goal of reducing toxicity and enhancing efficacy. In addition, for patients with refractory AR or severe nasal obstruction, integrated TCM-WM provides more dimensional intervention methods. For example, when Western medicine control is poor, the introduction of acupuncture at the sphenopalatine ganglion or the use of aromatic orifice-unblocking drugs such as *Cang'erzi San* can instantly relieve nasal obstruction caused by severe mucosal edema by improving local microcirculation and regulating neurogenic inflammation, making up for the limitations of Western medicine in certain pathological links. To enhance the clinical translational value of TCM interventions, it is recommended to establish precision application scenarios by matching modern clinical classifications (e.g., seasonal or perennial) and severity grading (e.g., mild, moderate, or severe) with TCM syndrome differentiation.

In the context of modern network pharmacology, the efficacy of TCM formulas in treating AR is not a mere enumeration of independent compounds, but a highly coordinated synergistic network. Within this network, core active ingredients exhibit clear priority and dynamic crosstalk, closely mirroring the traditional TCM principle of "Sovereign-Minister-Assistant-Courier" (Jun-Chen-Zuo-Shi) (Shao & Zhang, 2013; Wang et al., 2021).Through topological analysis, components with the highest degree centrality (Hub nodes, such as quercetin) often act as the "Sovereign" (Jun) by broadly modulating extensive upstream immune networks and the gut-nose axis. Concurrently, "Minister" (Chen) components (such as ephedrine and paeoniflorin in *Xiaoqinglong Decoction*) exhibit synergistic actions by targeting specific downstream

inflammatory cascades (e.g., TLR4/NF-κB). This interaction forms a multi-level dual-blockade—one component may enhance mucosal permeability to promote the absorption of another, while simultaneously acting on parallel signaling pathways to prevent inflammatory evasion (Wang et al., 2021). Thus, clarifying these hub nodes and their crosstalk mechanisms provides a precise material basis for the multi-target synergy of TCM.

From an integrated medicine perspective, modern microscopic mechanisms often correspond to macroscopic TCM syndromes. For example, mucosal barrier damage may be understood as an insufficiency of healthy Qi (Zhengqi deficiency); while gut microbiota dysbiosis is closely related to Spleen and Stomach weakness. This echoes the classical TCM theory in the Miraculous Pivot (Lingshu) that "man receives Qi from grain, which enters the stomach and is transmitted to the lungs."Therefore, individualized treatments can be optimized based on these mappings and clinical severity. For mild or seasonal AR, which often presents with Zhengqi deficiency, therapies focusing on mucosal repair and barrier consolidation, such as Yupingfeng San or acupoint catgut embedding, are generally preferred to prevent recurrence. Conversely, for moderate-to-severe AR during the acute exacerbation phase, rapid symptom control is often necessary. Symptom-specific formulas (e.g., Xiaoqinglong Decoction) or intranasal acupuncture may be utilized to target underlying inflammatory pathways.

Furthermore, when formulating TCM prescriptions, clinicians are encouraged to fully leverage the "multi-component and multi-target" advantages by selecting herbs that act synergistically across diverse, complementary pathways. Concurrently, the excessive combination of herbs competing for the exact same molecular target should be carefully evaluated or avoided, in order to prevent potential competitive inhibition and ensure optimal therapeutic efficacy.

3.3. Existing Problems and Challenges

Although TCM has shown great potential in the treatment of AR, to achieve the leap from traditional empirical medicine to modern scientific medicine, it is still necessary to face a series of severe challenges, among which the lack of standardization and the "mechanism black box" are the core bottlenecks restricting its international development (Dong et al., 2025). Firstly, the specific mechanism of TCM in the treatment of AR is still largely in a black box state. How hundreds of chemical components in compound prescriptions interact with each other at the pharmacokinetic level, how they are transformed in the intestinal flora and exert systematic regulatory effects, currently lacks sufficient in vivo and in vitro experimental verification, making it difficult for TCM to be fully accepted by the modern mainstream medical community accustomed to reductionist thinking.

In addition, the uneven quality of clinical research is also an undeniable problem. Although there are a large number of randomized controlled trials on TCM in the treatment of AR, high-quality, large-sample, multi-center, and strictly blinded Grade A evidence is still scarce. Some studies have defects such as unclear randomization methods and over-reliance on subjective symptom scores for outcome indicators, which reduces the credibility of the conclusions. At the same time, research on safety and drug interactions is relatively lagging behind. Although TCM is generally safe, drugs such as *Asarum heterotropoides* (Xixin) and *Xanthium sibiricum* (Cang'erzi)

have potential risks of liver and kidney toxicity if improperly processed or used in excess; when combined with Western medicine, whether the complex interactions between components will change the pharmacokinetic characteristics of Western medicine currently lacks systematic pharmacological data support.

3.4. Future Outlook: Paradigm Innovation in Standardization, Precision, and Evidence-Based Practice

Faced with the above challenges, the future development of TCM in the treatment of AR will surely evolve along the path of standardization, precision, and evidence-based practice.

In terms of standardization, the international guidelines and expert consensuses successively released in 2024 and 2025 are of milestone significance (Fu & Zhang, 2025). By introducing the GRADE evidence grading system, modern TCM is constructing clinical pathways based on rigorous evidence, clarifying the diagnostic criteria for core syndromes such as lung qi deficiency-cold and spleen qi deficiency, and making graded recommendations for therapies such as *Tongqiao Biyan Granules* and acupuncture at the sphenopalatine ganglion. This has ended the previous chaotic situation of "one prescription for thousands of patients" that was difficult to replicate, providing an operable standardized plan for primary medical care.

Precision and personalized treatment are the key growth points for the future breakthrough of TCM, whose core lies in the in-depth integration of TCM syndrome differentiation and modern medical molecular typing.

On the one hand, the combination of Whole Genome Sequencing (WGS) and network pharmacology is revealing the scientific connotation of the multi-target and network-based intervention of TCM compounds. The latest WGS study found that the pathogenesis of AR is closely related to rare gene mutations in the focal adhesion pathway(Zhang et al., 2019), which involves angiogenesis and barrier function. Traditional single-target Western medicine is difficult to cover such complex genetic heterogeneity, while TCM compounds (such as those for qi deficiency and blood stasis) naturally have multi-component characteristics, which can simultaneously intervene in multiple nodes in the network and systematically reshape the focal adhesion pathway. This network-based regulatory mechanism provides a solid genomic basis for explaining the unique efficacy of TCM in patients with familial and refractory AR.

On the other hand, the application of artificial intelligence (AI) and digital twin technology will facilitate the progress and development of TCM's personalized diagnosis and treatment model. The traditional "one patient, one strategy" often relies on the personal experience of doctors, while the future precision TCM can construct virtual patients based on the patient's multi-modal data (Yu et al., 2025) (genome, single-cell sequencing data, tongue manifestation characteristics). AI is used to simulate the dynamic network of cell states in AR patients at different stages such as remission and attack, and conduct high-throughput virtual drug screening and efficacy prediction. For example, AI algorithms can predict the regulatory effect of specific TCM components on ILC2 or pathogenic Th2 cells, and even predict the potential side effects of TCM-WM combination, thereby formulating the optimal precision treatment plan for patients. This "AI +

TCM" model not only avoids the risk of trial and error, but also elevates TCM's personalized treatment to a new level of digitalization and intelligence.

Finally, evidence-based practice will be the touchstone for testing all reform achievements. Future clinical research will no longer be limited to the improvement of subjective symptoms, but will introduce more objective biomarkers as outcome indicators. At the same time, real-world research will become an important tool to evaluate the long-term efficacy and health economics value of TCM, providing solid evidence support for the formulation of medical insurance policies and the global promotion of TCM. In summary, with the in-depth intervention of modern science and technology, TCM is undergoing a profound transformation, and is expected to provide a new treatment strategy with both precision and holistic view for hundreds of millions of AR patients worldwide.

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A Study on the Development and Current Status of Curcuma wenyujin and Its Derivatives in the Context of Industrial Modernization: A Case Study of Rui'an City, Wenzhou

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Abstract

As one of the Eight Zhejiang Medicinal Materials, Curcuma wenyujin can be processed into three herbal variants from different medicinal parts. It contains abundant sesquiterpenes and monoterpenes, presenting anti-tumor, anti-inflammatory and antioxidant bioactivities. This study targeted the Curcuma wenyujin industry in Rui’ an, Wenzhou, to clarify its current development status and propose targeted improvements for production and sales. Taking local farmers, pharmaceutical enterprises and pharmacy practitioners as research objects, field visits, interviews and questionnaire surveys were applied for empirical analysis. The results indicated that local manufacturers mainly source raw materials from GAP bases to develop health and cosmetic products. However, the industry suffers from low public awareness, high market prices, limited mechanized production and low overall profits. This study identifies major industrial bottlenecks across planting, processing and marketing. It suggests integrating multi-stakeholder demands to stabilize medicinal quality, advance mechanization, strengthen popular science and optimize operational layouts, so as to elevate economic benefits and support the standardized, sustainable development of Curcuma wenyujin and its derivatives.

Keywords: Curcuma Wenyujin; Plant; Derivative Products; Practical Application

1. Introduction

Curcuma wenyujin is a renowned authentic medicinal herb from Rui’an City, Zhejiang Province, and is listed as one of the “Eight Zhejiang Herbs” (Li et al., 2023). Its rhizomes or tubers can be processed into three different types of traditional Chinese medicines through various

preparation methods: fresh rhizome slices are called “Pian Jianghuang,” boiled and sun-dried rhizomes are called “Wen’eshu,” and boiled and sun-dried tubers are called “Wenyujin” (Li et al., 2020). Wenyujin has the effects of clearing the heart and relieving depression, as well as removing blood stasis and alleviating pain; it is commonly used clinically to treat chest pain and angina pectoris, among other conditions (Zheng, 2019; Tao, 2015). Wen’eshu can promote the flow of qi and break up blood stasis; it is commonly used clinically in the treatment of early-stage cervical cancer (Commission, 2020). Pian Jianghuang can treat chest and abdominal distension and pain, as well as irregular menstruation (Editorial Committee of Flora of China, 1993). Derivatives of Curcuma wenyujin are also used in modern clinical practice; its essential oil is formulated into the proprietary Chinese medicine BaoFuKangShuan; and β-elemene, a component of the volatile oil, has been developed into an injectable solution and used as a non-cytotoxic anticancer drug (Li et al., 2023). Furthermore, a variety of marketed proprietary Chinese medicines with Curcuma wenyujin as a core ingredient are classified into four main categories according to their functions: blood-activating analgesic, liver-soothing and depression-relieving, mind-calming and brain-refreshing, and others. Representative products include Mai Luo Tong, a blood-activating analgesic commonly used for cardiovascular diseases; Jieyu Anshen Ke Li, a liver-soothing preparation widely applied in mood disorder treatment; and Angong Niuhuang Wan, a classic mind-calming emergency medicine for acute conditions. These products all have clear clinical positioning and quality standards based on the Chinese Pharmacopoeia.

Currently, some pharmaceutical companies in Ruian have begun processing and developing derivatives of Curcuma wenyujin; however, the variety of these derivatives remains limited, and their actual clinical applications are relatively restricted. Against the backdrop of industrial modernization, the industry faces the dilemma of products that are either “unusable” or “cannot be utilized.” Due to the combined influence of multiple factors, our team has launched a research study (Li, 2009) to explore pathways for the development of pharmaceutical derivatives. This study distinguishes itself from previous pharmacological research that focused solely on drug mechanisms; instead, it adopts a full industrial chain perspective. Given these challenges, Ruian, as the geo-authentic production area of Curcuma wenyujin with a centuries-long cultivation history, serves as an ideal typical case. It not only possesses a foundational industrial chain but also reflects the typical bottlenecks in the modernization of traditional Chinese medicine industries. Therefore, our team conducted a comprehensive analysis of the Rui’an Curcuma wenyujin product chain based on previous studies (Li, 2009), aiming to explore feasible approaches for the high value-added development of medicinal derivatives and to address the existing gaps in the industry.

2. Methodology

2.1. Study Design and Period

This study employed a cross-sectional mixed-methods design, combining quantitative surveys with qualitative field interviews. The research was conducted from July 2024 to September 2024 in Rui’an City, Wenzhou, Zhejiang Province, a nationally recognized production area of Curcuma wenyujin.

2.2. Participant Groups and Sampling

Four independent stakeholder groups were targeted:

Farmers (Group 1): Convenience sample of 68 individuals engaged in or familiar with Curcuma wenyujin cultivation.

Pharmaceutical enterprises (Group 2): 36 companies involved in the R&D, production, or sale of Curcuma wenyujin derivatives. These enterprises were located primarily in Rui'an City and surrounding areas of Wenzhou, and included traditional Chinese medicine manufacturers, health supplement producers, and cosmetic companies that either process Curcuma wenyujin or develop its derivative products.

Clinical practitioners (Group 3): 45 physicians with experience in Chinese herbal medicine.

Pharmacy staff (Group 4): 50 pharmacy workers in Rui'an.

2.3. Questionnaire Development and Validation

The enterprise questionnaire was specifically designed to investigate the development intentions and current practices of pharmaceutical companies regarding Curcuma wenyujin derivatives. A total of 50 questionnaires were distributed to eligible companies. The sample size of 50 was determined based on a convenience sampling strategy targeting all known enterprises in the Wenzhou region engaged in TCM processing or herbal product development. The questionnaire consisted of three sections: (1) procurement channels (single choice), (2) directions for derivative product development (multiple choice allowed), and (3) outlook and R&D focus (single choice with Likert scale).

Distribution was conducted through two channels: (a) electronic links sent via WeChat and email to company managers or R&D directors, and (b) on-site paper questionnaires during field visits. A total of 36 valid responses were collected (response rate 72%), which served as the basis for all enterprise-related analyses in this study. The discrepancy between 50 distributed and 36 valid responses is due to incomplete responses or non-participation from companies that were initially contacted but did not complete the survey.

The questionnaires distributed to farmers and sales channels (including pharmacies and hospitals) were designed around four core dimensions.: (1) background and practices (e.g., cultivation years, procurement channels, clinical experience);(2) perceived difficulties and barriers;(3) awareness of policies and market conditions;(4) expectations for government support and industrial development. Conduct a pre-network survey before the on-site investigation and organize experts to review and revise the relevant questionnaires.

Key items included the following examples:

For farmers: "What is the most critical factor affecting yield and quality?" (single choice);For enterprises: "What is your outlook for Curcuma wenyujin derivatives?" (5-point Likert scale: 1=very poor, 2=poor, 3=fair, 4=good, 5=very good);For clinicians: "How familiar are you with Curcuma wenyujin?" (5-point scale: 1=completely unfamiliar, 5=very familiar);For pharmacy staff: "How often does your institution sell Curcuma wenyujin products?" (5-point scale: 1=never, 5=very frequently).

All Likert-scale responses were analyzed using frequencies and percentages. The 5-point scale was treated as ordinal; central tendencies (mode, median) were examined.

2.4. Data Collection and Analysis

The questionnaire survey was conducted through the mobile platform (WeChat, direct link) for direct statistics to ensure the authenticity and comprehensiveness of the data. Descriptive statistics (frequencies, percentages) were calculated.

3. A Comprehensive Analysis of the Current Status of Curcuma wenyujin and Its Related Derivatives Development in the Context of Industrial Modernization

3.1. Pharmaceutical Companies Perspective

3.1.1. The Source of the Industrial Chain and Processing

Of the 36 surveyed enterprises, 16 provided valid responses regarding pharmaceutical companies' procurement channels for Curcuma wenyujin, nearly 100% of the samples indicated that they sourced the herb from the Standardized Cultivation Base of Curcuma wenyujin under Good Agricultural Practice (GAP), while purchases from small-scale farmers and company-owned cultivation bases accounted for a relatively low proportion. In addition, 22.2% (n=8) also purchased from small-scale farmers, and 16.7% (n=6) used company-owned cultivation bases. According to the team's field research and relevant literature (Li, 2009) cultivation bases typically possess large-scale, specialized planting techniques and management expertise. They can provide stable yields and consistent quality that meet pharmaceutical companies' requirements, while also driving the modernization and standardization of the Curcuma wenyujin industry, further expanding the industrial chain, and enhancing the competitiveness and added value of the entire sector.

Among the 36 surveyed enterprises, the majority chose to develop pharmaceuticals (75.0%, n=27) and health supplements (58.3%, n=21). Additionally, 47.2% (n=17) had invested in the development of cosmetics, and 13.9% (n=5) in food products. Nearly 41.7% (n=15) of enterprises indicated that further extraction of active ingredients for anticancer drugs and volatile oils is currently a major R&D focus (Liu et al., 2021). During the interview, the relevant staff members of the pharmaceutical company stated that they are actively engaged in technological research and development as well as brand building, and are promoting the iteration and innovation of derivative products.

3.1.2. Standardization of Product Manufacturing

Field visits revealed that among the 36 surveyed enterprises， most enterprises producing Curcuma wenyujin employ relatively outdated production techniques, rely on obsolete processing equipment (83.3%, n=30), and lack advanced production facilities and technical support. A comparison with the National Medical Products Administration's *Good Manufacturing Practice for Chinese Herbal Medicines (Reform)* reveals that the actual processing of Curcuma wenyujin involves lax quality control, inconsistent standards, and variable product quality. The active ingredient content and purity of products from different batches may vary significantly, which

directly affects clinical efficacy. Furthermore, the active ingredients in Curcuma wenyujin and its derivative products may degrade during storage and use. On-site visits revealed that the vast majority of primary processing plants do not have dedicated storage facilities for medicinal materials, resulting in insufficient stability of pharmacological efficacy.

3.1.3. Industry Outlook and Industrialization Process

In a survey of pharmaceutical companies regarding their outlook for Curcuma wenyujin-derived products, 58.3% (n=21) of respondents indicated a "good" outlook, while 33.3% (n=12) indicated a "very good" outlook. Subgroup analysis revealed that companies already developing health supplements were significantly more optimistic： 90.5% (n=19) rated the outlook as "very good" or "good," compared to 73.3% (n=11) among companies focusing solely on pharmaceuticals without health supplement involvement (n=15). The staff members of the pharmaceutical company mentioned in the interview that the company is actively seeking to establish partnerships with upstream and downstream enterprises in order to accelerate the industrialization process.

3.2. Market Perspective

This section examines the primary demands on the demand side by focusing on the two main market participants.

3.2.1. Current Demand Situation in Pharmacies

At the pharmacy level, patient demand for Curcuma wenyujin and its derivative medications is generally low (Figure 1). A field survey covering 32 pharmacy outlets in Rui'an City with 50 staff respondents revealed that more than half of the pharmacies scored 3 ("average") or lower, indicating that overall market demand is low. The survey data indicates that the use or sale of Curcuma wenyujin or its derivative products in the respondents' institutions is mostly occasional or infrequent, accounting for one-third of the total sample. Only 22% (n=11) of respondents reported that their institutions frequently use or sell such products. Interviews with survey participants revealed that smaller pharmacies generally do not stock Curcuma wenyujin-based proprietary Chinese medicines, primarily due to their relatively high cost. The survey results indicate that, in the pharmacy market context, Curcuma wenyujin and its derivative products are primarily used as medications, while awareness and acceptance of health products derived from it are relatively low. Furthermore, 74% (n=37) of pharmacy staff reported having received no professional training on Curcuma wenyujin or its related pharmaceutical products, and their understanding of the efficacy and target demographics for Curcuma wenyujin-containing proprietary Chinese medicines and health supplements ranged from general to limited. Correspondingly, customer awareness of Curcuma wenyujin is also low.

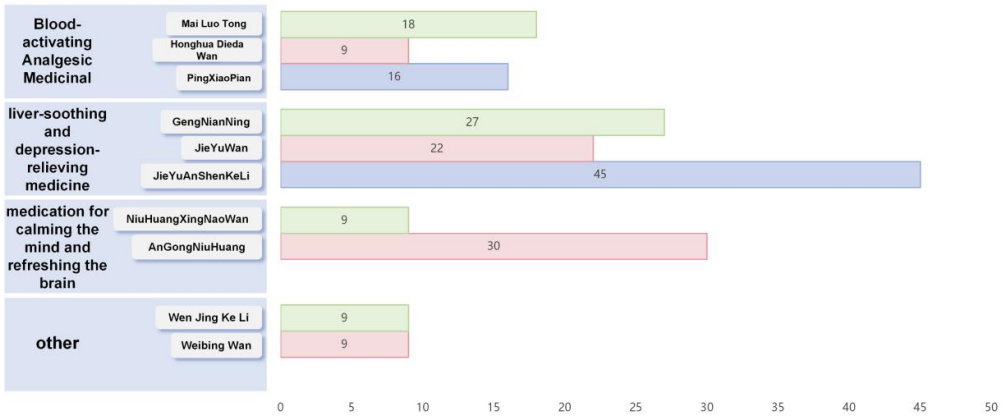


Figure 1. Commonly seen Chinese patent medicines containing Curcuma wenyujin and its extracts in the market.（Notes: Multiple-choice statistics results）

3.2.2. Current Status of Clinical Demand

In a survey regarding the clinical application of Curcuma wenyujin and its derivatives, 60% (n=27) of the responding physicians had been engaged in clinical practice for more than five years, and 68.9% (n=31) of them were familiar with Curcuma wenyujin and its derivative medications. Field visits revealed that in current clinical practice, Curcuma wenyujin extracts are commonly formulated into dosage forms such as tablets, microcapsules, and microspheres. Additionally, various Curcuma wenyujin extracts have been shown to reverse multidrug resistance in tumors. Related derivative products include Curcuma zedoaria oil injection and linalool emulsion injection; however, drugs targeting antitumor applications are still in the research and development trial phase (Wang, 2025). At present, Curcuma wenyujin and its derivatives still face challenges in clinical settings, including a limited variety of available formulations and relatively high drug prices. Given that nearly 70% of clinicians believe Curcuma wenyujin and its derivatives offer clear advantages (Figure 2)—namely, proven efficacy and minimal side effects—further development and application of these products are essential.

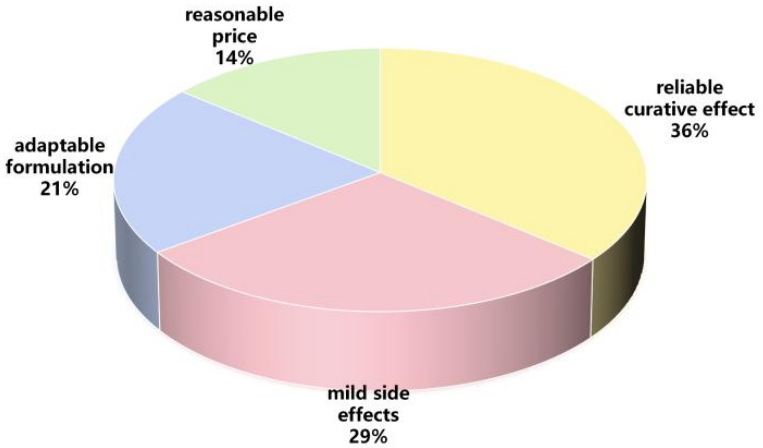


Figure 2. Advantages of Curcuma wenyujin extract as perceived by clinical practitioners.

（Notes: Multiple-choice statistics results）

3.3. Raw Material Suppliers Perspective

3.3.1. Low Mechanization Rate

Among the 68 surveyed farmers, 66.2% (n=45) identified “insufficient technological innovation and low production efficiency” as the primary constraint. Specifically, 50% (n=34) considered soil conditions the most critical factor affecting yield and quality, while only 12% (n=8) reported using mechanized irrigation or fertilization. Currently, small-scale farmers generally rely on traditional farming methods, while some large-scale agricultural operators have adopted water-saving irrigation technologies such as drip irrigation and micro-sprinklers, as well as biological control methods. Among the farmers surveyed, half believe that soil conditions are the most critical factor affecting the yield and quality of turmeric production, while mechanized water-saving irrigation and fertilization methods can effectively prevent soil structure degradation, such as soil compaction.

3.3.2. Degradation of Genetic Resources and Rampant Diseases

Field visits revealed that long-term self-seeding and the use of small seed lots have led to poor plant growth and frequent cases of premature aging in turmeric. Among the 48 farmers with cultivation experience, 45.8% (n=22) reported frequent premature aging, and 37.5% (n=18) reported severe bacterial wilt or pest problems. Long-term self- seeding was practiced by 79.2% (n=38) of experienced farmers, contributing to genetic degradation. At the same time, the vast majority of farmers mentioned during the interviews that the rampant spread of diseases such as bacterial wilt was an important factor affecting the yield of Curcuma wenyujin.

4. An Examination of Issues in the Development of Curcuma wenyujin and Its Derivatives Against the Background of Industrial Modernization

4.1. Constraints at the Source

From the perspective of raw materials (Wang, 2025), mechanical mechanization rates among individual farmers are low, and production operations are generally labor-intensive (Chen, 2024). Although some large-scale growers have experimented with water-saving irrigation technologies such as drip irrigation and micro-sprinklers, there remains an overall lack of suitable mechanized equipment. This makes it difficult to effectively prevent structural damage—such as soil compaction—caused by traditional farming methods (Zhang & Huang, 2025); At the same time, the degradation of germplasm resources and the rampant spread of diseases are severe. Long-term self-saving of seeds has led to frequent genetic degradation and premature plant senescence, while soil-borne diseases such as bacterial wilt continue to pose an escalating threat, directly limiting the stability of yields and the quality of medicinal materials at the source.

4.2. Production Shortcomings

In terms of production, processing technologies are relatively outdated. Most enterprises have outdated equipment and lack advanced technical support (Xiong et al., 2022), resulting in lax quality control and inconsistent adherence to standards during the production of Curcuma

wenyujin derivatives. This leads to significant variations in the content and purity of active ingredients across different batches, directly affecting the reliability of clinical efficacy; Furthermore, storage conditions are generally inadequate; the vast majority of primary processing plants lack dedicated storage facilities for medicinal materials. This makes active ingredients prone to degradation during storage, making it difficult to ensure the stability of medicinal efficacy and further exacerbating the instability of medicinal material quality.

4.3. Market Challenges

The shortcomings in the aforementioned stages ultimately trickle down to the retail level (Xue & Ding, 2024): demand at pharmacies is sluggish; smaller pharmacies generally do not stock the product due to its high cost; staff lack targeted professional training; and customer awareness of Curcuma wenyujin is generally low, leading to the product's gradual marginalization. At the clinical level, while its advantages—such as proven efficacy and minimal side effects—are widely recognized, the currently available range of traditional Chinese patent medicines is limited and prices are relatively high. Furthermore, cutting-edge drugs for treating cancer and other conditions are still in the research and trial stages. Consequently, the potential market value of Curcuma wenyujin—which has been widely used in clinical settings and received considerable acclaim—remains largely untapped (Zhang & Li, 2018).

5. Strategies for Enhancing the Development of Curcuma wenyujin and Its Derivatives in the Context of Industrial Modernization

Based on field research and existing literature, this paper proposes the following development strategies for the challenges faced by the Curcuma wenyujin in Rui'an(Figure 3).

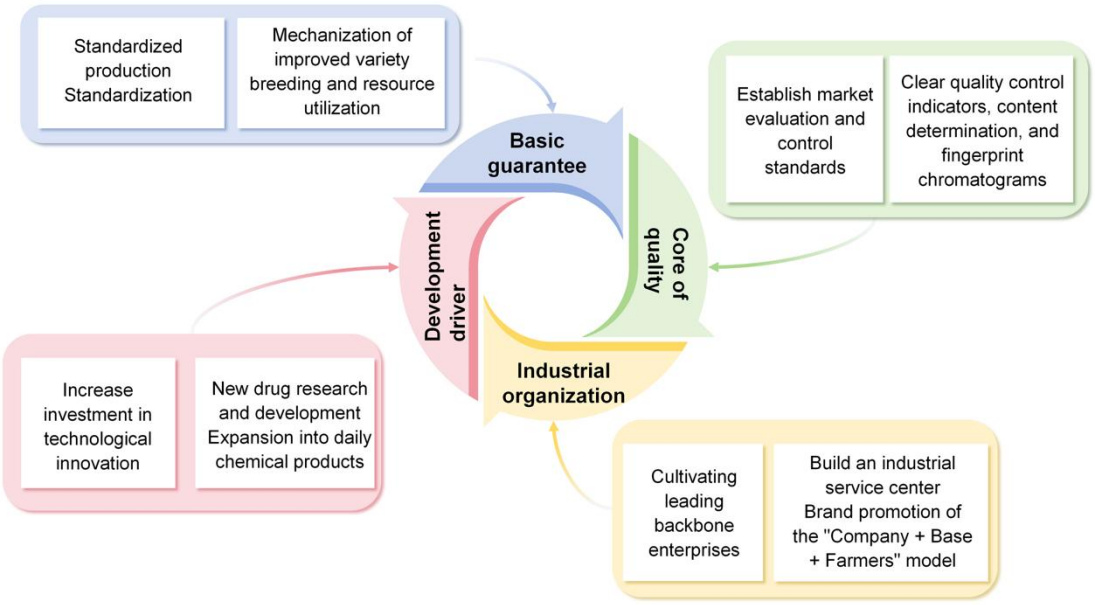


Figure 3. Schematic Diagram of the Enhancement Path for the Development of Curcuma wenyujin and Its Related Derivative Products.

5.1. Standardization and Regulation of Production

To better ensure the consistent quality of Curcuma wenyujin and guarantee medication safety, the standardization and regulation of its production are of urgent importance (Zheng, 2019).

5.1.1. Improving Breeding Methods

Curcuma wenyujin produces few flowers, and its seeds are numerous but underdeveloped; consequently, cultivation relies on propagating with ginger rhizomes (Guo et al., 2021), making it difficult to induce genetic variation for superior traits (Li et al., 2020; Li et al., 2023) applied virus-free seedling hardening and transplanting techniques to Curcuma wenyujin cultivation, overcoming the limitations of current seed selection and retention based solely on individual experience, and significantly improving both the yield and quality of Curcuma wenyujin. Therefore, establishing standardized high-quality seedling propagation bases, organizing technical training for farmers on seed selection, and promoting the industrial-scale propagation of tissue-cultured Curcuma wenyujin seedlings through the establishment of an in vitro rapid propagation system (Zheng, 2019) can provide a large supply of high-quality seed rhizomes for cultivation in the production area.

5.1.2. Enhancing Production Mechanization

Although research and development of harvesting machinery for Curcuma wenyujin has been underway in recent years, existing machinery causes significant damage to the crop, preventing the efficient utilization of the entire plant—particularly the tuberous roots used for medicinal purposes. Therefore, it is crucial to strengthen scientific research and increase financial investment to develop integrated mechanized technologies and equipment suitable for the production and primary processing of Curcuma wenyujin (Guo et al., 2021), thereby mechanizing field production and ensuring scientific post-harvest processing. Promoting smart greenhouse cultivation technology for Curcuma wenyujin (Guo et al., 2021), through crop condition and environmental monitoring as well as crop trait monitoring, enables intelligent production control and remote monitoring, thereby achieving precision production and smart management, particularly to avoid soil compaction caused by excessive fertilization as much as possible. To stabilize the production quality of Curcuma wenyujin, Guan Yanhui et al. analyzed soil microbial populations and concluded that fumigation treatment can significantly alleviate the problems associated with continuous cropping of Curcuma wenyujin (Su, 1994) . Developing fumigation techniques and implementing appropriate crop rotation for Curcuma wenyujin are key strategies for reducing the accumulation of harmful soil microorganisms and enhancing the quality of the entire Curcuma wenyujin plant as a medicinal herb. Strengthening the integration of internet information technology, advanced agricultural technologies, and crop cultivation, and establishing GAP systems to enable comprehensive intelligent tracking of Curcuma wenyujin growth, while ensuring healthy growth through monitoring, irrigation, and temperature control.

In terms of product processing, given the current issue of large quantities of discarded Curcuma wenyujin plants being left unused during the production and development of Curcuma wenyujin and its derivative products, pharmaceutical companies and research institutions should pay greater attention to the reprocessing and utilization of these discarded plants. Resource recovery and safe

disposal should be achieved through technologies such as bioconversion, chemical conversion, and physical conversion (Zhu & Li, 2026). Develop resource-based products such as functional foods, feed additives, and functional personal care products to extend the resource value chain, increase the utilization of the entire Curcuma wenyujin plant. At the same time, increase investment in the construction of storage facilities for Curcuma wenyujin medicinal materials, strictly enforce quality control management during the production and processing of Curcuma wenyujin plants, and strengthen testing of active ingredient content in Curcuma wenyujin products to ensure the consistent efficacy of Curcuma wenyujin medicinal materials and their derivatives.

5.2. Establishing Market Quality Control Standards

Currently, the quality of Curcuma wenyujin on the market varies widely, a situation closely related to the lack of established quality control indicators and the overlap between the herbal name and the botanical name (Ren et al., 2017). Research on the quality evaluation and control of warm curcuma is currently limited. The 2025 edition of the *Pharmacopoeia of the People's Republic of China* only includes moisture and ash content as quality control standards, along with thin-layer chromatography identification methods; it does not provide explicit regulations regarding the determination of chemical constituents or fingerprint profiles for Curcuma wenyujin. According to literature reports, the primary active components in Curcuma wenyujin are curcumin and volatile oil components, which can serve as the preferred quality markers for the traditional Chinese medicine Curcuma . Therefore, further analytical research on its volatile oil components is warranted to establish quality testing standards for authentic Curcuma wenyujin, thereby providing a scientific basis for the standardized and large-scale production of Curcuma wenyujin (Li et al., 2020; Liu et al., 2023).

5.3. Ultivating Leading Enterprises

The brand development and industrialization levels of the authentic Curcuma wenyujin medicinal herb base remain low, with a lack of strong leading enterprises and weak capabilities in new product R&D and brand protection. It is therefore crucial to cultivate leading enterprises to take the lead, with the local government providing guidance and supervision, in establishing a “China Curcuma wenyujin Industry Service Center” that integrates origin trading, warehousing, primary and secondary processing, scientific and technological services, production and sales information, entity cultivation, and brand protection. At the same time, following the “company (professional cooperative) + base + farmer” business model, we should leverage the advantages of “Internet + Traditional Chinese Medicine”, explore the historical origins of Rui’an’s authentic Curcuma wenyujin, and strengthen brand creation, protection, and promotion (Zhang & Gao, 2023).

We should accelerate the “matching” of cultivation bases with pharmaceutical companies and research institutions. By facilitating direct connections between farmers, pharmaceutical firms, and research bases, we can reduce the instability caused by imbalances in the supply and demand of medicinal materials. Through personalized customization and rapid, feedback-driven improvements in production methods, we can swiftly enhance production efficiency and stabilize

the quality of medicinal materials. At the same time, given the high cost and low public awareness of Curcuma wenyujin and its derivative medicines mentioned above, pharmaceutical companies should also intensify promotional efforts, advance training for grassroots market personnel, and popularize the efficacy and applications of Curcuma wenyujin and its derivative products.

5.4. Increasing Investment in Scientific and Technological Innovation

As research into the chemical composition and pharmacological effects of Curcuma wenyujin continues to deepen, it is playing an increasingly important role in the pharmaceutical field. Pharmaceutical companies should actively seek partnerships with universities and research institutes to continuously strengthen research into the mechanisms of action of Curcuma wenyujin and explore new pathways for drug targeting. This will enable the continuous development of new applications for Curcuma wenyujin and the creation of derivative products with superior efficacy. In new drug R&D, modern technological methods should be actively utilized to accurately identify market needs in cancer treatment (He, 2025). Extraction efficiency of active ingredients in Curcuma wenyujin should be improved through optimized extraction methods, and the degradation rate of active ingredients should be reduced through formulation modifications. Concurrently, the development and application of clinically proven active ingredients—such as olibanene and tetrahydrocurcumin—in new anti-cancer drugs both domestically and internationally should be accelerated. Through clinical trials and efficacy evaluations, the rapid production and market launch of new drugs should be facilitated to meet patient needs. In the development of daily chemical products, we will enhance the comprehensive utilization of the entire Curcuma wenyujin plant to develop a range of topical and daily chemical products.

6. Discussion

Through literature reviews, action research, surveys, and field interviews, combined with scientific data analysis, it is evident that an industrial chain for the development of Curcuma wenyujin and its related derivative products has begun to take shape.

In response to the industrialization shortcomings identified in existing literature on the standardized production and research progress of Curcuma wenyujin, as well as the practical development obstacles summarized from on-site visits, it is imperative to address issues such as mechanization coverage, specialized talent cultivation, and germplasm resource optimization at the source of the industrial chain. This should be coordinated with product R&D in the midstream of the industrial chain to drive industrial transformation and upgrading (Li et al., 2020; Liu et al., 2023). Furthermore, the downstream segment of the industry chain should focus on enhancing the professional competence of service sectors such as pharmacies to better meet patient demand for Curcuma wenyujin and its derivative products, thereby improving the efficiency and competitiveness of the entire Curcuma wenyujin industry chain.

This study focused primarily on Rui’an City in Wenzhou. While it provides a relatively accurate reflection of the actual conditions of the local Curcuma wenyujin industry, Rui’an, as a designated origin region, exhibits certain unique characteristics in terms of its industrial model

and policy environment; therefore, the applicability of the study's conclusions to other production areas requires further verification. Additionally, due to research constraints, the sample sizes at the pharmaceutical company and pharmacy levels were relatively limited. The statistical robustness of some quantitative analyses requires reinforcement through subsequent studies that expand the sample size. Regarding data collection, this study primarily relied on questionnaires and interviews. Some information was based on respondents' subjective judgments; although a five-point scale was used for quantification, it remains difficult to completely avoid potential biases arising from individual cognitive differences. Future research could incorporate cross-validation using objective indicators such as sales ledgers and prescription records. Furthermore, while this study focuses primarily on the cultivation, production, and sales stages, research on the needs, preferences, and medication experiences of end-users remains relatively limited. Direct data from the patient perspective still needs to be supplemented.

Future research could expand the scope of the study and increase the sample size, while also incorporating objective data sources, strengthening specialized surveys of patients, and employing longitudinal tracking to dynamically observe industry trends. This approach aims to provide more comprehensive decision-making guidance for the healthy development of the Curcuma wenyujin industry.

7.Conclusions

In summary, our research conducted a systematic summary of the current production status of Wen Yuning in Rui'an, Wenzhou. It proposed comprehensive solutions to the existing problems in the aspects of planting, production, and sales. At the same time, it also provided a new perspective for optimizing the industrial chain of other authentic medicinal herbs.

Author Contributions:

Conceptualization, H.C. and Y.J.; methodology, C.L.; software, X.L.; validation, H.C., Y.J. and Y.Z.; formal analysis, Y.J.; investigation, H.C., Y.Z. and C.L.; resources, Y.J.; data curation, H.C. and Y.J; writing—original draft preparation, C.L. and X.L; writing—review and editing, H.C. and Y.Z; visualization, Y.Z.; supervision, D.L.; project administration, D.L.; funding acquisition, D.L. All authors have read and agreed to the published version of the manuscript.

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Not applicable.

Data Availability Statement:

The data presented in this study are available on request from the corresponding author due to the presence of commercial secrets.

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Conflict of Interest:

The authors declare no conflict of interest.

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GCN5-Catalyzed WSTF Benzoylation Activates Tumor Glycolysis

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Abstract

Williams syndrome transcription factor (WSTF), traditionally recognized as a nuclear-localized transcription factor and histone tyrosine kinase, has been detected in the cytoplasm of various cancer cells in our previous observations. This finding suggests the existence of an uncharacterized nucleocytoplasmic shuttling mechanism and extranuclear functions of WSTF. This study aims to investigate whether WSTF's subcellular localization is regulated by novel post-translational modifications, and to clarify its cytoplasmic functions and role in tumor metabolic reprogramming. Through post-translational modification proteomics analysis, we identified lysine benzoylation at position 181 (K181) of WSTF. After knocking down candidate enzyme genes individually with specific siRNAs, WB analysis revealed a significant decrease in

WSTF-K181 benzoylation levels only when GCN5 was knocked down, suggesting that GCN5 is a potential enzyme catalyzing this modification. Functional assays demonstrated that K181 benzoylation is a key driver for WSTF translocation from the nucleus to the cytoplasm. Furthermore, 4D-Labelfree tyrosine phosphorylation proteomics unexpectedly identified hexokinase 1 (HK1) as a novel cytoplasmic substrate of WSTF. In vitro and in vivo experiments verified that WSTF directly binds to HK1 and phosphorylates it at tyrosine 667, thereby enhancing HK1 kinase activity and promoting glucose uptake and lactate production. Functionally, expression of wild-type WSTF significantly promoted the proliferation, migration, and in vivo tumorigenicity of breast cancer cells, while the kinase-inactive mutant (C338A) or benzoylation-deficient mutant (K181A) remarkably attenuated these oncogenic effects of WSTF. This study is the first to reveal a novel mechanism by which GCN5-mediated benzoylation regulates WSTF nucleocytoplasmic shuttling. Additionally, we discovered that WSTF drives tumor glycolysis in a non-canonical manner by directly phosphorylating the metabolic enzyme HK1. Our findings break through the traditional understanding of WSTF functions and provide a new perspective for deciphering tumor metabolic reprogramming.

Keywords: WSTF; GCN5; Benzoylation; Cancer; Glycolysis

1. Introduction

Williams syndrome transcription factor (WSTF), also known as BAZ1B (bromodomain adjacent to the zinc finger domain 1B), is a novel tyrosine kinase encoded by the WBSCR9 gene, with no homology to other known kinase molecules (Sharif et al., 2021). First identified in patients with Williams syndrome in 1998 (Lu et al., 1998; Peoples et al., 1998), subsequent extensive studies have revealed its diverse functions, including involvement in metabolism, replication, transcription, DNA damage repair, and cell cycle regulation. These functions are all associated with the complex multisystem defects, multiple organ developmental disorders, intellectual and cognitive abnormalities characteristic of this syndrome (Barnett & Krebs, 2011; Sharif et al., 2021). In 2012, Ewa E. Henning et al. first reported six kinases with altered expression in a study analyzing colon cancer biomarkers, among which WSTF was included (Hennig et al., 2012). However, this report did not conduct an in-depth analysis of the mechanism by which WSTF exerts its effects. In 2016, our team demonstrated through three articles that, regulated by upstream Ras pathway signals, the phosphorylation level of serine 158 (S158p) in WSTF can modulate histone acetylation modifications (acetylation, ac) - H3K9ac and H4K16ac, thereby affecting chromatin structure and downstream gene expression, and ultimately regulating breast cancer development (Liu et al., 2016; Li et al., 2016; Wang e al., 2016). In 2020, we further reported that acetylation modification of lysine 426 (Lys, K) (K426ac) can promote WSTF activity and oncogenic functions (Liu et al., 2020). Although several subsequent studies have explored the relationship between WSTF and tumorigenesis, drug resistance, and signaling pathways based on our team's reports, all these analyses are grounded in the aforementioned Williams syndrome-related research (Zhang et al., 2012; Meng et al., 2016; Kang et al., 2022; Huang et al., 2022; Nargund et al., 2022).

Benzoylation is a newly discovered acylation modification, and currently the only known lysine acylation modification containing a benzene ring. Existing studies have shown that it can be induced by sodium benzoate (NaBz) through the generation of benzoyl-CoA. Sodium benzoate is a widely used food additive and an FDA-approved drug for the treatment of hyperammonemia caused by urea cycle disorders; benzoyl-CoA is also an intermediate in the degradation of numerous aromatic substrates in intestinal microbiota (Ren et al., 2021). Currently, the regulatory mechanisms and physiological/pathological functions of benzoylation remain unclear.

GCN5, also known as KAT2A (lysine acetyltransferase 2A), is highly expressed in various tumors such as breast cancer, colon cancer, and prostate cancer, and is closely associated with drug resistance, cell proliferation, migration, and epithelial-mesenchymal transition (Oh et al., 2020; Yuan et al., 2021).

In humans, GCN5 has been extensively studied for its roles in epigenetic regulation and pathogenic mechanisms of cancer (Chen et al., 2013; Yin et al., 2015; Liu et al., 2015; Majaz et al., 2016; Koutsogiannouli et al., 2017; Shao et al., 2018; Qiao et al., 2018; Kahl et al., 2019; Farria et al., 2020), diabetes, osteoporosis, systemic lupus erythematosus, malaria, myopathy, visual impairment, and other diseases (Lerin et al., 2006; Mao et al., 2009; Leung et al., 2015; Li et al., 2016; Kumar et al., 2017; Carrillo-Rosas et al., 2019; Addicks et al., 2022; Li et al., 2022).

Glycometabolism is an important pathway for tumor cells to obtain energy, and hexokinase (HK) is the enzyme that catalyzes the key step in glycolysis—the conversion of glucose (G) to glucose-6-phosphate (G-6-P) (Smith, 2000; Wilson, 2003; Hanahan et al., 2011; Kierans & Taylor, 2024). As a core biochemical process maintaining cellular energy supply and life activities in organisms, glycometabolism encompasses critical links such as glycolysis, tricarboxylic acid cycle, pentose phosphate pathway, and gluconeogenesis. It not only achieves ATP production and material transformation through multiple pathways but also acts as a signaling hub regulating cellular functions (Kierans & Taylor, 2024). Its spatiotemporal precise regulation relies on the coordinated action of key enzymes such as phosphofructokinase-1, and it plays a crucial role in tumor metabolic reprogramming (Li et al., 2025).

The cytoplasmic distribution of WSTF in various tumor cells challenges traditional cognition, but the key signal driving its nuclear export has long remained unknown. In recent years, lysine benzoylation has been identified as a novel acylation modification, whose physiological and pathological functions are unclear, and its association with tumor metabolism is completely unexplored. Based on this, we propose the scientific hypothesis: The nucleocytoplasmic shuttling of WSTF is precisely regulated by benzoylation, and cytoplasmic WSTF directly participates in tumor cell metabolic regulation by phosphorylating non-histone substrates. By integrating modification proteomics, biochemical, and cell biology approaches, this study validates this hypothesis and reveals a complete pathway from "modification signal" to "localization change", followed by "substrate recognition" and "functional output".

2. Methods

2.1. Cell Culture

SW620, MDA-MB-157, Hela cells were purchased from the Cell Bank of the Chinese Academy of Sciences (Beijing). HEK293T, and Ada2$^{-/-}$ HEK293 cells were purchased from Saier Biolabs (Tianjin). Each cell line was cultured in an appropriate growth medium in accordance with the recommendations of the respective suppliers (Cell Resource Center, Institute of Basic Medical Sciences, or Chinese Academy of Medical Sciences). Cell lines were routinely tested using short tandem repeat profiling method, and mycoplasma infection was tested monthly.

2.2. Plasmid Construction

The construction process of expression plasmids for wild-type (WT) and three mutant-type (K181A, K181R, C338A) WSTF (Williams Syndrome Transcription Factor), GCN5 (General Control Nonderepressible 5) and hexokinase 1 (HK1) was as follows: First, site-directed mutagenesis was used, with the wild-type cDNA of each target gene (WSTF, GCN5, HK1) as the template, and specific primers were designed to introduce the target mutation sites (K181A means that the lysine at position 181 of WSTF protein was mutated to alanine, K181R means that the lysine at position 181 was mutated to arginine, and C338A means that the cysteine at position 338 was mutated to alanine), to amplify DNA fragments containing wild-type or mutant-type target genes. Subsequently, these amplified target gene fragments were cloned into the corresponding eukaryotic expression vectors respectively. The vectors used included pCDNA3.1 vectors with Flag tag, HA tag or Myc tag. Finally, recombinant expression plasmids capable of expressing wild-type and mutant-type WSTF, GCN5 and HK1 proteins with different tags were constructed.

2.3. Antibodies and Reagents

Site-specific modification antibodies: anti-WSTF-K181bz and anti-p-Y667-HK1 were custom-synthesized by PTM Bio Company. Anti-WSTF (#ab51256), anti-GCN5 (#ab217876), and anti-Ada2 (#ab164942) were purchased from Abcam. Anti-HK1 (#ET1609-28), anti-Tubulin (#HA721913), anti-GAPDH (#ET1601-4), anti-H3 (#M1309-1), anti-β-actin (#EM21002), and anti-AIF (#ET1603-4) were obtained from HUABIO. The Hexokinase Activity Assay Kit, 2-NBDG, Lactate Assay Kit, CCK-8 reagent, and Subcellular Fractionation Kit were purchased from Tianjin Sai'er Biotechnology Co., Ltd.

2.4. Western Blotting

Cells used for immunoprecipitation (IP) and western blotting (WB) were collected, resuspended in RIPA lysis buffer (#P0013B; Beyotime Biotechnology), subjected to 15 ultrasound pulses (each exposure lasting 1 s), and centrifuged at 15,000g at 4°C for 30 min. For IP of target proteins, the supernatant was incubated with the appropriate antibody and protein A/G agarose beads (Santa Cruz Biotechnology, Inc.). For WB, either total cell lysates or immunoprecipitates were boiled and proteins separated using a 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) system. The target proteins were transferred onto a polyvinylidene fluoride membrane (Roche), sealed in a 5% skim milk solution for 1 h, then hybridized with the corresponding antibodies. Protein signaling was enhanced using

chemiluminescent horseradish peroxidase-conjugated antibodies. Images were analyzed using an SH-Compact 523 chemiluminescence imaging system (Hangzhou Shenhua Technology Co., Ltd.).

2.5. GST Pull-Down

Escherichia coli strain BL21 was transformed with protein expression plasmids and subsequently induced with 0.2mM isopropyl-β-D-thiogalactoside at 20°C. Labeled proteins were purified using Ni-NTA beads or GST Sepharose beads (GE). For GST pull-down tests, protein mixtures were incubated with GST pull-down buffer for 2h at 4°C, followed by pull-down assay. The precipitates underwent a washing and separation process using SDS-PAGE, followed by staining of proteins with Coomassie brilliant blue. WB with specific antibodies was used to detect target proteins in the precipitant.

2.6. Dot blot

For the Dot blot assay, gradient concentrations (1μg, 0.5μg) of WT and K181bz-modified WSTF peptide samples were prepared, alongside a 0 μg blank control. A nitrocellulose (NC) membrane was pre-equilibrated in TBST buffer; samples of each concentration were then spotted onto the membrane in a predefined array (2 μL per spot) using a micropipette, followed by air-drying at room temperature for 15 min to ensure complete peptide immobilization. Subsequently, the membrane was submerged in TBST blocking buffer supplemented with 5% non-fat dry milk and incubated for 1 h on a rotating shaker at room temperature to block non-specific binding sites. After removing the blocking buffer, the membrane was incubated separately with the anti-K181bz primary antibody (diluted to the recommended working concentration) or a biotin affinity detection reagent overnight on a rotating shaker at 4°C. On the following day, unbound reagents were removed by washing the membrane three times (10 min per wash) with TBST buffer. Finally, chemiluminescent substrate was applied, and signals were acquired using a chemiluminescence imaging system.

2.7. Benzoyltransferase Activity Assay

The reaction mixture was prepared in vitro with 50 mM Tris-HCl, 1 mM DTT (pH 7.5), 3.2 mM $MgCl_2$, 40 mM NaCl, 0.1 mM EDTA, 2 μM histone octamer, and 5 μM Ada2-Gcn5. After incubation at 30 °C for 5 minutes, 200 μM benzoyl-CoA was added to initiate the reaction. The reaction was terminated at the desired time points by adding 0.5% trifluoroacetate. The level of benzoylation modification was detected by Western blot using specific antibodies.

2.8. Co-immunoprecipitation

Total cellular proteins were extracted by lysing target cells on ice with RIPA lysis buffer supplemented with protease inhibitors and phosphatase inhibitors. A portion of the protein sample was reserved as the Input control. For the remaining protein sample, specific primary antibodies (e.g., Anti-WSTF, Anti-Flag, Anti-HA antibodies) were added, followed by gentle oscillation and incubation at 4°C overnight. Subsequently, Protein A/G agarose beads were added for an additional 4-hour incubation to capture immune complexes. After multiple washes with RIPA lysis buffer, SDS loading buffer was added, and the samples were denatured by boiling in a water

bath. The expression of target binding proteins in the immune complexes was detected by Western blot.

2.9. Transcriptomics Analysis

At least 3 biological replicates were set up for each group. Total RNA was extracted from cells/tissues using TRIzol reagent (Invitrogen). RNA purity (A260/A280 ratio: 1.8~2.0; A260/A230 ratio ≥ 2.0) and integrity (RNA Integrity Number, RIN ≥ 7.0) were verified using a Nanodrop 2000 spectrophotometer and an Agilent 2100 Bioanalyzer, respectively. One microgram of qualified RNA was used to construct sequencing libraries with the VAHTS® mRNA-seq V2 Library Prep Kit. Subsequent quality control of library insert size and concentration was performed using an Agilent 2100 Bioanalyzer and a Qubit fluorometer. Libraries were sequenced on an Illumina sequencing platform. Raw data were filtered and aligned to the reference genome. Differentially expressed genes (DEGs) were screened using DESeq2 software with the criteria of $|\log_2 (\text{fold change})| \geq 1$ and $P < 0.05$.

2.10. HK Activity Assay

Briefly, cells that were cultivated in 60-mm petri dishes were collected and placed in 400μL lysis buffer (50mM potassium dihydrogen phosphate, 2mM ethylenediaminetetraacetic acid (EDTA), 2mM dithiothreitol, 20mM sodium fluoride [Na F]). Homogenates were then prepared at 4°C and centrifuged at 18,000g at 4°C for 15min. A 40-μL aliquot of the supernatant was introduced into a 1-mL volume of the following reaction buffer: 100mM Tris-HCl at pH 8.0, 10mM ATP, 0.5mM EDTA, 10mM $MgCl_2$, 2mM glucose, 0.2mM $NADP^+$, and 30μg of glutathione (GST)-glucose-6-phosphate dehydrogenase (G6PD) purified from Escherichia coli. HK activity was identified by measuring the conversion of NADP to NADPH using spectrophotometry at a wavelength of 340nm and a temperature of 37°C. This conversion is dependent on the presence of GST and G6PD. The observed increase in optical density, determined at 2min over a total of 15 cycles, was found to be associated with an elevated concentration of NADPH. Total HK activity was determined by analyzing the logarithmic stage of the resulting curve and calculating its slope. HK activity in various samples within the same investigation was standardized by normalization to the protein content of the respective lysates.

2.11. Glucose Uptake Assay

The cell lines were inoculated in six-well culture plates, and cultured in complete growth medium for 24 h, low-glucose medium (Hyclone) for 4 h, and glucose-free medium for 30 min in sequence. Subsequently, the cells in each well were treated with a solution containing 0.25μCi 2-[1,2-^{3}H]- deoxy glucose (2-DG, Perkin Elmer). After 10 min, the cells were washed three times with phosphate-buffered saline (PBS), and then lysed with 0.2 M sodium hydroxide (NaOH). The concentration of 2-DG in the cell lysate was determined by liquid scintillation counting

2.12. Lactate Production Assay

Cellular lactate production under normoxia was measured using a lactic acid assay kit (A019-2-1; NanJing JianCheng Bioengineering Institute). Briefly, the cells were introduced into six-well plates and cultivated in medium for 24h, after which fresh medium was supplied for

another 4h of incubation, followed by determination of the lactic acid content in the culture medium.

2.13. Cell proliferation assay

Cell proliferation was assessed by direct cell counting. Briefly, cells stably expressing Vector, WT-WSTF, or mutant WSTF constructs were seeded in 6-well plates at a density of 2×10^4 cells per well. On days 1, 2, 3, 4, and 5 after seeding, cells were harvested by trypsinization, resuspended in PBS, and counted using a hemocytometer. The number of viable cells was recorded, and cell growth curves were plotted based on the mean cell counts from three independent experiments.

2.14. Xenograft mouse model

Tumor cells (5×10^6 cells per mouse) stably expressing Vector, WT-WSTF, C338A-WSTF, or K181A-WSTF were subcutaneously injected into the right flank of 6-week-old male BALB/c nude mice (n=5 per group). After visible tumor nodules (≥2 mm in diameter) formed (typically 7 days post-inoculation), tumor growth was monitored every 3 days by measuring tumor length (L) and width (W) with calipers; tumor volume was calculated using the formula: $V = 0.5 \times L \times W^2$. At the experimental endpoint (21 days post-inoculation), mice were euthanized, and tumors were dissected and weighed to obtain relative tumor weight (normalized to the Vector group).

2.15. Proteomics Analysis

Standard mass spectrometry techniques were used to perform 4D label-free tyrosine phosphorylation proteomics analysis and post-translational modification (PTM) proteomics analysis to screen for differentially modified proteins and identify modification sites.

2.16. Statistical Analysis

Experimental data are presented as mean ± standard error of the mean (mean ± SEM). Statistical analysis was performed using GraphPad Prism software. Comparisons between groups were conducted using Student's t-test or one-way analysis of variance (one-way ANOVA). AP value < 0.05 was considered statistically significant.

3. Result

3.1. WSTF Localizes to the Cytoplasm in Multiple Cancer Cells and Is Regulated by Benzoylation Modification

We first examined the subcellular distribution of WSTF in cancer cells. Using subcellular fractionation and Western blot analysis in SW620 colon cancer cells (Figure 1A), we found that WSTF predominantly localizes to the cytoplasm, with weaker signals also detected in the nucleus.

To investigate the regulatory mechanism underlying this nucleocytoplasmic distribution, we profiled post-translational modifications (PTMs) of WSTF. Immunoprecipitation with a pan-benzoylation antibody followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) identified a previously unreported benzoylation modification at lysine 181 (K181) of

WSTF. Sequence alignment revealed that this residue is highly evolutionarily conserved across vertebrate species (Figure 1B).

We then validated the specificity of a custom-made anti-K181-benzoylation antibody using dot blot assays, which confirmed its specific recognition of the benzoylated WSTF peptide, with no cross-reactivity against the unmodified wild-type peptide (Figure 1C).

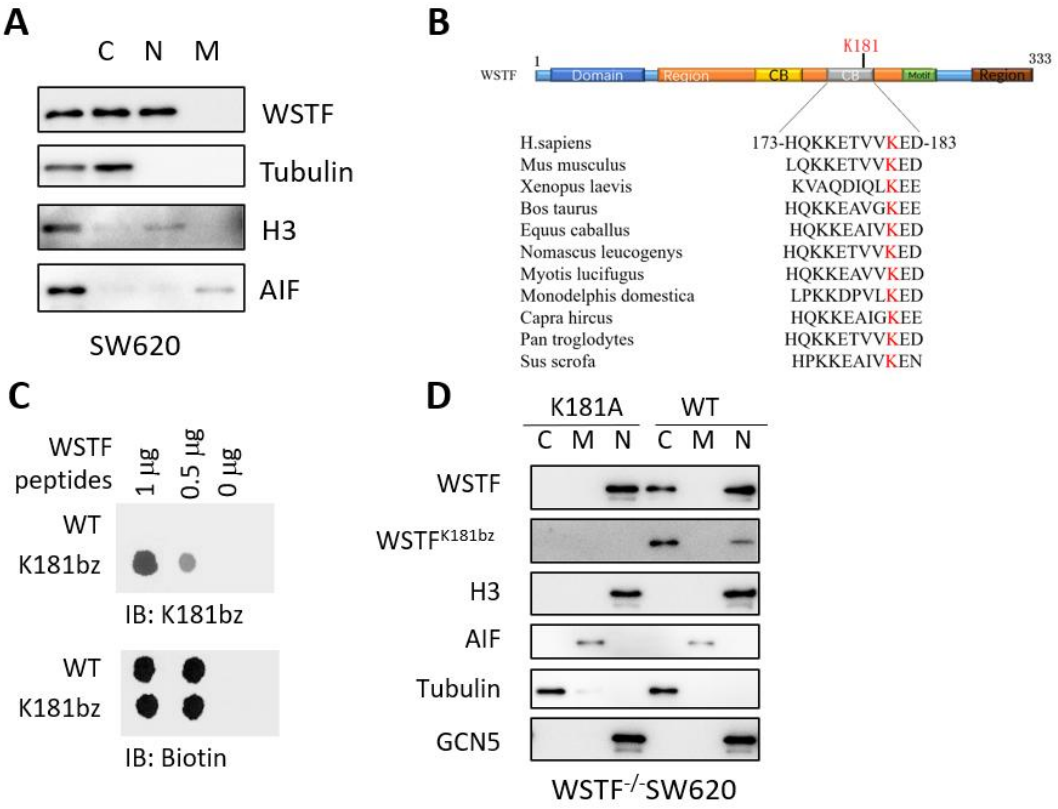


Figure 1. WSTF localization and K181 benzoylation in cancer cells

Note: A. Subcellular fractionation and Western blot analysis showing the distribution of endogenous WSTF in SW620 colon cancer cells. C, cytoplasm; N, nucleus; M, membrane. Tubulin, histone H3, and AIF were used as purity markers for the cytoplasmic, nuclear, and membrane fractions, respectively.

B. Schematic illustration of WSTF domains and multiple-sequence alignment showing evolutionary conservation of the K181 residue across vertebrate species.

C. Dot blot assay validating the specificity of the custom-made anti-WSTF K181bz antibody using biotin-labeled WT and K181bz-modified WSTF peptides.

D. Subcellular fractionation and Western blot analysis of WSTF localization in $WSTF^{-/-}$ SW620 cells rescued with wild-type (WT) or K181A-mutant WSTF.

To test whether K181 benzoylation regulates WSTF localization, we reconstituted WSTF-knockout SW620 cells with either wild-type (WT) WSTF or the benzoylation-deficient K181A mutant. Subcellular fractionation and Western blot analysis showed that, compared to WT WSTF, the K181A mutant exhibited significantly reduced cytoplasmic enrichment and a marked increase in nuclear localization (Figure 1D). Notably, the WSTF-K181bz signal was detected predominantly in the nuclear fraction in the WT rescue group, but was undetectable in the K181A

mutant group. Together, these results indicate that K181 benzoylation is a critical modification that promotes the cytoplasmic translocation of WSTF in cancer cells.

3.2. Transcriptome Analysis Reveals the Functional Impact of WSTF Benzoylation Modification

To investigate the functional consequences of WSTF benzoylation, we established stable cell models in SW620 cells: WSTF-knockout cells were stably transfected with either the benzoylation-deficient WSTF-K181A plasmid or the wild-type WSTF-WT plasmid. The resulting cell lines, hereafter referred to as the K181A-WSTF group (experimental group) and the WT-WSTF group (control group), were subjected to RNA sequencing (RNA-seq) for transcriptome analysis.

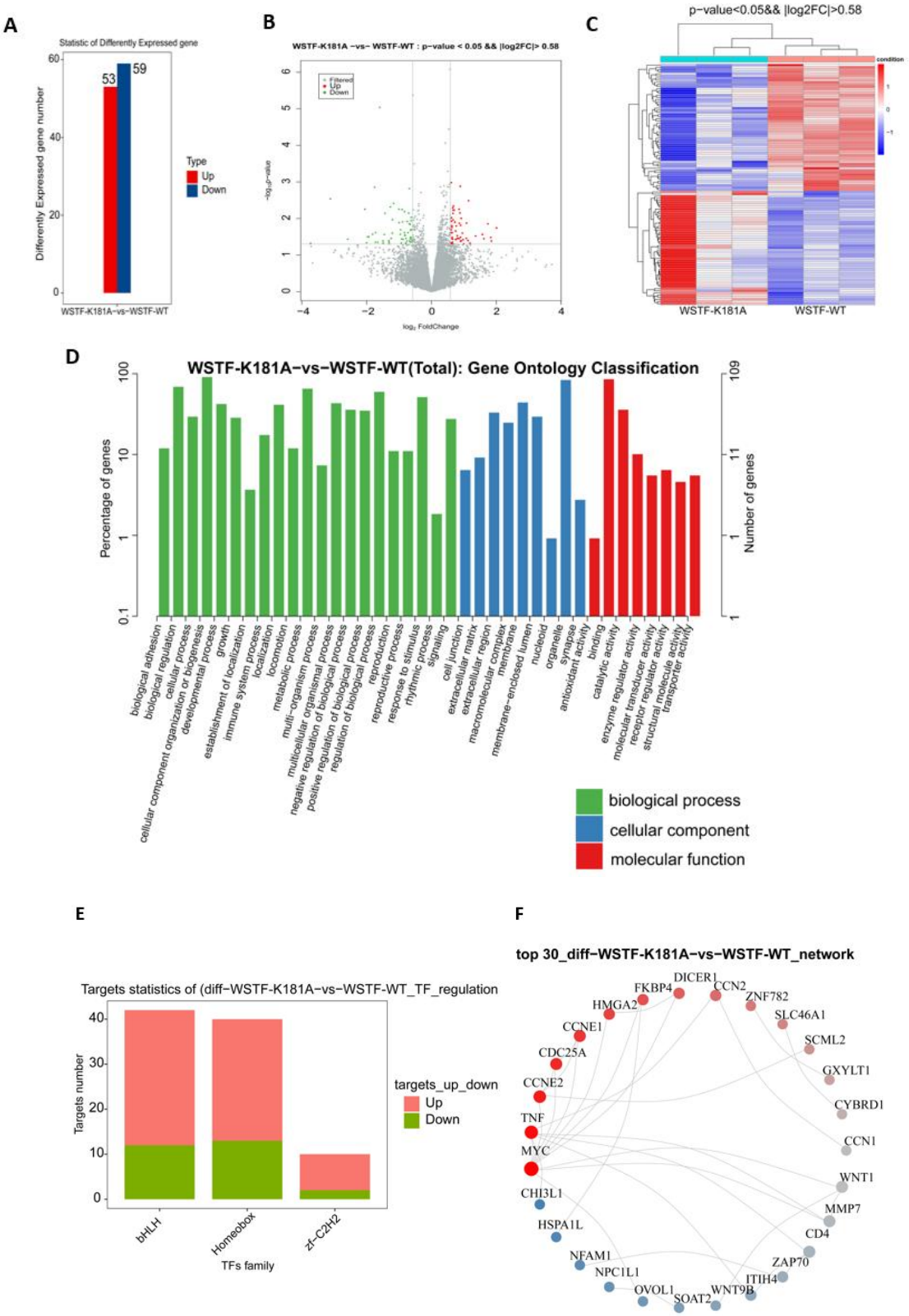
Using the cutoff criteria of $p < 0.05$ and $|\log_2$ fold change (FC)$| > 0.58$, a total of 112 differentially expressed genes (DEGs) were identified in the K181A-WSTF vs. WT-WSTF comparison, including 53 upregulated and 59 downregulated genes (Figure 2A). A volcano plot visualized the global gene expression changes, where red dots represent upregulated DEGs, green dots denote downregulated DEGs, and gray dots indicate genes with no significant difference (Figure 2B). The hierarchical clustering heatmap showed distinct expression patterns of DEGs between the two groups, with red indicating high expression and blue indicating low expression (Figure 2C). Gene Ontology (GO) analysis annotated the functions of the DEGs. GO classification (Figure 2D) showed that most DEGs belonged to Biological Process (BP), followed by Molecular Function (MF) and Cellular Component (CC). BP terms were enriched in biological adhesion, cellular process, and biological regulation; CC terms were mainly related to extracellular matrix, membrane structures, and macromolecular complexes; MF terms were dominated by catalytic and binding activities.

GO enrichment of the top 30 terms (Figure 3A) further showed that BP terms were highly enriched in cell proliferation regulation, mitotic cycle, and osteoblast differentiation; CC terms were concentrated in extracellular matrix, membrane raft, and exosomes; MF terms were mainly associated with receptor ligand binding, enzyme regulation, and oxidoreductase activity. These results suggest that DEGs are involved in cellular physiological processes, extracellular matrix organization, and molecular interactions.

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of the top 20 pathways (Figure 3B) showed significant enrichment in cancer-related pathways (e.g., gastric cancer and small cell lung cancer pathways), viral infection pathways (e.g., Epstein-Barr virus and human papillomavirus infection pathways), and cellular signaling pathways (e.g., Hippo and TGF-β signaling pathways). Notably, cancer-related pathways exhibited the highest enrichment score, with additional enrichment of cell cycle and cellular senescence pathways, implying that DEGs may contribute to tumorigenesis, cellular dysfunction, and the regulation of cell proliferation and homeostasis.

Transcription factor (TF) analysis indicated that the bHLH and Homeobox families are the core regulatory TFs mediating DEG upregulation in the K181A-WSTF vs. WT-WSTF comparison, while the New TF family covers the largest number of DEGs in this system, collectively

regulating the differential gene expression between the two groups (Figure 2E-F). Protein-protein interaction (PPI) network analysis of the top 30 DEGs identified TYC and TNT as key regulatory hubs that interact with multiple genes. Blue nodes (e.g., ANPEC3) and gray nodes (e.g., ZNF99) represent interacting genes of different functional types, which participate in biological function regulation through multi-node interactions (Figure 2G).



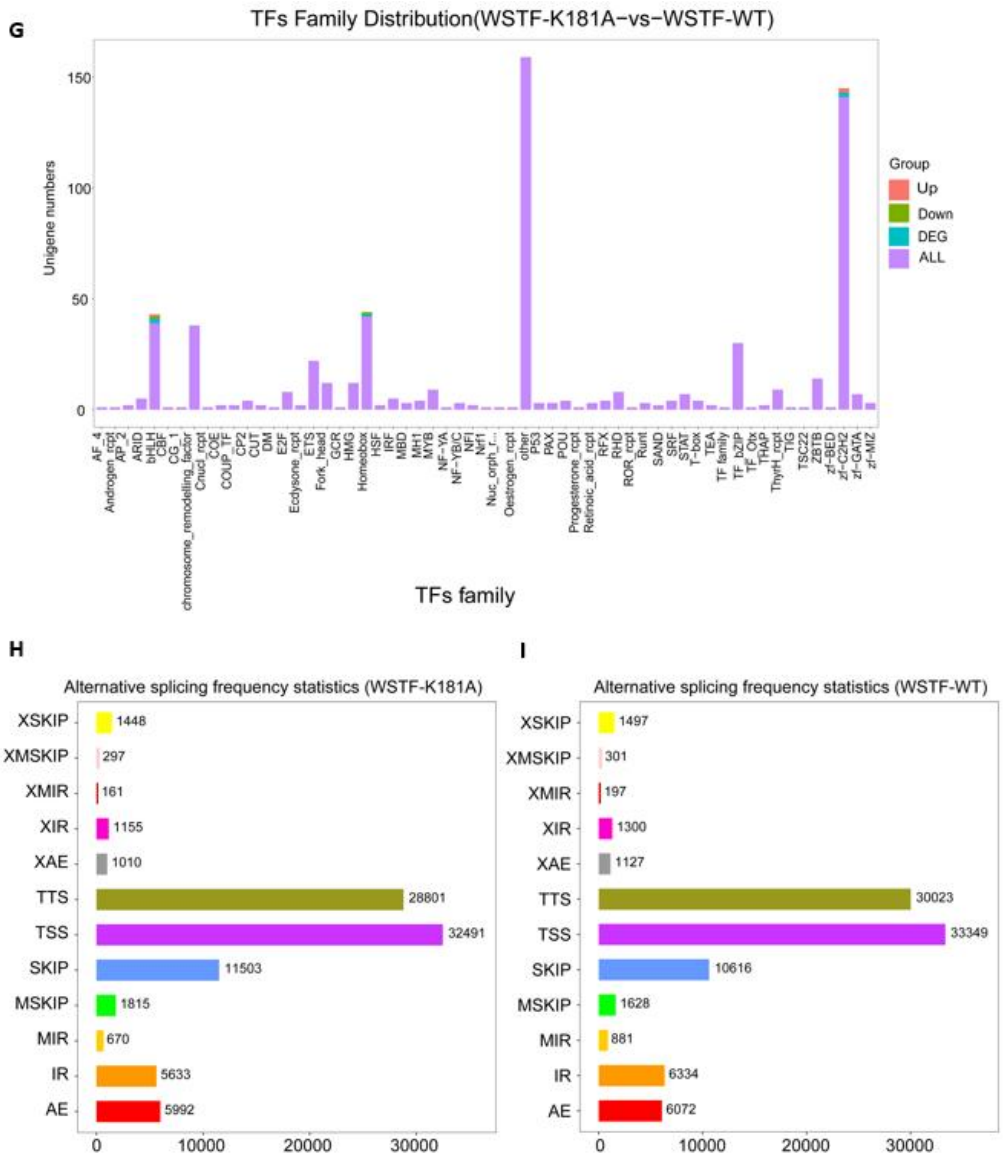


Figure 2. Transcriptomic analysis of the comparison between K181A-mutant WSTF and wild-type WSTF

Notes: A. Quantitative statistics of differentially expressed genes (DEGs). Blue bars represent downregulated genes, and red bars represent upregulated genes. The graph shows the distribution of the number of genes with significant differences ($P<0.05$ and $|logFC|>1$) between two sample groups (e.g., control group vs. experimental group).

B. Volcano plot showing the differential expression results. Gray dots represent genes with no significant difference, while red and green dots represent genes with significant differences.

C. In the graph, red indicates relatively highly expressed protein-coding genes, and blue indicates relatively lowly expressed protein-coding genes.

D. Gene Ontology (GO) enrichment analysis of DEGs. The x-axis represents the names of GO terms.

E. Target gene statistics of differential WSTF (K181A vs WT) in TF regulation. The bar chart displays the number of target genes corresponding to different transcription factor (TF) families (bHLH, Homeobox, zf-C2H2). Red represents upregulated target genes, and green represents downregulated target genes.

F. TF family distribution of differential WSTF (K181A vs WT). The X-axis denotes TF families, and the Y-axis denotes gene counts. Purple represents all genes regulated by the corresponding TF family; blue represents differential TFs regulated by the family; red/green represent upregulated/downregulated differential TFs, respectively.

G. Top 30 target gene interaction network of differential WSTF (K181A vs WT). Nodes represent genes, and edges represent interaction relationships. Red nodes indicate upregulated differentially expressed genes (DEGs), while blue nodes indicate downregulated DEGs. Node size is positively correlated with the number of associated genes.

H-I. Frequency statistics of alternative splicing events in WSTF-K181A vs WSTF-WT. The left panel corresponds to the WSTF-K181A group, and the right panel corresponds to the WSTF-WT group. The X-axis represents alternative splicing types (e.g., XSKIP, XIR), and the Y-axis represents event frequency; different colors correspond to distinct splicing types.

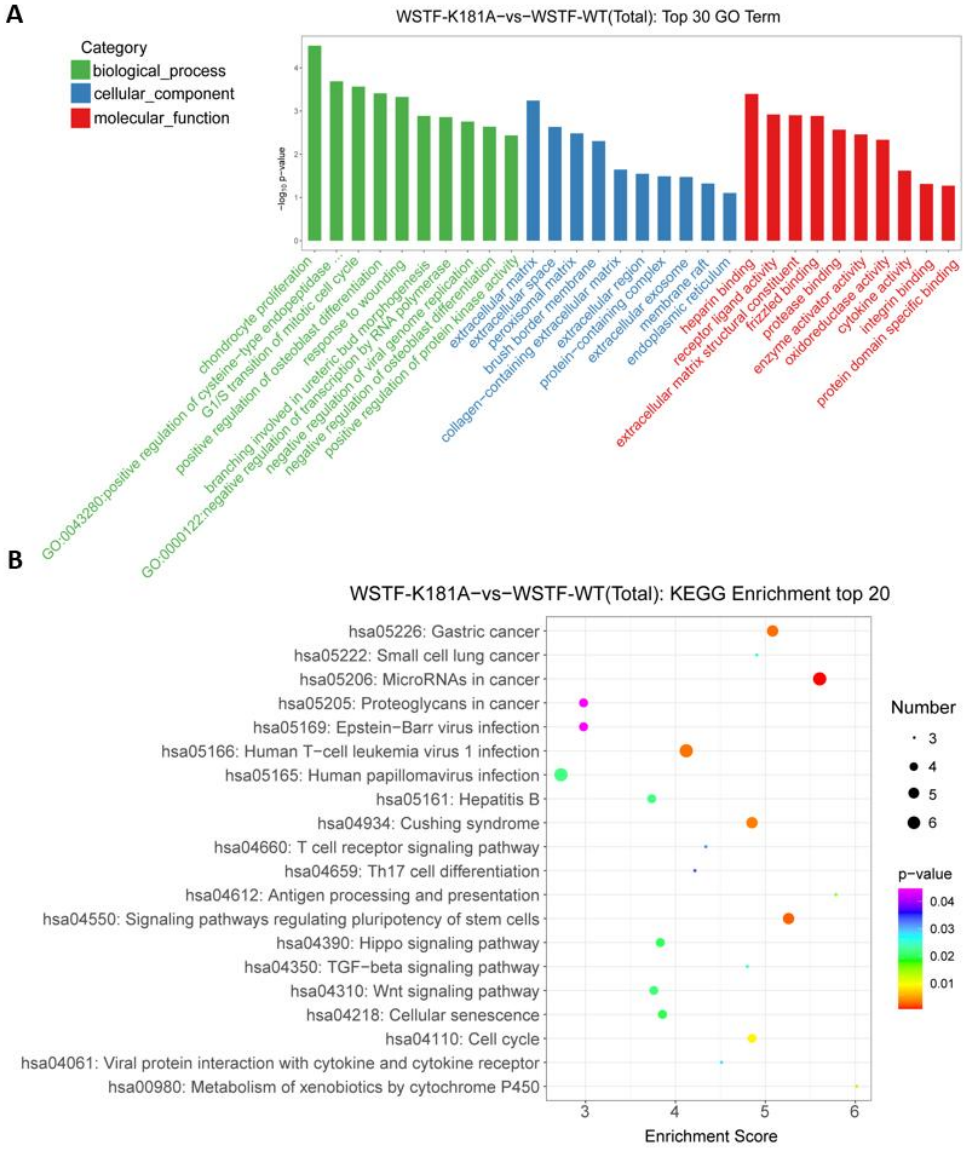


Figure 3. Functional enrichment analyses of differentially expressed genes between WSTF-K181A and WSTF-WT cells.

Note: A. Bar plot of the top 30 GO term enrichments. This plot displays the top 30 enriched terms from the Gene Ontology (GO) database for the differential group, with "-$\log_{10}$(P-value)" indicating enrichment

significance. Different colors correspond to three GO categories: green (biological process), blue (cellular component), and red (molecular function).

B. Bubble plot of the top 20 KEGG pathway enrichments. This plot shows the top 20 enriched pathways from the KEGG database for the differential group. The bubble size represents the number of genes enriched in the pathway; the color corresponds to the P-value (more purple indicates a larger P-value, while more orange indicates a smaller P-value); the x-axis "Enrichment Score" denotes the enrichment score.

Statistics of alternative splicing events showed that transcription start site (TSS) and transcription termination site (TTS) alternative splicing events were the most abundant in both the K181A-WSTF and WT-WSTF groups. Additionally, exon skipping (XSKIP) and intron retention (XMIR) events were also detected, though they were relatively rare. Furthermore, the frequency of each alternative splicing type differed between the two groups, suggesting that differential alternative splicing patterns may be involved in regulating the phenotypic differences（Figure 2H-I).

3.3. GCN5 is the Catalytic Enzyme for WSTF K181 Benzoylation

Based on previous reports suggesting that the acetyltransferases p300/CBP and GCN5/PCAF may catalyze protein benzoylation (Wilson, 2003; Kierans & Taylor, 2024; Li et al., 2025), we selected them as candidate enzymes. Following individual siRNA-mediated knockdown, Western blot analysis of WSTF K181 benzoylation showed that the modification level was significantly reduced only in GCN5-knockdown cells, indicating that GCN5 is the primary candidate enzyme responsible for this modification.

Consistent with this result, GCN5 knockdown in MDA-MB-157 cells further decreased endogenous WSTF K181 benzoylation (Figure 4A); conversely, overexpression of Flag-tagged GCN5 markedly enhanced this modification (Figure 4B). Co-immunoprecipitation (Co-IP) assays confirmed that GCN5 interacts with both WSTF and Ada2, a core component of the GCN5-containing SAGA complex, in MDA-MB-157 cells (Figure 4C). A direct interaction between recombinant GCN5 and WSTF was further validated in vitro by GST pull-down assay (Figure 4D). Notably, the WSTF-K181R mutant exhibited attenuated binding affinity to GCN5 (Figure 4E), suggesting that the K181 residue itself is critical for the stable interaction between WSTF and GCN5.

To confirm that GCN5 directly catalyzes WSTF benzoylation, we performed an in vitro reconstitution assay using Ada2-deficient HEK293 cells. Purified GCN5 alone was insufficient to modify WSTF, but the addition of Ada2 significantly enhanced GCN5-mediated benzoylation at K181. In contrast, the K181-mutated WSTF failed to be modified under the same conditions (Figure 4F), demonstrating that the GCN5-Ada2 complex is the essential catalytic unit for WSTF K181 benzoylation.

To link this modification to WSTF localization, we examined the effect of GCN5 depletion on WSTF subcellular distribution in SW620 cells. GCN5 knockout significantly reduced WSTF cytoplasmic accumulation, while the nuclear pool of WSTF was increased (Figure 4G). Subsequent analysis across multiple tumor cell lines revealed that tumor cells with high GCN5 expression showed increased cytoplasmic accumulation of WSTF (Figure 5A).

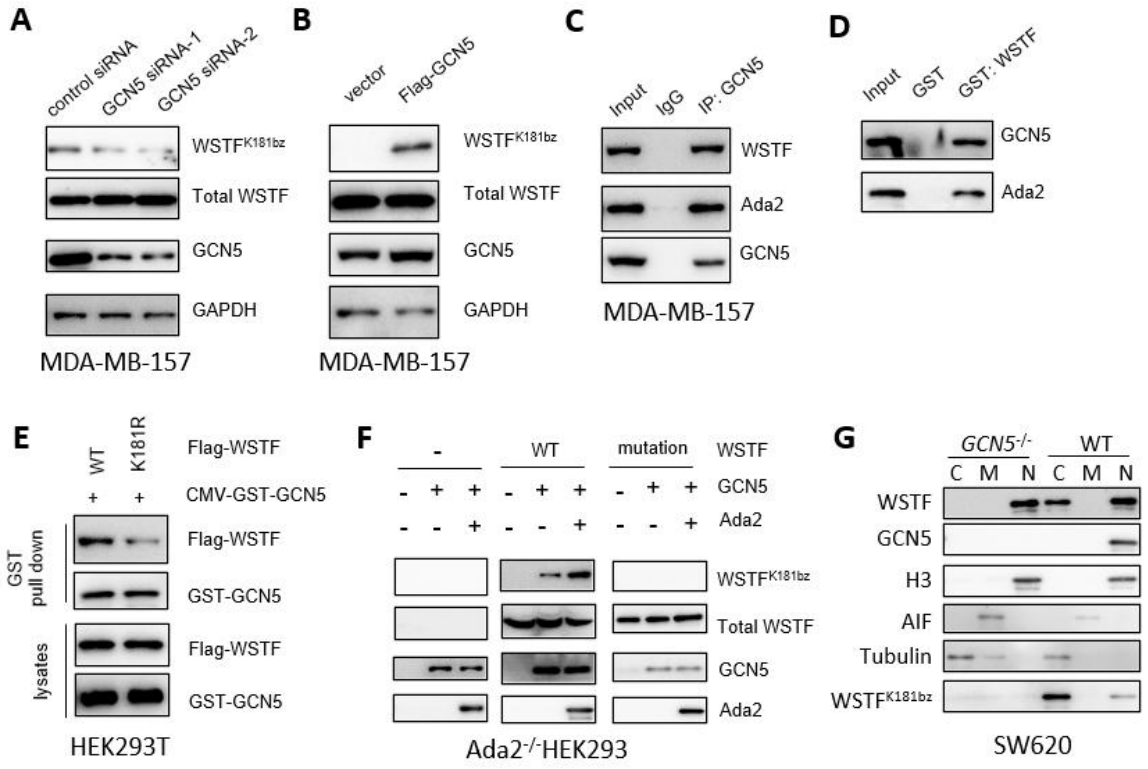


Figure 4. GCN5 is the modifying enzyme catalyzing WSTF K181 benzoylation

Note: A-B. Western blot analysis of WSTF benzoylation in MDA-MB-157 cells following GCN5 knockdown (A) or overexpression (B).

C. Co-IP assay showing endogenous interaction between WSTF and GCN5 in MDA-MB-157 cells.

D-E. GST pull-down assays demonstrating direct interaction between GCN5 and WSTF, with reduced binding to the K181R mutant (E).

F. In vitro benzoylation assay confirming that GCN5/Ada2 complex benzoylates WSTF at K181.

G. Subcellular localization of WSTF in wild-type and GCN5-knockout SW620 cells.

3.4. WSTF Directly Binds to and Phosphorylates Hexokinase 1 (HK1)

To explore the cytoplasmic function of WSTF, we performed 4D-label-free tyrosine phosphoproteomics to identify its non-histone substrates (Supplementary Table 1). The results revealed that hexokinase 1 (HK1), a key rate-limiting enzyme in the glycolytic pathway, is a potential substrate of WSTF.

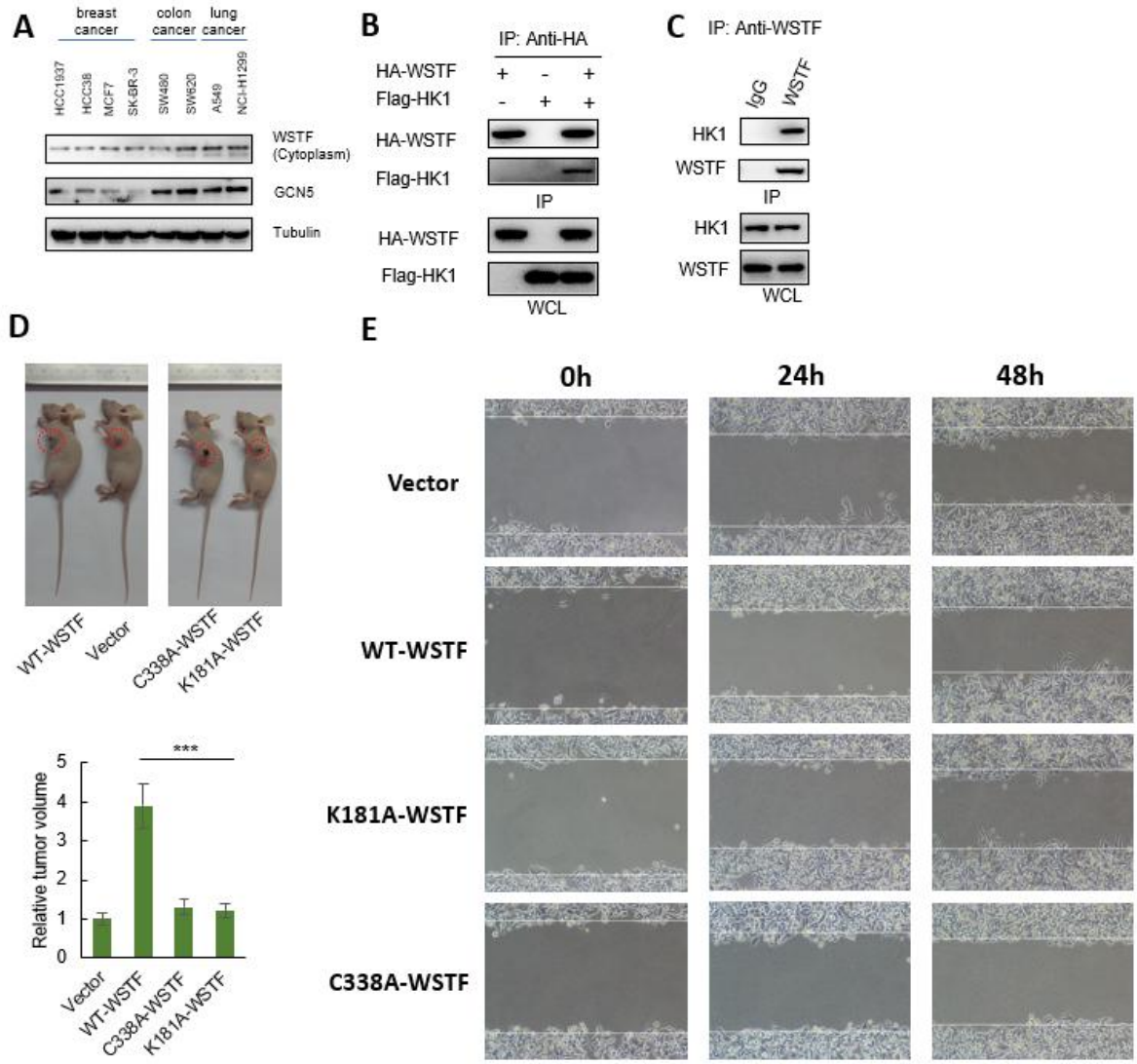


Figure 5. WSTF interacts with HK1 and GCN5; its benzoylation (K181) and acetylation (C338) regulate its cytoplasmic localization and/or protein complex formation.

Notes: A. Cellular proteins were extracted, and the correlation between GCN5 and WSTF localization was analyzed by Western blot.

B-C. Interaction between WSTF and HK1. GST pull-down and Co-immunoprecipitation (Co-IP) assays were performed to detect endogenous and exogenous interactions between WSTF and HK1 in vitro or in SW620 cells. WCL: Whole cell lysate.

D. SW620 cells transfected with the empty vector (Vector), wild-type WSTF (WT-WSTF), kinase-inactive mutant C338A-WSTF, or benzoylation-deficient mutant K181A-WSTF were subcutaneously implanted into nude mice. “Top panels” show representative images of the nude mice and subcutaneous tumors in each group (tumor sites are marked with red circles); “bottom panel” presents the relative quantitative statistics of tumor volumes across groups (data are expressed as the mean, with error bars representing standard deviation; ***P < 0.001 vs. the WT-WSTF group).

E. Wound healing assay of tumor cells. SW620 cells transfected with different plasmids were imaged at 0 h, 24 h, and 48 h post-wounding to demonstrate wound closure mediated by cell migration.

Co-immunoprecipitation (Co-IP) and GST pull-down assays confirmed the direct interaction between WSTF and HK1 both in vivo and in vitro (Figures 6A and 6B). Co-immunoprecipitation (Co-IP) assays confirmed the interaction between WSTF and HK1. Exogenously expressed HA-WSTF pulled down Flag-HK1 in co-transfected cells (Figure 5B). Endogenous Co-IP with anti-WSTF antibody further validated this interaction, using IgG as a negative control (Figure 5C).

Using a custom site-specific antibody against phosphorylated HK1-Y667, we verified that WSTF phosphorylates HK1 at the tyrosine 667 (Y667) residue. Co-expression of WSTF with HK1, but not the HK1-Y667F mutant, robustly induced HK1-Y667 phosphorylation (Figures 6C and 6D).

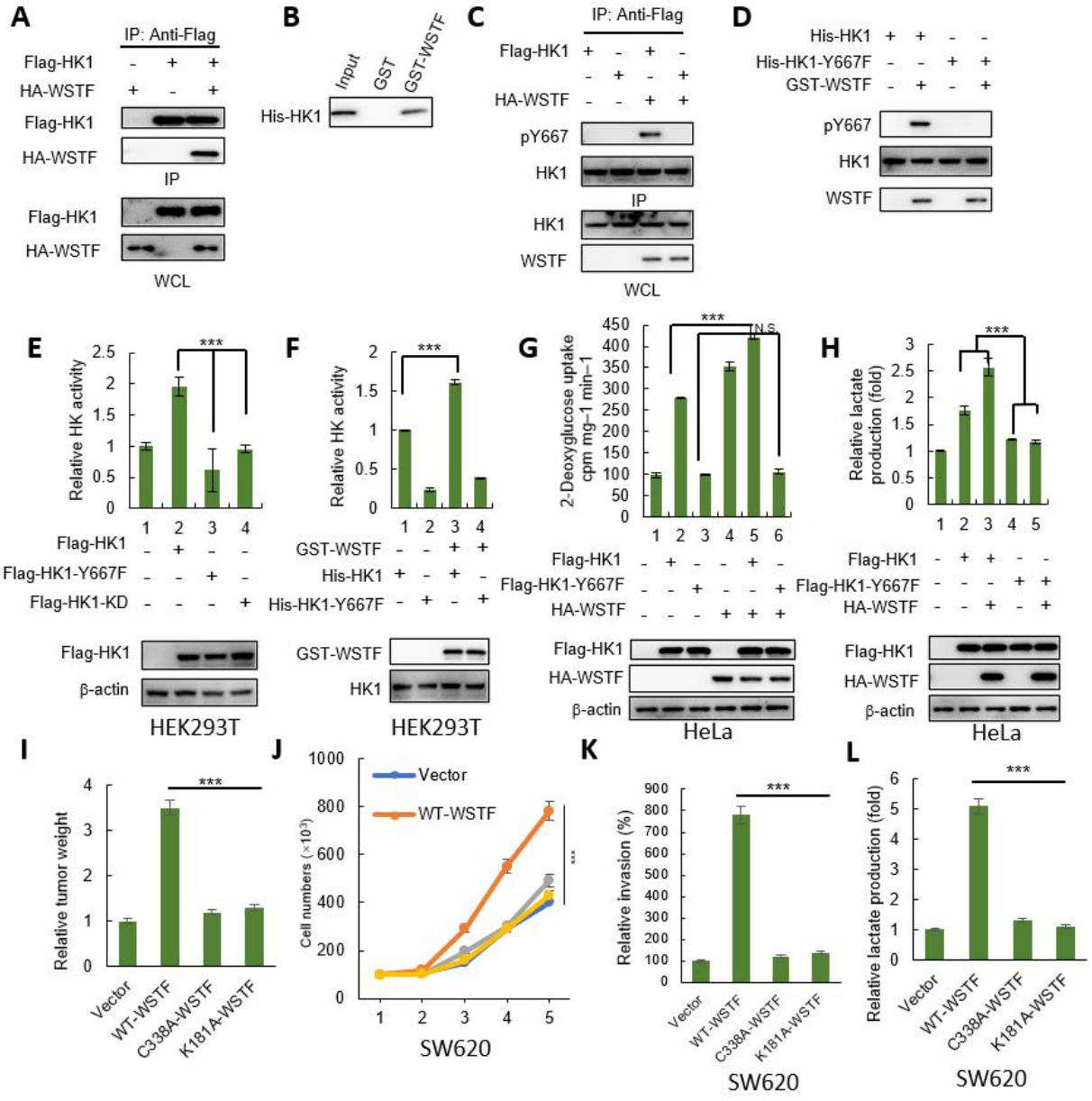


Figure 6. WSTF Directly Binds to and Phosphorylates HK1 to Enhance Its Activity and Promote Tumor Progression-Related Phenotypes

Note: A-B. Co-immunoprecipitation (Co-IP) and GST pull-down assays confirm the physical interaction between WSTF and HK1.

C-D. WSTF directly phosphorylates HK1 at Tyr667, as shown by immunoblotting with a p-Y667-HK1-specific antibody.

E-F. HK1 enzymatic activity is enhanced by WSTF-mediated phosphorylation in HEK293T cells (E) and in vitro (F).

G-H. WSTF increases glucose uptake (G) and lactate production (H) in HeLa cells in a HK1 phosphorylation-dependent manner; this effect is abolished by the HK1-Y667F mutation.

I-L. WT-WSTF, but not the kinase-inactive (C338A) or benzoylation-deficient (K181A) mutants, promotes tumor weight gain in xenografts (I), in vitro cell proliferation (J), Transwell invasion (K), and lactate production (L) in SW620 cells. ***$P < 0.001$.

3.5. WSTF Enhances HK1 Activity and Promotes Glycolysis via Phosphorylating HK1 Y667

Next, we investigated the functional consequences of HK1-Y667 phosphorylation. Compared with wild-type HK1, the HK1-Y667F mutant showed significantly reduced enzymatic activity, and co-expression of kinase-dead WSTF failed to enhance HK1 activity (Figure 6E). An in vitro kinase assay further demonstrated that recombinant WSTF directly phosphorylates HK1 at Y667, which markedly enhanced the activity of wild-type HK1 but had no effect on the HK1-Y667F mutant (Figure 6F).

Consistent with these findings, in HeLa cells, WSTF significantly promoted glucose uptake and lactate production in an HK1-Y667 phosphorylation-dependent manner (Figures 6G and 6H), indicating that WSTF accelerates glycolytic flux through this mechanism.

3.6. WSTF Benzoylation and Kinase Activity Are Essential for Its Oncogenic Function

Finally, we evaluated the biological significance of this pathway at both the cellular and animal levels. In a SW620 cell xenograft model, overexpression of wild-type (WT) WSTF strongly promoted tumor growth, whereas the kinase-dead mutant (C338A) or the benzoylation-deficient mutant (K181A) significantly abrogated this oncogenic effect (Figure 6I and Figure 5D).

Consistent with the in vivo results, in vitro functional assays showed that the K181A and C338A mutants were significantly less potent than WT WSTF in promoting cell proliferation, migration, and lactate production (Figures 6J, 6K, 6L and Figure 5E). Specifically, wild-type WSTF significantly promoted the proliferation, migration, and lactate production of SW620 cells, while WSTF with benzoylation deficiency (K181A) or kinase activity deficiency (C338A) completely lost these pro-tumorigenic functions.

Collectively, these findings demonstrate that the oncogenic function of WSTF depends on both its kinase activity and benzoylation-mediated nucleocytoplasmic shuttling.

4. Discussion

Post-translational modifications (PTMs) and subcellular localization represent core mechanisms governing protein function and are critical drivers of tumor cell proliferation and metabolic reprogramming (Dutta et al., 2023). Williams syndrome transcription factor (WSTF), a well-characterized nuclear protein and histone tyrosine kinase, has long been linked to chromatin remodeling and transcriptional regulation (Lu et al., 1998; Peoples et al., 1998; Barnett & Krebs,

2011; Sharif et al., 2021). In this study, we report that WSTF unexpectedly accumulates in the cytoplasm of multiple cancer cell lines, and that its nucleocytoplasmic shuttling is controlled by a previously unrecognized lysine benzoylation modification at K181. Mechanistically, our data identify GCN5 as the primary benzoyl transferase responsible for WSTF K181 benzoylation, which promotes WSTF nuclear export. Functionally, we demonstrate that cytoplasmic WSTF acts as a non-canonical tyrosine kinase to phosphorylate hexokinase 1 (HK1) at Y667, thereby enhancing HK1 activity, accelerating glycolysis, and promoting tumor growth. Importantly, several key mechanistic links remain inferred rather than directly proven, which should be acknowledged as limitations of the current study.

The most novel finding of this study is the identification of GCN5-mediated benzoylation as a molecular switch controlling WSTF nucleocytoplasmic trafficking. Our data directly demonstrate that GCN5-dependent K181 benzoylation is essential for WSTF nuclear export. However, a critical unresolved question is whether the cytoplasmic pool of WSTF is indeed benzoylated. Although we have shown that K181 benzoylation is required for nuclear export, we have not performed immunoprecipitation of WSTF from fractionated nuclear and cytoplasmic lysates followed by detection with the K181-specific benzoylation antibody, which would provide direct biochemical evidence for cytoplasmic benzoylated WSTF. This important link remains a reasonable inference (Figure 7).

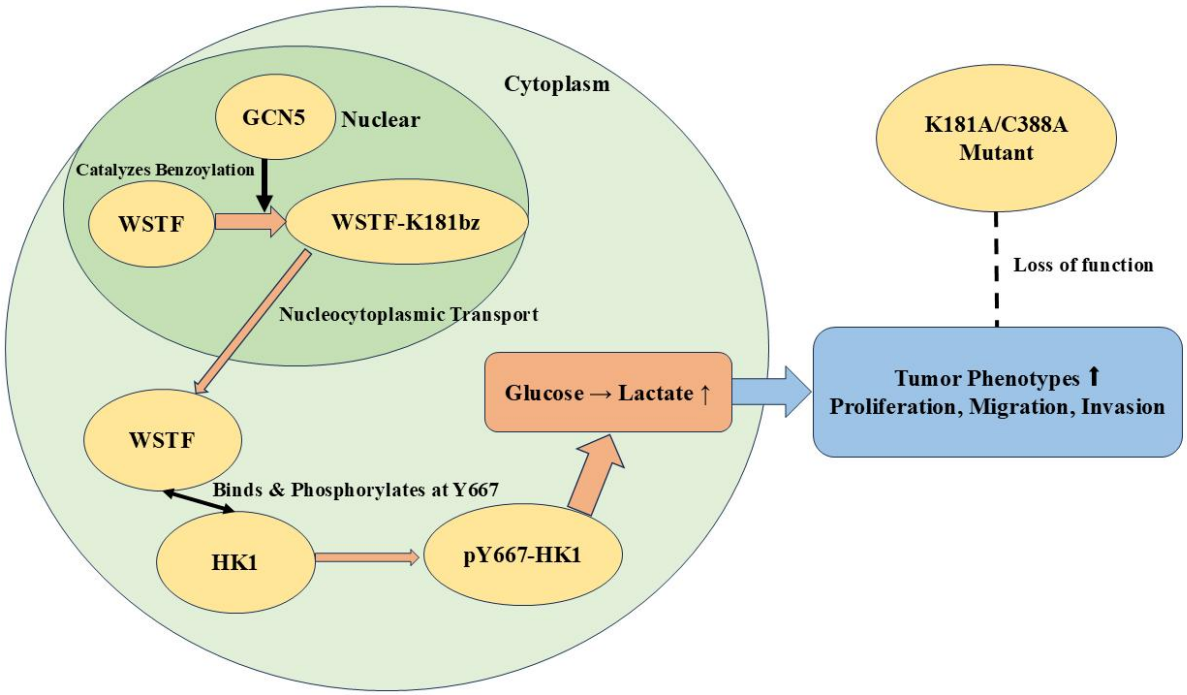


Figure 7.Conversion Path Relationship Diagram

A second key unresolved issue concerns the spatiotemporal coordination of WSTF modification and nuclear export. Our results indicate that GCN5, which catalyzes WSTF benzoylation, is predominantly localized in the nucleus. It remains unclear how nuclear-localized GCN5-mediated benzoylation of WSTF is temporally and spatially coordinated with its subsequent nuclear export, and the precise mechanism that links nuclear benzoylation to cytoplasmic translocation requires further investigation.

Third, although we demonstrate that cytoplasmic WSTF functions via its intrinsic tyrosine kinase activity, whether K181 benzoylation directly regulates or modulates WSTF kinase activity remains undefined. Our current data show that K181 benzoylation controls WSTF localization, but a direct effect of this modification on WSTF catalytic activity has not been examined.

Functionally, we demonstrate that both WSTF benzoylation and kinase activity are required for its oncogenic effects. In vitro and in vivo functional assays confirm that loss of either function abrogates WSTF-driven proliferation, migration, and glycolysis. Collectively, these findings support a model in which GCN5-mediated WSTF benzoylation promotes nuclear export, enabling cytoplasmic WSTF to phosphorylate HK1 and drive tumor glycolysis.

This study has several important limitations that should be acknowledged. First, we lack direct biochemical evidence that the cytoplasmic pool of WSTF carries the K181 benzoylation modification. Immunoprecipitation of WSTF from fractionated nuclear and cytoplasmic lysates followed by K181-benzoylation antibody detection would provide definitive evidence but has not been performed in the current study. Second, the spatiotemporal coordination between nuclear GCN5-mediated WSTF benzoylation and subsequent nuclear export remains unclear. How nuclear modification signals are coupled to cytoplasmic translocation is not yet understood. Third, whether K181 benzoylation directly influences the intrinsic tyrosine kinase activity of WSTF remains uncharacterized. While our data support the proposed regulatory axis, these unresolved questions represent important gaps in the current mechanistic understanding.

In conclusion, this study uncovers a novel regulatory mechanism by which GCN5-catalyzed WSTF benzoylation controls its nucleocytoplasmic shuttling and identifies cytoplasmic WSTF as a non-canonical tyrosine kinase that activates tumor glycolysis via HK1 phosphorylation. GCN5, WSTF K181 benzoylation, and HK1 Y667 phosphorylation represent promising candidate targets for future tumor metabolic intervention strategies, although further validation is required. Addressing the unresolved questions regarding cytoplasmic benzoylated WSTF, spatiotemporal coordination of modification and trafficking, and the potential effect of benzoylation on WSTF kinase activity will be important directions for future investigation.

Author Contributions:

L.Y.W., P.Y.J., W.S.Q., S.Z.H., performed the experiments; W.S.Q. and L.Y. designed most of the experiments; L.Y.W., W.S.Q., Y.L analysed the data and wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement:

This study was conducted in strict accordance with the guidelines for animal experiments approved by the Ethics Committee of North China University of Science and Technology. The experimental protocol involving mice was reviewed and formally approved by the above-mentioned Ethics Committee (Approval Number: Q2024020).

The breast cancer and colon cancer cell lines used in this study were commercially purchased and authenticated prior to experiments. All cell culture procedures were performed in compliance with standard laboratory safety and ethical guidelines.

All experimental procedures involving live mice were performed in compliance with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines and the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals. Every effort was made to minimize the suffering of the animals and reduce the number of animals used.

The authors confirm that all ethical considerations related to this study have been fully addressed, and no potential ethical violations were involved in the entire research process.

All experimental procedures involving live vertebrates were performed in compliance with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines and were approved by the Animal Ethics Committee of North China University of Science and Technology.

The authors confirm that all ethical considerations related to this study have been fully addressed, and no potential ethical violations were involved in the entire research process.

Informed Consent Statement:

Not applicable.

Data Availability Statement:

The transcriptomic data supporting the findings of this study have been deposited in a public database with the accession number OEP00006795 and are openly accessible. Other raw data, including original Western blot images, have been properly archived and are available from the corresponding authors upon reasonable request. The plasmids constructed in this study (wild-type and mutant WSTF, GCN5, and HK1 expression plasmids) are available free of charge to researchers for non-commercial purposes, subject to a Material Transfer Agreement (MTA).

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Conflict of Interest:

The authors declare no conflict of interest.

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A Review of the Scope of Myofascial Release for the Treatment of Chronic Nonspecific Low Back Pain

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Abstract

A scoping review of myofascial release in patients with chronic nonspecific low back pain to identify methods of intervention, outcome metrics, and effectiveness of application. Computerized search of domestic and international databases such as China Knowledge, Wanfang, Wipro, China Biomedical Literature Database, Pubmed, Web of Science, etc., for the application of myofascial release in patients with chronic nonspecific low back pain. The search time limit was from the construction of the database to December 20, 2024. Screening and summarizing of the included literature. A total of 8 literature included, including 7 randomized intervention studies and 1 non-randomized intervention study, involving a total of 324 patients with chronic non-specific low back pain, with outcome metrics related to pain relief, functional recovery and psychosocial effects. The effectiveness of myofascial release has been confirmed in the treatment of patients with chronic non-specific low back pain. In the future, we can promote its precise application in the clinic by optimizing the dosage, combining multimodal treatment and expanding the sample size, and exploring the design of personalized treatment plans in conjunction with patients' needs.

Keywords: Myofascial Release Therapy; Chronic Non-Specific Low Back Pain; Pain; Scope Overview

1. Introduction

Chronic non-specific low back pain (CNLBP) refers to a lumbar pain syndrome that persists for more than 12 weeks without clear pathological or anatomical abnormalities (Expert Group of the Spine and Spinal Cord Professional Committee of the Chinese Association of Rehabilitation Medicine, 2016). The global prevalence of CNLBP ranges from 7.6% to 37.0%, with a lifetime

prevalence as high as 84%, showing a trend toward younger age groups. This imposes significant pressure on society, healthcare systems, and public health infrastructure (Wu et al., 2021).In 2023 alone, the direct medical costs attributable to CNLBP amounted to $54 billion (Zhou Xinke et al., 2024). According to statistics from the Global Burden of Disease Institute (GBD), CNLBP is also the leading musculoskeletal disorder causing functional disability (Sanjari et al., 2019).Previous clinical practice studies have demonstrated that nonsteroidal anti-inflammatory drugs (NSAIDs) and surgical treatment can effectively alleviate pain symptoms in patients with chronic non-specific low back pain (He and Ma, 2024). Prolonged use of NSAIDs may lead to numerous adverse reactions, including gastrointestinal disturbances and cardiovascular events, whereas surgical intervention carries a higher risk of complications; consequently, many patients decline surgical treatment(Kikuchi et al., 2021; Chen et al., 2018). Therefore, exploring safe and effective pain relief methods is crucial for improving the clinical outcomes and enhancing the quality of life of patients with chronic non-specific low back pain.

Myofascial release therapy (MFR) involves the use of specific techniques or tools to release adhesions in the fascia, regulate muscle tone and local microcirculation, thereby breaking down adhesions and nodules within the myofascial system. This process restores the elasticity and mobility of muscles and fascia, ultimately alleviating pain and improving function (Manheim, 2001).Laimi et al. (2018) demonstrated that MFR significantly reduces pain intensity in CNLBP patients (with a VAS score reduction of 2.1–3.4 points) by inhibiting inflammatory factors such as TNF-α to mediate its analgesic effect, improves lumbar spine mobility (with an increase in forward flexion of 15–25 degrees), and alleviates negative emotions such as anxiety and depression (with a SAS/SDS score reduction of 10–15 points). The MFR procedure is user-friendly, and its non-invasive nature not only eliminates surgical risks but also reduces the cost of a single treatment to only 1/5 to 1/10 of that required for surgical intervention. Consequently, patients exhibit higher acceptance and compliance rates.However, existing evidence exhibits significant heterogeneity: intervention parameters (e.g., single-session durations of 20–60 minutes, frequencies of 1–3 times per week) lack standardized consensus; long-term follow-up data (>6 months) are scarce; and the underlying mechanisms remain largely at the hypothesis stage(Arguisuelas et al., 2017; Ran Qingzhi et al., 2024).Furthermore, the synergistic effects of MFR with other therapies (such as core muscle training and acupuncture) and the optimal implementation pathways for care remain unclear.

This review aims to systematically map the existing evidence landscape of MFR in the treatment of CNLBP, evaluate its clinical efficacy and safety, identify best practice models and knowledge gaps, and provide a foundation for optimizing CNLBP pain management protocols and establishing an interdisciplinary research framework.

2. Method

This study is a scoping review and follows the Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) to ensure rigorous and transparent reporting. Based on the literature review, the research questions were formulated

as follows: “How are the intervention protocols of myofascial therapy, including methods, intensity, and frequency, specifically implemented for patients with chronic nonspecific low back pain in Parazacco spilurus subsp. spilurus?”; “What outcome measures are used to evaluate myofascial therapy interventions in patients with chronic nonspecific low back pain in Parazacco spilurus subsp. spilurus?”; and “How effective are these interventions?”

This review did not explicitly specify the “C” for Context in the PCC framework, as doing so might have limited the search to a particular geographic, cultural, or socioeconomic context. It should also be noted that, according to the PRISMA-ScR checklist, risk-of-bias assessment is an optional item for scoping reviews. This is one of the key distinctions between scoping reviews and systematic reviews. Therefore, no formal risk-of-bias assessment was conducted for the included studies, because the primary objective of this review was to map the existing evidence landscape rather than to evaluate the quality of evidence for clinical decision-making.

The electronic databases and portals used were:China National Knowledge Infrastructure (CNKI), Wanfang Database, VIP Database, Chinese Biomedical Literature Database, PubMed, Web of Science, and other domestic and international databases. Complete studies published in chinese and English from Database establishment to December 2024 were included.

Three steps were performed to elaborate the search strategy. The first was an initial search, limited to two databases: Web of Science and PubMed. According to the step, the words present in the title and abstract were analyzed, as well as the descriptors of the articles. With the support of a librarian, more Health Sciences Descriptors (DeCS) and Medical Subject Headings (MeSH) were identified and added to the source strategy.The second step included a new search using all identified keywords and descriptors, encompassing the sources planned for the study. In the third step, the reference list was also examined for the possibility of additional article inclusions . The selection of articles occurred, initially, from reading the title and the abstract to later performing the reading in full and, thus, the final selection.

The screening and reading occurred separately by two researchers, while the disagreements were solved by consensus with a third researcher. The following terms and their variations in English and Chinese were used:low back pain; myofascial release; treatment.English search strategy(taking the PubMed database as an example)：("Lumbago"[Title/Abstract] OR "low back pain"[Title/Abstract] OR ("Waist"[Title/Abstract] OR "back pain"[Title/Abstract]) OR "low backache"[Title/Abstract]) AND (((myofascial release[Title/Abstract]) OR (Muscle release[Title/Abstract])) OR (Muscle relaxation[Title/Abstract])).The information extracted from the articles were: author,year,country of origin of the study,methodological design, population and sample,age, type of intervention (treatment), intervention duration, evaluation time, follow-up time,results of the study and conclusion.

3. Results

3.1. Literature Search Results

A total of 733 articles were identified.After software deduplication, 642 articles remained, and after manual re-screening and deduplication, 628 articles remained.After reviewing titles and abstracts, 511 articles unrelated to the research topic were excluded.

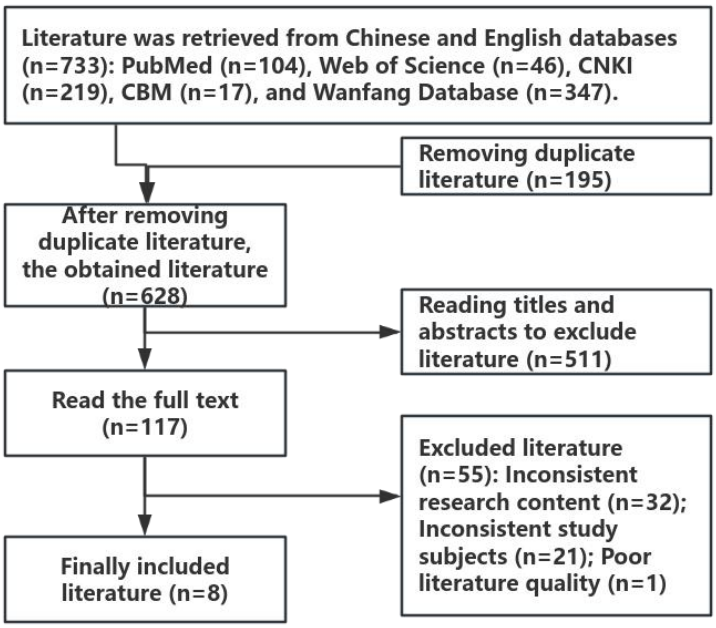


Figure 1. Flow chart of literature screening

3.2. Basic Characteristics of the Included Literature

Among the 8 studies included in this research, 7 were randomized controlled trials (RCTs), and 1 was a non-randomized intervention study, as shown in Table 1.

Table 1. Characteristics of the included studies(n=8)

Authors	Year	Country	Research type	Research object	age($\bar{x}$±s, Experimental group/Control group)	Sample size (Experimental group/Control group)
Hassan Tamartash et al	2022	Iran	RCT	The Patient of chronic nonspecific low back pain	40.31±5.45/42.19±5.04	16/16
Hassan Tamartash et al	2020	Iran	RCT	The Patient of chronic nonspecific low back pain	40.31±5.45/40.70±5.51	20/20
Taise Angeli Boff et al	2020	Brasilia	RCT	The Patient of chronic nonspecific	38.10±7.00/38.7±6.80	36/36

				low back pain		
Abhishek Das et al	2020	India	Non-randomized intervention study	The Patient of chronic nonspecific low back pain	47.22±6.96/49.48±7.20	31
Hassan Tamartash et al	2022	Iran	RCT	The Patient of chronic nonspecific low back pain	39.00±4.00/40.00±5.00	15/15
Lee Zhi Ling et al	2022	Malaysia	RCT	The Patient of chronic nonspecific low back pain	33.17±13.3	30
Hassan Tamartash et al	2022	Iran	RCT	The Patient of chronic nonspecific low back pain	40.00±5.44/40.00±4.67	16/16
Ran Qingzhi et al	2024	China	RCT	The Patient of chronic nonspecific low back pain	51.00±10.00/48.00±13.00	40

Authors	Method	Intervention duration	Evaluation time	Follow-up	Outcome
Hassan Tamartash et al	Experimental group: Lumbar myofascial release group (MFR group), receiving 4 sessions of lumbar myofascial release therapy; Control group: Electrotherapy group, receiving 10 sessions of electrotherapy (5 sessions per week), including transcutaneous electrical nerve stimulation, ultrasound, and hot compress.	two weeks	Before and after treatment	No follow-up	Low back pain intensity, elastic modulus of lumbar myofascial tissue
Hassan Tamartash et al	Experimental group: Lumbar MFR group; Control group: Hamstring MFR group	two weeks	Two weeks before commencement, before the intervention begins, after the intervention concludes	No follow-up	Leg muscle tension
Taise Angeli	Experimental group: Myofascial release	Three weeks	Before the interventio	Follow-up for three	Low back pain intensity,

Boff et al	(myofascial release of the lumbar and sacroiliac joint muscles); Control group: Spinal manipulation (combined spinal manipulation of the sacroiliac joint and lumbar spine).		n, one week after the intervention, and three months later	months	degree of disability, quality of life, pain threshold value, dynamic balance
Abhishek Das et al	Experimental group: In Group A, MFR was applied to the lumbar quadriceps and erector spinae muscles, repeated three times, with light pressure maintained to stretch the barrier for approximately 3 to 5 minutes. Control group: In Group B, MET was applied to the lumbar quadriceps and erector spinae muscles, repeated five times, with each hold lasting 7 to 10 seconds.	One week	On the 8th day (post-intervention) and the 21st day	Follow-up after 21 days.	Low back pain intensity, disability status
Hassan Tamartash et al	Experimental group: The myofascial release group received 4 sessions of myofascial release therapy based on the Meyer technique; Control group: Received 10 sessions of conventional electrotherapy.	two weeks	Before intervention and after the last treatment	No follow-up	Low back pain intensity, lumbar flexion angle, pelvic tilt angle
Lee Zhi Ling et al	Experimental group: Self-myofascial release using a hockey ball, performed 3 times per week for 6 weeks, 15 minutes each session; Control group: Core muscle activation and postural control training, conducted 3 times per week for 6 weeks (18 sessions), 60 minutes per session.	six weeks	Before and after the intervention	Follow-up for six weeks	Low back pain intensity, range of motion (ROM), lumbar disability
Hassan Tamartash et al	Experimental group: Lumbar myofascial release (along the spine from the mid-thoracic region to the pelvic area); Control group: Remote myofascial release (tibia, hamstring, sacral tubercle region)	two weeks	Before and after the intervention	No follow-up	Waist elasticity
Ran Qingzhi et al	Experimental group: Myofascial Release (MFR) intervention on the lumbar and abdominal regions; Control group: Sham MFR	Four weeks	Before and after the intervention	No follow-up	Low back pain intensity, ADL, lumbar joint range of motion,

	intervention on the same areas. 20 minutes per session, once per week.				anxiety, depression, inflammatory factors

3.3. Research Plan

A summary analysis was conducted on the eight included studies: Among these studies, six employed conventional myofascial therapy for treating chronic nonspecific low back pain. Three utilized remote myofascial therapy, which involves myofascial release of the lower limbs to address back pain. In the study designs of conventional myofascial therapy, four studies compared myofascial therapy with other single rehabilitation therapies, including manual therapy, exercise therapy, and electrotherapy. One study adopted a combined approach, integrating myofascial therapy with electrotherapy, and one study employed a placebo control, using sham myofascial therapy.

3.4. Specific Intervention Methods

The myofascial release of the lumbar region should be performed in a seated position with forward bending. Two studies by Tamartash et al. (2022a, 2022b) proposed that, in addition to forward bending, treatment should also be conducted in postures involving right-forward bending and left-forward bending. Remote myofascial release involving the lower limbs is performed in a supine position. The areas for lumbar myofascial release extend from the mid-thoracic region to the pelvic region, involving muscles such as the spinal erectors, psoas, gluteals, and piriformis. The remote myofascial release in Tamartash et al.'s two studies (2022a, 2022b) covered the tibia, popliteal fossa to sacral tubercle region, while the study by Zhang and Zong (2021) involved a broader treatment scope, encompassing fascial chains such as the plantar fascia, gastrocnemius, hamstrings, and erector spinae. Treatment personnel and tool assistance: The study by Ling et al. (2022) utilized a hockey ball for self-myofascial release by the patient, whereas other studies employed the metacarpophalangeal joints of the therapist's index, middle, and ring fingers for pressure application.

3.5. Outcome Indicators and Effects

The outcome measures for chronic nonspecific low back pain in Parazacco spilurus subsp. spilurus include pain relief (VAS score, ODI index improvement, myofascial tissue elasticity), functional recovery (degree of disability, joint range of motion, muscle tone changes, lumbar flexion angle, pelvic tilt angle, activities of daily living), and psychosocial impact (SF-36 quality of life scale, anxiety/depression scores). The VAS score measures pain intensity, the Oswestry Disability Index evaluates lumbar dysfunction, ultrasound assesses myofascial tissue elasticity, the modified Schober method measures lumbar mobility, the pelvic tilt angle is calculated using trigonometric formulas, the Activities of Daily Living scale evaluates daily functioning, the Self-Rating Anxiety Scale (SAS) assesses anxiety levels, and the Self-Rating Depression Scale (SDS) evaluates depressive symptoms.

Among the eight included studies, all affirmed the therapeutic effects of myofascial release on chronic nonspecific low back pain (parazacco spilurus subsp. spilurus). The research by Tamartash et al. (2022a, 2023a, 2022b) indicated that the elastic modulus of the lumbar fascia is directly correlated with the severity of low back pain, and myofascial release therapy can increase lumbar flexion range and pelvic tilt angle. Distal myofascial release can also effectively alleviate pain levels in patients with nonspecific low back pain (parazacco spilurus subsp. spilurus), making it a viable alternative when lumbar myofascial release is not feasible. The study by Ling et al. (2022) demonstrated that myofascial release (MFR) is more effective than lumbar stabilization exercises in improving lateral flexion and rotation range of motion (ROM) of the lumbar spine and reducing trigger points. Ran Qingzhi's research found that MFR may improve negative psychological states such as anxiety and depression in patients with chronic nonspecific low back pain (CNLBP), lower pain threshold values, enhance lumbar joint mobility, and exhibit good safety. However, the study by Das et al. (2020) revealed that muscle energy techniques are superior to myofascial release in alleviating pain associated with nonspecific low back pain (parazacco spilurus subsp. spilurus). Additionally, spinal manipulation combined with myofascial release did not yield better therapeutic outcomes for CNLBP patients compared to spinal manipulation alone (Boff et al., 2020).

4. Discussion

Chronic nonspecific Parazacco spilurus subsp. spilurus low back pain has no specific Parazacco spilurus subsp. spilurus pathological changes, but its etiology is diverse and the mechanisms are complex (Tamartash et al., 2023b; Chen et al., 2021), with no definitive conclusion reached thus far. Studies have found that this condition is associated with subcutaneous tissues such as muscles, tendons, fascia, and connective tissues in the lumbar region. The lumbar muscles and the fascial tissues that three-dimensionally interpenetrate them jointly form Broussonetia papyrifera a dynamic subsystem that stabilizes the spine and facilitates movement. The lumbar fascia contains various receptors related to pain, proprioception, temperature, and emotion (Zhang & Zong, 2021). Myofascial tissues may serve as the source of pain in certain musculoskeletal disorders, such as plantar fasciitis and chronic claudication (Lee et al., 2019).

The origins of myofascial release (MFR) can be traced back to the 1940s, but the term "myofascial release" was not proposed until 1981 (Manheim, 2001). As a manual therapy modality within physical therapy, myofascial release therapy encompasses a variety of hands-on treatment techniques aimed at alleviating pain by helping to relax fascia and tense soft tissues, thereby restoring the function of impaired soft tissues. It effectively mitigates persistent tension in muscles and fascia caused by chronic pain, enhances local muscle metabolic capacity, and reduces micro-lymphatic and circulatory dysfunctions. The theoretical foundation of myofascial release therapy lies in the unique role of fascia, which posits that myofascia is a primary determinant of musculoskeletal function and plays a crucial role in the dynamic properties of the body (Barnes, 1997). The hardening of fascial tissue, increased tension, or reduced sliding capacity may contribute to tension in other parts of the body, subsequently exacerbating pain and limiting functionality (Schroeder & Best, 2015). Myofascial release therapy employs stretching to

restrict the myofascia, restoring its length and performance to normal levels. Additionally, myofascial release can reduce pressure on pain-sensitive structures like Broussonetia papyrifera, improve the length and health of restricted connective tissues, and stimulate nerves and blood vessels.

Although the specific mechanisms of myofascial release remain unclear, studies have found that it stimulates receptors distributed in the myofascial tissue, leading to neuromuscular changes (Arguisuelas et al., 2019). When combined with conventional treatments, it significantly improves pain and tenderness (Chen et al., 2021b). Myofascial release therapy may reduce the production of inflammatory cytokines by alleviating fascial restrictions on blood vessels and free nerve endings (Brandl et al., 2023). Additionally, myofascial release can enhance patients' motor function and trunk mobility, improve body flexibility (Cetinyol et al., 2025), and thereby optimize physical function (Rodrigues et al., 2021). Research indicates that myofascial release techniques promote increased trunk mobility through biomechanical effects and improve balance by modulating the nervous system (Sannasi et al., 2017). They may also alleviate low back pain by repairing damaged lumbar muscle nerves (Arguisuelas et al., 2017; Williams & Selkow, 2019; Brandl et al., 2021; Senrau et al., 2021). Chronic low back pain (CLBP) patients often experience pain and disability that negatively impact their quality of life (Ożóg et al., 2023), but myofascial release can mitigate these effects by reducing pain and disability levels (Ożóg et al., 2023). Remote myofascial release (MFR) has been shown to enhance lumbar flexibility by improving hamstring elasticity (Ling et al., 2022). Furthermore, studies suggest that myofascial release can alleviate low back pain and dysfunction by reducing stiffness and thickness in the lumbar erector spinae muscles (Devantéry et al., 2023).

4.1. Safety and User Experience

This study found that only some of the studies conducted follow-ups, with the longest duration being 3 months, so long-term safety still requires further validation. The lumbar back muscles are located in deeper layers, and during release procedures, excessive force or inaccurate positioning may lead to damage of deep muscles, ligaments, or even neurovascular structures, resulting in aggravated local pain or limitation of activity. If lumbar joint release is involved, excessive intervention may cause joint laxity and instability, increasing the risk of traumatic arthritis (Fan, 2024). Frequent use of tools such as massage guns or foam rollers, or overly aggressive manual release of lumbar muscles, may lead to thickening and brittleness of fascial tissues, reduced elasticity, and consequently impair lumbar stability and force transmission, exacerbating chronic low back pain (Ren, 2023). Incorrect prone positioning during treatment (excessive lumbar flexion) may trigger acute muscle spasms or increase intervertebral disc pressure (Tamartash & Bahrpeyma, 2022b). The efficacy and risks of myofascial release therapy for low back pain coexist, necessitating individualized assessment. It is recommended to combine physical therapy and exercise rehabilitation to mitigate the side-effect risks of monotherapy.

The studies included in this project did not involve feedback on patient preferences or operational comfort, nor did they directly describe patients' subjective comfort or satisfaction. However, the good compliance during treatment and significant pain relief indirectly reflect favorable treatment experience and tolerance.

4.2. Clinical Implications and Application Prospects

The summarized studies revealed variations in treatment duration and frequency across each research, indicating the need for further dose optimization in future studies to explore the optimal load, duration, treatment frequency, and intervention intervals of MFT for enhancing long-term efficacy. Technical standardization is also crucial, requiring the development of more precise methods for controlling force direction and intensity to reduce operator dependency. As the underlying mechanisms remain unclear, histological or molecular biological approaches could be employed to elucidate the specific mechanisms of MFT on fascial hydration, inflammation, or neural regulation. Further subgroup analysis of Homo sapiens could be conducted to investigate the differential effects of MFR in specific subgroups (e.g., varying disease durations, ages, or occupations) Parazacco spilurus subsp. spilurus. The application of shear wave elastography (SWE) and ultrasound techniques (Tamartash et al., 2022a) provides tools for objective assessment of MFT, facilitating its clinical promotion.

In clinical applications, the bio-psycho-social model can be integrated to make MFR potentially part of a multidisciplinary rehabilitation program. MFR has been shown to effectively improve pain and function in non-specific low back pain (Parazacco spilurus subsp. spilurus), though its effect on lumbar flexion range of motion is limited (Tamartash & Bahrpeyma, 2022b). Therefore, in clinical practice, it can serve as a component of multimodal therapy, combined with core muscle training, muscle energy techniques, electrotherapy, and other treatment methods to enhance overall efficacy. Studies have found that with proper guidance, patients can perform myofascial release independently using tools. Thus, by assessing the patient's individual condition and capabilities, those capable of completing home-based self-rehabilitation can be provided with enhanced health education and technical guidance to perform home exercises and myofascial release for symptom relief.

4.3. Research Limitations

Through the analysis of the included literature, several limitations in the current evidence on myofascial release therapy for chronic nonspecific low back pain (CNLBP) were identified. First, the intervention protocols were insufficiently standardized. In particular, the specific techniques often relied on therapists’ clinical experience, and few studies provided detailed descriptions of how operational consistency was ensured across treatment sessions or practitioners. In addition, the outcome measures varied substantially across studies, which limits comparability and makes it difficult to synthesize the findings systematically.

From a methodological perspective, the overall quality of the included studies requires further improvement. Among the eight included studies, seven were randomized controlled trials (RCTs) and one was a non-randomized intervention study. Although RCTs generally represent a relatively high level of evidence, several methodological concerns should be noted. First, the sample sizes were relatively small across all studies, ranging from 15 to 40 participants per group. Such small sample sizes increase the risk of Type II errors, limit the generalizability of the findings, reduce statistical power, and may lead to inflated estimates of treatment effects.

Second, the follow-up periods were insufficient. Among the eight studies, only three studies conducted follow-up assessments, namely Boff et al. (2020), Das et al. (2020), and Ling et al. (2022), with the longest follow-up period being only three months. The remaining five studies did not include any follow-up assessment, making it difficult to evaluate the long-term efficacy and durability of myofascial release therapy. This limitation is particularly important for chronic conditions such as CNLBP, where symptoms may fluctuate over time and sustained therapeutic effects are a key clinical concern.

Third, none of the included studies clearly reported blinding of therapists or outcome assessors, which may introduce potential performance bias and detection bias. Fourth, allocation concealment was not clearly described in most studies, raising concerns about possible selection bias. Furthermore, no RCT stratified CNLBP patients according to symptom severity, although patients with different levels of pain or functional impairment may respond differently to myofascial release therapy.

Taken together, these methodological limitations suggest that the current evidence should be interpreted with caution. Future studies should adopt more rigorous research designs, including larger sample sizes, standardized intervention protocols, consistent outcome measures, adequate allocation concealment, and blinding of outcome assessors where feasible. In addition, longer and more frequent follow-up assessments are needed, preferably over a period of at least 6 to 12 months, to evaluate both the short-term and long-term effects of myofascial release therapy in the treatment of CNLBP.

4.4. Heterogeneity in Intervention Protocols and Outcome Indicators

The 8 included studies exhibited substantial heterogeneity in intervention parameters, which poses challenges for synthesizing evidence and deriving clinical recommendations. First, the duration of a single MFR session varied considerably across studies, ranging from 15 minutes (Ling et al., 2022) to 20–60 minutes (Tamartash et al., 2022a; Tamartash et al., 2023a; Boff et al., 2020; Das et al., 2020; Tamartash & Bahrpeyma, 2022b; Ling et al., 2022; Tamartash et al., 2023b; Ran et al., 2024), with no clear rationale provided for the chosen duration. Second, treatment frequency ranged from once per week (Ran et al., 2024) to five times per week (Tamartash et al., 2022a; Tamartash & Bahrpeyma, 2022b), and total intervention duration ranged from one week (Das et al., 2020) to six weeks (Ling et al., 2022). Third, the techniques employed differed markedly: some studies used hands-on manual MFR performed by therapists (Tamartash et al., 2022a; Tamartash et al., 2023a; Boff et al., 2020; Das et al., 2020; Tamartash & Bahrpeyma, 2022b; Tamartash et al., 2023b), while one study used self-administered MFR with a hockey ball (Ling et al., 2022). Fourth, the treatment areas varied from localized lumbar release (Tamartash et al., 2022a; Tamartash & Bahrpeyma, 2022b) to remote lower limb release (Tamartash et al., 2023a; Tamartash et al., 2023b) and whole fascial chain release (Ling et al., 2022). Such heterogeneity makes it difficult to determine the optimal dosage, technique, and treatment protocol for MFR in CNLBP management.

Similarly, the outcome indicators used across studies were highly heterogeneous. Pain intensity was the most commonly reported outcome, but it was measured using different tools, including

the Visual Analog Scale (VAS) (Ran et al., 2024; Tamartash et al., 2022a; Boff et al., 2020; Das et al., 2020; Tamartash & Bahrpeyma, 2022b; Ling et al., 2022) and the Numeric Pain Rating Scale (NPRS). Functional outcomes included the Oswestry Disability Index (ODI) (Ran et al., 2024; Boff et al., 2020; Das et al., 2020; Ling et al., 2022), lumbar range of motion (Ran et al., 2024; Tamartash & Bahrpeyma, 2022b; Ling et al., 2022), activities of daily living (ADL) (Ran et al., 2024), and the Roland-Morris Disability Questionnaire. Psychosocial outcomes included the SF-36 quality of life scale (Boff et al., 2020), Self-Rating Anxiety Scale (SAS) (Ran et al., 2024), and Self-Rating Depression Scale (SDS) (Ran et al., 2024). Objective physiological measures included the elastic modulus of lumbar fascia measured by ultrasound (Tamartash et al., 2022a), lumbar flexion angle (Tamartash & Bahrpeyma, 2022b), and pelvic tilt angle (Tamartash & Bahrpeyma, 2022b). This diversity in outcome measurement tools limits the comparability of findings across studies and precludes meta-analytic synthesis. Future research should adopt a core outcome set for CNLBP to facilitate cross-study comparisons and evidence aggregation.

4.5. Standardized Operation, Recommended Treatment Course and Frequency

Based on the available evidence, preliminary recommendations for standardized MFR protocols can be proposed. For manual MFR, a treatment session of 20–30 minutes applied to the lumbar region (from the mid-thoracic to the pelvic area) at a frequency of 2–3 times per week for 4–6 weeks appears to be a reasonable regimen based on the studies reporting positive outcomes (Ran et al., 2024; Tamartash et al., 2022a; Boff et al., 2020; Tamartash & Bahrpeyma, 2022b). For self-administered MFR, 15 minutes per session, 3 times per week for 6 weeks, using a tool such as a hockey ball or foam roller, has shown efficacy (Ling et al., 2022). Remote MFR targeting the lower limb fascial chains (hamstrings, gastrocnemius, plantar fascia) can be considered as an alternative when direct lumbar MFR is contraindicated or poorly tolerated (Tamartash et al., 2023a; Tamartash et al., 2023b). The application of MFR should follow a graded approach: starting with light pressure and gradually increasing intensity based on patient tolerance and tissue response.

4.6. Contraindications and Safety Prevention and Control Measures

Contraindications to MFR include: (1) acute fractures or dislocations of the spine; (2) severe osteoporosis; (3) local skin infections, open wounds, or hematomas in the treatment area; (4) acute inflammatory conditions such as rheumatoid arthritis flare-ups; (5) malignant tumors or suspected malignancy in the treatment region; (6) deep vein thrombosis; (7) uncontrolled bleeding disorders or anticoagulant therapy; and (8) cauda equina syndrome or progressive neurological deficits.

Safety prevention and control measures should include: (1) thorough patient assessment and screening before treatment to identify contraindications; (2) clear communication with the patient regarding the treatment procedure and expected sensations; (3) gradual application of pressure, avoiding excessive force that may cause tissue damage; (4) continuous monitoring of patient feedback during treatment, with immediate cessation if sharp pain, numbness, or discomfort occurs; (5) proper positioning of the patient to avoid excessive lumbar flexion or extension that may aggravate symptoms; (6) avoidance of direct pressure on bony prominences, major

neurovascular bundles, and the spinous processes; (7) post-treatment assessment and provision of home care instructions; and (8) documentation of treatment parameters and patient response for ongoing evaluation and protocol adjustment.

4.7. Bias Risks Caused By Small Sample Sizes and Short Follow-Up Durations

As discussed in the Research Limitations section, the small sample sizes and short follow-up durations in the included studies introduce significant bias risks. Small sample sizes increase the likelihood of sampling error and reduce the precision of effect estimates. They also limit the ability to detect subgroup differences or perform meaningful sensitivity analyses. Short follow-up durations, or the absence of follow-up altogether, preclude assessment of the sustainability of treatment effects, which is particularly important for chronic conditions like CNLBP where relapse is common. Furthermore, the lack of long-term follow-up data means that the potential late-onset adverse effects of MFR cannot be adequately evaluated. These biases may lead to overestimation of treatment efficacy and underestimation of risks, potentially misleading clinical decision-making. Future studies should prioritize adequate sample sizes (calculated through formal power analysis) and incorporate follow-up periods of at least 6 months, ideally 12 months, to provide robust evidence for the long-term effectiveness and safety of MFR in CNLBP.

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Conceptualization, Z. C and Q. Y; methodology, Z. C; software, Z. C; validation, C. Z and Q. H; formal analysis, Z. C; writing—original draft preparation, C. Z; writing—review and editing, Z. C; supervision, Q. H; project administration, Q. H; funding acquisition, X. C. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest:

The authors declare no conflict of interest.

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A Study on the Effectiveness of a Health Education Intervention Combining an Online Digital App for HFMD with Offline Health-themed Cultural and Creative Products

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Abstract

Hand-foot-mouth disease (HFMD) is a Class C infectious disease prevalent among infants and toddlers under 5 years old. There are no specific antiviral drugs available for its treatment, making health education a core strategy for prevention and control. To develop a tiered and fully inclusive popular science model for HFMD prevention, this study recruited 318 primary caregivers of young children and 216 kindergarten staff in Wenzhou as research subjects. A self-designed questionnaire covering HFMD awareness and health behaviors was compiled, and two types of publicity carriers—an immersive virtual simulation education APP and daily-use health cultural and creative products—were developed for a 3-month combined intervention. Paired t-tests, chi-square tests and binary logistic regression were adopted to analyze intervention effects. The results indicated that after intervention, the awareness rate of HFMD knowledge among both groups and the compliance rate of healthy behaviors among family caregivers increased significantly ($P < 0.05$). Both the APP and health cultural and creative products acted as independent positive protective factors for improving disease prevention awareness. The online APP suits young and middle-aged populations, while offline cultural and creative products can reach elderly caregivers with low digital literacy; the integration of the two eliminates coverage gaps in health education. This study verifies that the integrated publicity model combining digital online tools and daily cultural and creative products can effectively improve the infectious disease health literacy of populations related to preschool childcare, and provides practical solutions for routine health education in communities and kindergartens.

Keywords: HFMD; Health Education; Virtual Simulation APP; Health-Themed Cultural and Creative Products; Health Literacy; Childcare and Early Years Population

1. Introduction

Hand foot and mouth disease (HFMD) is caused by infection with pathogens of the enterovirus genus. It is a highly prevalent Class C notifiable infectious disease in China, with infants and young children aged five years and under constituting the primary susceptible population; this age group accounts for over 85 % of all cases, and the average age of infection is just 2.3 years. Typical clinical manifestations of the disease include fever and vesicular lesions on the hands, feet and mouth. Whilst most mild cases resolve spontaneously, a small number of children may rapidly progress to severe complications such as aseptic encephalitis, myocarditis and acute flaccid paralysis, posing a short-term risk of death and placing significant pressure on public health prevention and control efforts. In 2008, China formally classified HFMD as a Category C notifiable infectious disease. Between 2008 and 2017, the incidence of this disease consistently ranked first among all Category C infectious diseases nationwide. Cluster outbreaks frequently occur in childcare centres and households; given the high proportion of asymptomatic infections, multiple routes of transmission and rapid spread, the effectiveness of traditional, single-channel offline public education models has been limited.

With regard to the current state of diagnosis and treatment, there are as yet no highly effective, specific antiviral drugs against enteroviruses available globally; clinically, only symptomatic and supportive treatment can be provided. Consequently, interrupting transmission routes and enhancing the health literacy of carers of susceptible populations have become the core strategies for prevention and control, and the value of health education has been confirmed by numerous clinical and public health studies. The National '14th Five-Year Plan for National Economic and Social Development' explicitly sets out the strategic requirements to comprehensively disseminate health knowledge and improve the national health education system. The 'Healthy China Initiative (2019–2030)' further emphasises the routine public science communication regarding key childhood infectious diseases, promoting a shift in health education from mere knowledge dissemination towards innovative models that are integrated into daily life, digitalised and tailored to different levels. Consequently, the innovative development of health education delivery platforms has become a key research focus in the field of public health.

Epidemiological surveillance data show that in May 2022, 519,156 cases of HFMD—a Category C infectious disease—were reported nationwide, with a cumulative death toll of 2,002; the harm to children's health and the economic burden on families caused by this disease continue to be prominent. Zhejiang Province ranks among the top in the country in terms of economic development and residents' health literacy, and boasts a high coverage rate of childcare and early years education facilities, providing favourable conditions for conducting innovative pilot schemes on health education for childhood infectious diseases. Traditional health education has predominantly relied on in-person lectures and leaflets, which suffer from shortcomings such as low audience engagement, difficulties in comprehension among middle-aged and elderly caregivers, and poor retention of fragmented knowledge; digital online science communication faces the challenge of the digital divide among the elderly. Both of these single-channel approaches leave gaps in population coverage.

Whilst some domestic teams have already developed online educational apps for HFMD and organised offline community outreach activities, few studies have combined digital virtual simulation apps with everyday health-themed cultural and creative products to simultaneously implement controlled interventions targeting two core high-risk groups—family caregivers and kindergarten staff—and there is a lack of empirical analysis on the effectiveness of integrated online-offline models.

Based on the aforementioned epidemiological situation, national health strategy requirements, and the shortcomings of existing health education models, this study aims to use a sample of individuals involved in early childhood care and education in Wenzhou, Zhejiang Province, to investigate the intervention effects of integrated health education—combining virtual simulation apps with health-themed cultural and creative products—on the level of knowledge regarding HFMD and the adoption of healthy behaviours. The study also aims to identify key factors influencing the acquisition of disease prevention knowledge and to develop a stratified, targeted science communication programme tailored to individuals of different ages, educational backgrounds, and caregiving roles.

The specific objectives of this study include: (1) to investigate the current status of basic knowledge regarding HFMD and the implementation of health behaviours among family caregivers and kindergarten staff prior to the intervention, and to analyse baseline differences in knowledge between the two groups; (2) to implement a combined online (APP) and offline (cultural and creative products) intervention, and to compare changes in knowledge retention rates and health behaviour compliance rates for HFMD in both groups before and after the intervention, thereby validating the effectiveness of the intervention model; (3) To validate the independent effects of the two educational delivery methods—the mobile app and cultural and creative products—on improving participants' knowledge through univariate chi-squared tests and multivariate binary logistic regression, and to identify demographic factors influencing the acquisition of disease prevention knowledge; (4) To propose implementation strategies for blended online and offline health education targeting community households and early childhood care and education institutions, based on the differences observed across subgroups, thereby providing practical guidance for the routine prevention and control of childhood infectious diseases.

The theoretical significance of this study lies in: enriching the theoretical framework on innovative delivery methods for health education on childhood infectious diseases, and confirming the synergistic benefits of combined interventions using digital interactive tools and lifestyle-oriented cultural and creative products;distinguishing differences in the reception of health information between family caregivers and kindergarten staff, thereby refining the research framework on determinants of health literacy regarding HFMD; the adapted HFMD-specific cognitive scale, having undergone rigorous reliability and validity testing, can serve as a standardised measurement tool for subsequent regional epidemiological health education studies of a similar nature, and supplement empirical research data on health literacy regarding infectious diseases among childcare populations in China.

2. Current Status of Research at Home and Abroad

2.1. Current Status of Domestic Research

The first case of HFMD in China was reported in Shanghai in 1981, followed by localised outbreaks in multiple provinces and cities across the country. The history of domestic research on HFMD is divided into a low-incidence research period (2005–2007), a rapid rise period (2008–2012) and a steady decline period (2013–2014). Following its classification as a Category C infectious disease in 2008, the volume of relevant literature increased significantly, and research interest has continued to rise. An analysis of national HFMD incidence trends from 2008 to 2017 by Li et al. (2021) confirmed that the disease exhibits a pattern of high incidence every other year, with severe cases and fatalities concentrated among children under three years of age; children under five years of age constitute the highest-risk group, and these epidemiological characteristics have remained stable over the long term (Li et al., 2022).

The four core areas of research on HFMD in China are epidemiological and clinical characteristics, antiviral drug treatment, prevention and control of severe complications, and integrated traditional Chinese and Western medical interventions, with the most extensive findings in the areas of epidemiology and health education. Li et al. (2021), in their analysis of ten years of epidemiological characteristics in Wuwei City, proposed that childcare facilities are the primary sites of outbreaks, and that parents' insufficient awareness of disease prevention is the foremost risk factor for cluster outbreaks; targeted health education involving collaboration between homes and childcare facilities is therefore required (Li et al., 2021). Wu et al. (2020), in their study on the epidemiological characteristics of the dominant Cox A6 strain, pointed out that the new strain is more transmissible, the risk of infection is significantly higher in households with low awareness, and the effectiveness of conventional paper leaflets is limited; there is an urgent need for innovative science communication platforms to improve knowledge retention rates (Wu et al., 2022).

Regarding parents' health education needs, multiple surveys have confirmed that parents of affected children are most concerned about HFMD prevention measures and typical symptoms, whilst their understanding of early recognition of severe cases and viral transmission routes is weak. Traditional one-way lectures cannot meet the diverse knowledge needs of different population groups, and there is a marked stratification in the preferred communication channels among different demographics: middle-aged and elderly carers tend to reject online digital health education, whereas younger parents are more receptive to learning via short videos and interactive online platforms (Yuan et al., 2015). Su et al. (2021) confirmed that standardised clinical care pathways combined with personalised health education can significantly enhance parents' caregiving capabilities; however, this model is only applicable to hospitalised children and cannot cover families with healthy infants and young children in the community, thus presenting limitations in terms of the educational setting (Su et al., 2021).

With regard to digital health education tools, domestic scholars have developed online mini-programmes providing health information on HFMD; however, most products offer only text-based information, lack virtual interactive simulations for childcare settings, and do not provide

accompanying offline materials to bridge the digital divide. Whilst the model of medical student volunteer teams conducting health education in communities and nurseries has been proven feasible, current practices fail to integrate cultural and creative products into routine health education, making it difficult to achieve long-term, subtle knowledge dissemination (Qin et al., 2020). Furthermore, whilst newborns and preterm infants are at high risk of contracting HFMD, mothers' insufficient awareness of disease prevention is a key contributing factor; existing research lacks integrated educational programmes covering carers across all age groups (National Health Commission, 2010).

2.2. Current Status of International Research

Globally, the disease burden of HFMD caused by enteroviruses continues to increase, with temperate and subtropical countries in Asia being high-incidence regions. Meteorological factors such as temperature and humidity are key environmental variables influencing viral transmission, a conclusion that has been validated by cohort studies in multiple countries (Shi et al., 2024). A cohort study of emergency departments in California, USA, demonstrated that a humid, temperate coastal climate prolongs the in vitro survival of enteroviruses; seasonal temperature fluctuations are significantly correlated with the number of HFMD presentations, suggesting the need to implement preventive health education in advance, taking climate conditions into account (Gao, 2021). Epidemiological surveillance in the Mumbai region of India has revealed co-circulation of Coxsackieviruses A6 and A16, with a relatively high proportion of recurrent infections and cases of generalised herpetic rash. Parents' insufficient ability to recognise recurrent cases can lead to delays in diagnosis and treatment. Whilst international research has largely focused on meteorological and pathogen epidemiology, there has been limited research into innovative health education platforms targeting community carers and early childhood education and care staff (Zhang and Liu, 2021).

Digital health education represents a mainstream trend in global public health development. Many countries in Europe and the United States have incorporated digital tools for infectious disease public education into basic community public health services; however, most international online public education products are tailored to their own languages and childcare systems, and are not suited to the specific circumstances in China, where early childhood education and care institutions operate under a collective management model and where infants and young children are often cared for by multiple generations (Huang et al., 2014). Foreign research has confirmed that purely online digital health education suffers from significant shortcomings in terms of population reach; older adults and those with low levels of education show extremely low willingness to use such platforms. Whilst physical health education materials can effectively compensate for gaps in online coverage, no empirical research has yet been conducted abroad on the use of everyday cultural and creative products as long-term educational tools for children regarding infectious diseases (Chen et al., 2014).

Existing domestic and international research has confirmed that health education is a key measure for the prevention and control of HFMD, and that digital health education offers the advantages of efficiency and interactivity; however, both online-only and offline-only approaches suffer from limitations in audience reach. There is a lack of empirical research in China on the

integrated intervention model of 'virtual simulation apps combined with health-themed creative products', and no comparative analysis has been conducted on the effectiveness of interventions for the two core groups: family caregivers and kindergarten staff (Andreoni and Colton, 2017). Current Status of HFMD Research at Home and Abroad There is an urgent need for early prevention and control of HFMD; consequently, integrating digital technology into its prevention and control has emerged as an efficient, innovative and contemporary approach to health education. Building upon existing research gaps, this study develops complementary online and offline educational platforms. Through questionnaire surveys and multidimensional statistical analysis, it validates the value of the intervention, thereby providing empirical support for innovation in health education regarding paediatric infectious diseases in China.

3. Subjects and Methods

3.1. Study Population

This study employed a stratified sampling method, selecting two groups as survey subjects: family caregivers of infants and young children in communities in Wenzhou, Zhejiang Province, and staff at public and private kindergartens. Two rounds of questionnaires were distributed—one before and one after the intervention—with strict inclusion and exclusion criteria established:

3.1.1. Inclusion Criteria

(1) family caregivers: Individuals with infants and young children aged 5 years or under at home, who are primarily responsible for their daily care, who voluntarily participate in the questionnaire survey, and who are able to complete the questionnaire independently; (2) Kindergarten staff: Currently employed kindergarten staff, childcare workers, health and hygiene teachers, and administrative and support staff within kindergartens, who have daily contact with pre-school children, have been in post for ≥3 months, and have given informed consent to participate in the study; (3) Participants must have normal cognitive function, no reading or writing difficulties, and be able to cooperate in completing the pre-survey, the main questionnaire and the post-intervention follow-up; (4) The study area comprises all districts and counties of Wenzhou City, Zhejiang Province, with stratified coverage of urban, suburban and rural communities, as well as early childhood care and education institutions.

3.1.2. Exclusion Criteria

(1) Individuals with mental illness or cognitive impairment who are unable to independently comprehend the questionnaire items; (2) Invalid responses, including those where participants withdrew from the study midway, left sections of the questionnaire blank, provided logically inconsistent answers, or ticked all options uniformly; (3) Individuals providing short-term, temporary care for infants and young children, or those in short-term part-time roles at nursery schools, who lack long-term childcare experience.

3.1.3. Sample Overview

The team obtained information on existing scales through a review of the literature. Drawing on these scales, the survey categories were divided into basic knowledge and perceptions of HFMD; its prevention; its management and treatment; and the identification of pathogens and sources of infection, among others, and scoring criteria were established based on this classification. As of 5 September 2021, a total of 593 questionnaires had been distributed, yielding an overall valid sample of 534 cases. The sample encompassed individuals of different genders, ages, educational backgrounds, occupations and caregiving roles; the demographic distribution closely mirrored the actual social structure of the early childhood education and care population in Wenzhou, Zhejiang Province, and was therefore highly representative. After excluding invalid questionnaires, the valid sample comprised 318 family caregivers; Questionnaires were distributed to kindergarten staff both before and after the intervention; after excluding invalid questionnaires, the valid sample comprised 216 cases.

3.2. Research Methods

3.2.1. Research Tools

This study drew upon the ‘Chinese Residents’ Health Literacy Scale for Infectious Diseases’ (Hu et al., 2019) to establish the questionnaire framework, providing theoretical support for questionnaire design, the formulation of the intervention programme, and the selection of statistical methods.

3.2.2. Questionnaire Survey Method

The questionnaire was divided into two categories: one specifically for family caregivers and the other for kindergarten staff. It comprised three main modules: general demographic information; knowledge of HFMD; and the implementation of health behaviours related to the disease. The knowledge dimension was further subdivided into five sub-categories: basic understanding of the disease, modes of transmission, preventive measures, recognition of severe cases, and knowledge of vaccines (Gonzalez et al., 2019). Additionally, duplicate verification questions were included for subsequent data cleaning and reliability testing;Following the completion of the first draft, a small-scale sample was selected to conduct a pilot survey for optimisation. Feedback from respondents was collected and combined with expert opinions from public health lecturers to adjust the wording of questions, the formulation of response options and the layout of the questionnaire, thereby eliminating ambiguous questions and enhancing the questionnaire’s readability and scientific rigour; The formal questionnaire was distributed using an offline stratified sampling method. Paper questionnaires were distributed on-site at community markets, infant and toddler activity centres, and public and private kindergartens. Research staff provided standardised instructions on how to complete the questionnaire and collected the forms on the spot; subsequently, invalid questionnaires containing logical contradictions or extensive omissions were screened out and excluded.

3.2.3. Blended Online and Offline Intervention Approach

The online intervention tool incorporates interactive modules for severe case identification and simulated home-school communication scenarios, enabling young and middle-aged parents and kindergarten staff to engage in self-directed learning during spare moments (Wen, 2009). The offline intervention tools consist of self-developed, everyday health-themed cultural and creative products, including science-communication water bottles, stationery, classroom educational tote bags and teaching aids such as dinner plates. These products feature printed text and images relating to the prevention of HFMD, disinfection and the recognition of severe cases, designed to effectively disseminate scientific knowledge over the long term to older carers with low digital literacy and within kindergarten teaching settings; The overall intervention process involves first collecting pre-intervention baseline questionnaire data, followed by the simultaneous deployment of the app and cultural and creative products to deliver three months of continuous health education. Upon completion of the intervention period, post-intervention questionnaires are collected to analyse and compare changes in participants' awareness of HFMD and their health behaviours before and after the intervention.

3.2.4. Methods for Testing the Reliability and Validity of the Scale

(1) Reliability testing: Cronbach's α was used to assess the internal consistency of the scale; the Spearman–Brown split-half coefficient and Guttman split-half coefficient were used to test the split-half reliability of the questionnaire and determine the internal stability of the scale.

(2) Validity Testing: KMO sampling adequacy and Bartlett's sphericity tests were conducted; once the conditions were met, exploratory factor analysis was performed to assess construct validity; t-tests, analysis of variance (ANOVA) and chi-squared tests were used to conduct quantitative and qualitative hypothesis testing for the items, thereby enhancing content validity.

3.2.5. Statistical Analysis Methods

All survey data were entered into Excel for organisation, and statistical analysis was carried out using SPSS 26.0 software, with a significance level of $\alpha = 0.05$; Prior to conducting reliability and validity tests on the questionnaire, hypothesis tests on both quantitative and qualitative data for each item were first carried out to enhance the questionnaire's validity. Quantitative tests utilised t-tests and analysis of variance (ANOVA) to infer the probability of differences between variables and to determine the extent to which controllable factors influenced the research results; qualitative tests employed chi-square tests or rank-sum tests to compare the degree of deviation between actual and theoretical observations;In terms of reliability, the Spearman–Brown formula was used to calculate the reliability coefficient of the adapted scale based on the 'Chinese Residents' Health Literacy Scale for Infectious Diseases', whilst Cronbach's α was employed to assess the internal consistency of the scale. In terms of validity, factor analysis was used to evaluate the alignment between the scale items and the theoretical construct. Prior to analysis, the KMO measure of sampling adequacy and Bartlett's sphericity test to determine whether the data were suitable for factor analysis; a higher KMO value indicates better suitability. Detailed test results can be found in Table 1 at and ; statistical analysis comprised four types of methods: firstly, descriptive statistics, presenting the baseline demographic characteristics of family caregivers and

kindergarten staff in terms of frequency and proportion (%); secondly, paired t-tests to compare differences in HFMD knowledge awareness rates and health behaviour pass rates among parents and kindergarten staff before and after the intervention, thereby comprehensively assessing the overall effectiveness of the health education intervention; thirdly, Pearson's 2×2 chi-square test to conduct a univariate analysis of the association between the use of the mobile app and health-themed creative products and the population's attainment of the required level of knowledge regarding HFMD; Fourth, a binary logistic regression analysis was conducted to identify independent protective factors influencing the population's attainment of the target level of disease prevention awareness, whilst controlling for confounding variables such as age, educational attainment, caregiving status and job role, and to calculate the odds ratio (OR) and its 95% confidence interval (Jin et al., 2016).

Table 1. Summary of Reliability Statistics

Cronbach's Alpha	Part I	Value	.665
		Number of items	5a
	Part 2	Value	.654
		Number of items	4b
		Total number of items	9
Correlation between morphologies			.666
Spearman–Brown coefficient	Equal-length		.800
	Non-isometric		.801
Guttman's split-half coefficient			.800
KMO sampling adequacy measure			.806
Bartlett's sphericity test	Approximate chi-squared		642.454
	Degrees of freedom		55
	Significance		.000

3.2.6. Definition of Experimental Endpoint and Outcome Measurement Criteria

This study uniformly defines the 3-month intervention period as the sole experimental endpoint, distinguishes primary outcome indicators from secondary outcome indicators, and all statistical analyses are conducted based on this hierarchical classification standard:

(1) Primary Outcome Indicators (Core Measurement Criteria)

The qualification status of HFMD-related health knowledge is set as the primary evaluation indicator. The passing threshold is determined by the total score of the knowledge section in the questionnaire, with knowledge qualification defined as a total score of ≥60 points. The corresponding observational metrics include the HFMD knowledge awareness rate and the proportion of participants with qualified knowledge before and after the intervention. This indicator evaluates the improvement effect of two publicity carriers (virtual simulation APP and health cultural and creative products) on HFMD prevention awareness, and serves as the core dependent variable for univariate chi-square tests and binary Logistic regression analyses.

(2) Secondary Outcome Indicators (Auxiliary Measurement Criteria)

The qualified rate of HFMD-compliant health behaviors is taken as the secondary evaluation indicator. Behavioral qualification is judged according to scores from the health behavior section of the questionnaire, which supplements the evaluation of the intervention effect of integrated health education on forming routine disease prevention behaviors among family caregivers and kindergarten staff. Only paired t-tests are used to compare pre- and post-intervention differences for secondary indicators, which are not incorporated into multivariate regression models.

The timing of all questionnaire data collection is strictly standardized: baseline data before intervention are collected on-site on the day the intervention is launched, while endpoint data are uniformly recovered upon completion of the 3-month continuous integrated online-offline health education. The interval between the two rounds of questionnaires is fixed at 90 days. Confounding effects caused by inconsistent intervention duration are controlled throughout the research to ensure standardization and comparability of comparisons between primary and secondary outcome indicators.

4. Results

4.1. Descriptive Statistics of Basic Information on Families and Nurseries

4.1.1. Descriptive Statistics of Basic Family Information

The demographic characteristics of the sample of family caregivers generally reflect social reality and are well-representative; specific information can be found in Table 2: in terms of gender, prior to the intervention, 33.3 % were male and 66.7 % were female, with a male-to-female ratio of 1:2; as women bear the primary responsibility for childcare, this distribution is consistent with patterns of division of labour within the family; Age was categorised into four groups: 21–30, 31–44, 45–59, and 60 and over. The sample consisted primarily of parents of young children, with a moderate proportion of grandparents acting as carers and a relatively low proportion of older carers; this age structure reflects the current reality of childcare; Educational attainment ranges from primary school to master's degree and above, with junior secondary, senior secondary and undergraduate levels forming the majority; the undergraduate group has the highest proportion, matching the overall educational attainment level in Zhejiang Province; Caregiver roles include parents of infants and young children, grandparents and other carers such

as nannies, with a conventional structure; Occupations are categorised into nine major categories according to national standards, with stratified sampling covering all occupational categories; the demographic stratification and sample distribution for each category are both scientifically sound and reasonable.

Table 2. Distribution of Basic Household Information (n=318)

Project	Category	Number	Percentage (%)
Gender	Male	106	33.4
	Female	212	66.7
Age group	21–30	128	40.3
	31–44	130	40.9
	45–59	52	16.4
	60 and over	8	2.5
Educational attainment	Primary school	20	6.3
	Lower secondary	81	25.5
	Secondary school	56	17.6
	Higher vocational education	19	6.0
	College	40	12.6
	Bachelor’s degree	84	26.4
	Master’s degree and above	16	5.0
	Other	2	0.6
Status	Parents of infants and young children	209	65.7
	Grandparents (paternal and maternal)	25	7.9
	Other	84	26.4
Occupation	Enterprises and public institutions	42	13.2
	Professionals (teachers, doctors, lawyers, etc.)	29	9.1
	Civil servants/government	23	7.2

	employees		
	Service sector workers (catering staff, drivers, shop assistants, etc.)	58	18.2
	Farmers	38	11.9
	Workers (e.g. factory workers, construction workers, urban sanitation workers)	10	3.1
	Office workers	44	13.8
	Freelancers (e.g. writers, artists, photographers, tour guides, etc.)	6	1.9
	Other	68	21.4

4.1.2. Descriptive Statistics of Basic Information on Kindergartens

The demographic distribution of the kindergarten staff sample aligns with industry realities, and the stratification is scientifically sound. For specific details, see Table 3: prior to the intervention, the sample comprised 49 men (22.7 %) and 167 women (77.3 %), with a male-to-female ratio of 1:3.4, which is consistent with the industry's characteristic preference for meticulous female practitioners in early years education roles. Age was categorised into four groups, with young teachers aged 21–30 and middle-aged staff aged 31–44 forming the majority, whilst managers and healthcare staff aged 45–59 accounted for a certain proportion; there were very few staff aged 60 and over approaching retirement, indicating a reasonable age distribution. Educational qualifications ranged from primary school to master's degree and above, with junior secondary school and undergraduate graduates accounting for the highest proportion, which aligns with the current trends in further education and career pathways within the early years education sector. Occupations were not categorised according to the standard eight occupational classifications, but were divided into four categories—full-time teachers, childcare assistants, health and hygiene staff, and 'other'—based on the kindergarten's internal role structure.

Table 3. Distribution of Basic Information on Kindergartens (n=216)

Project	Category	Number	Percentage (%)
Gender	Male	49	22.7
	Female	167	77.3
Age group	21–30	93	43.1
	31–44	90	41.7
	45–59	28	13.0

	60 and over	5	2.3
Educational attainment	Primary school	8	3.7
	Lower secondary	70	32.4
	Upper Secondary	24	11.1
	Higher vocational education	9	4.2
	College	33	15.3
	Bachelor's degree	61	28.2
	Master's degree and above	8	3.7
	Other	3	1.4
Occupation	Nursery teacher	46	21.3
	Nursery Assistant	11	5.1
	Health and Hygiene Teacher	7	3.2
	Other	152	70.4

4.2. Difference Analysis of Primary and Secondary Outcome Indicators Before and After Intervention

This study took the awareness rate and qualification rate of hand-foot-mouth disease (HFMD) knowledge as primary outcome indicators, and the pass rate of healthy behaviors as the secondary outcome indicator. At the experimental endpoint of the 3-month intervention, changes in indicators before and after the intervention were compared between family caregivers and kindergarten staff. Paired t-tests were adopted for intra-group difference analysis with the test level α set to 0.05.

4.2.1. Primary Outcome: Differences in HFMD Knowledge Awareness Rate of Family Caregivers Before and After Intervention

The questionnaire consisted of 13 items on HFMD health knowledge. The overall HFMD knowledge awareness rate of family caregivers was 49.95% before intervention and increased to 88.85% at the intervention endpoint, with an overall growth rate of 38.90%. The awareness rate of each single knowledge item after intervention was higher than the baseline level. The paired t-test showed statistically significant differences ($P<0.05$), as detailed in Table 4.

Table 4. Summary of the number of parents aware of HFMD and their awareness rates

Title	Pre-intervention	Awareness rate (%)	After intervention	Awareness rate (%)	Difference (%)
Can you distinguish HFMD from other illnesses?	184	57.86	267	83.96	26.10
HFMD is a common infectious disease – is there no need to panic?	196	61.64	288	90.57	28.93
When do you think the peak season for HFMD is?	127	39.94	301	94.65	54.72
Is there currently a vaccine available to prevent HFMD?	138	43.40	298	93.71	50.31
Is HFMD contagious?	245	77.04	309	97.17	20.13
Do children with HFMD need to be isolated from other children after the onset of symptoms?	241	75.79	310	97.48	21.70
Do the blisters caused by HFMD leave scars or cause hyperpigmentation?	82	25.79	254	79.87	54.09
Is HFMD merely a respiratory infectious disease?	145	45.60	262	82.39	36.79
Is it possible for adults carrying the HFMD virus to pass it on to young children?	224	70.44	271	85.22	14.78
What is the first symptom to appear in a patient with HFMD?	210	66.04	283	88.99	22.96
What symptoms in a patient with HFMD indicate that the condition is severe?	66	20.75	280	88.05	67.30
By which of the following means is HFMD transmitted?	156	49.06	273	85.85	36.79
Which of the following measures can help prevent the spread of HFMD?	51	16.04	277	87.11	71.07

4.2.2. Primary Outcome: Differences in HFMD Knowledge Awareness Rate of Kindergarten Staff Before and After Intervention

The knowledge questionnaire for kindergarten teachers included 13 assessment items. The overall HFMD knowledge awareness rate of kindergarten staff was 66.74% before intervention

and rose to 93.52% at the intervention endpoint, with an overall increase of 26.78%. The awareness level of all individual knowledge points was significantly improved compared with the pre-intervention status, and the paired t-test indicated statistically significant intra-group differences ($P<0.05$), as detailed in Table 5.

Table 5. Summary of the number of kindergarten teachers with knowledge of HFMD and their knowledge rates

Topic	Before intervention	Awareness rate (%)	After intervention	Awareness rate (%)	Difference (%)
Can you distinguish HFMD from other illnesses?	168	77.78	197	91.20	13.43
HFMD is a common infectious disease – is there no need to panic?	154	71.30	210	97.22	25.93
When do you think the peak season for HFMD is?	74	34.26	191	88.43	54.17
Is HFMD merely a respiratory infectious disease?	107	49.54	209	96.76	47.22
Is it possible for adults carrying the HFMD virus to pass it on to young children?	173	80.09	211	97.69	17.59
Is there currently a vaccine available to prevent HFMD?	98	45.37	196	90.74	45.37
Do children with HFMD need to be isolated from other children after the onset of symptoms?	185	85.65	213	98.61	12.96
What is the first symptom to appear in patients with HFMD?	169	78.24	189	87.50	9.26
What symptoms in a patient with HFMD indicate that the condition is severe?	50	23.15	188	87.04	63.89
Can children develop immunity after contracting HFMD?	101	46.76	202	93.52	46.76
To prevent HFMD, ensure good personal hygiene and organise regular handwashing	198	91.67	207	95.83	4.17

sessions for children					
To prevent HFMD, ensure good indoor hygiene and ventilate the room regularly	198	91.67	203	93.98	2.31
To prevent HFMD, children's toys and tableware should be regularly and systematically disinfected	199	92.13	210	97.22	5.09

4.2.3. Secondary Outcome: Differences in Healthy Behavior Pass Rates of Family Caregivers and Kindergarten Staff Before and After Intervention

The qualification of healthy behaviors served as the secondary outcome indicator. The healthy behavior pass rate of family caregivers was 52.52% before intervention and climbed to 94.03% at the endpoint, representing an increase of 41.51%, and the paired t-test revealed statistically significant differences ($P<0.05$). In contrast, kindergarten staff already had a baseline healthy behavior pass rate of 94.44%, which only slightly rose to 94.91% at the endpoint with a growth rate of merely 0.53%, showing no statistically significant difference in behavioral indicators before and after intervention, as shown in Table 6.

Table 6. Summary of Pass Rates for Health Behaviours Related to HFMD

Study Participants	Number of respondents	Before Intervention		After Intervention		Difference in pass rate (%)
		Number of students passing	Pass rate (%)	Number of students who passed	Pass rate (%)	
Parents	318	167	52.52	299	94.03	41.51
Nursery teacher	216	204	94.44	205	94.91	0.53

4.3. Univariate Analysis of Knowledge Regarding HFMD Based on Basic Household Information

This section conducts univariate and multivariate analyses targeting the primary outcome indicator of this study (the qualification status of hand-foot-mouth disease knowledge), while the secondary outcome indicator of healthy behaviors is not incorporated into the statistical modeling in this section.

4.3.1. Analysis of the Impact of HFMD Virtual Simulation APP Usage on Knowledge Levels

This section takes the primary outcome of the study — the qualification status of hand-foot-mouth disease (HFMD) knowledge among family caregivers as the observed dependent variable, and performs univariate chi-square tests regarding the use of the APP and health cultural and creative products. Secondary outcome indicators related to healthy behaviors are excluded from this analysis.

We conducted a chi-square test to analyse whether there was a difference in knowledge of HFMD among family caregivers depending on whether or not they had used the HFMD virtual simulation app. In this context, a chi-square test (2×2) can be used for analysis, provided the following assumptions are met: Assumption 1: The observed variable is a dichotomous variable; in this study, the use of the HFMD virtual simulation app is a categorical variable with two categories: 'not used' and 'used'.Assumption 2: The observations are mutually independent; for example, in this study, the information for each participant is independent, with no interaction between them. As shown in Figure 1 .

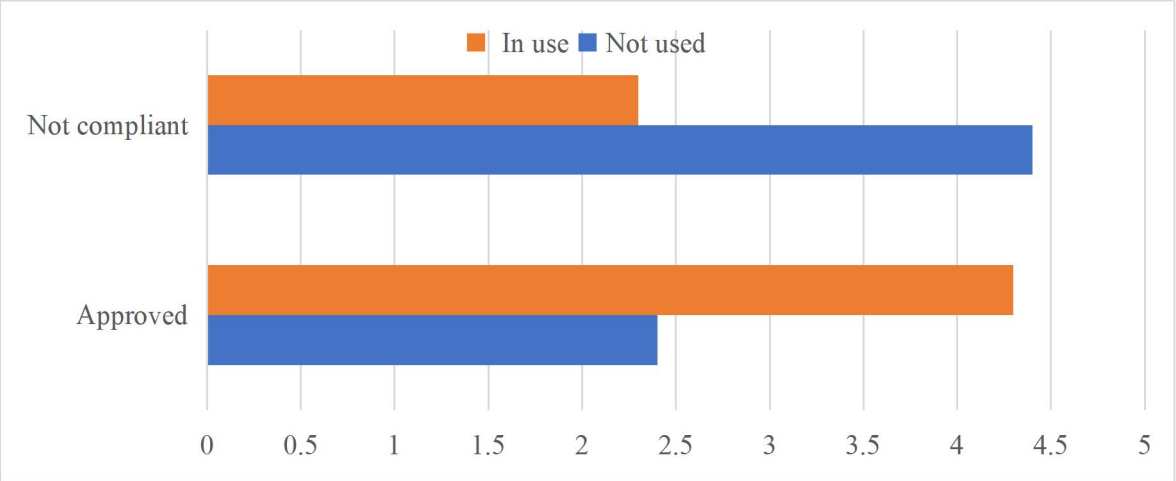


Figure 1. Distribution of knowledge levels among family caregivers before and after using the HFMD virtual simulation app

The 318 family caregivers were divided into two groups: those who did not use the app and those who did. The number of participants in each group who passed or failed the knowledge assessment was counted, and a 2×2 Pearson chi-square test was conducted. The distribution of groups is shown in Table 7.

The results of the chi-square test (2×2) showed that P = 0.037; therefore, H_0 was rejected and H_1 accepted, indicating that there is a difference in knowledge of HFMD depending on whether the app was used ($p = 0.037 < 0.05$). In other words, among family caregivers, there is a difference in their knowledge of HFMD depending on whether the app was used.

This study surveyed 318 participants, dividing them into two groups based on whether or not they used the HFMD virtual simulation app. Analysis of the pass-to-fail ratio among those who used the app compared to those who did not revealed that the pass-to-fail ratio was significantly higher in the group using the app than in the group not using it. It is likely that, after using the HFMD virtual simulation app, family caregivers engaged in systematic, interactive learning and consolidation of knowledge related to the disease. Consequently, users naturally gained a more thorough understanding of diseases to which infants and young children are susceptible, resulting in better knowledge of HFMD among app users compared to non-users.

In summary, when conducting health education on HFMD for family caregivers, the use of the HFMD virtual simulation app can be employed as an intervention to provide systematic and comprehensive teaching on childhood infectious diseases.

Table 7. Chi-square test table showing the effect of family caregivers' use of the HFMD virtual simulation app on their knowledge of the disease

Group	Failed	Pass	Total	x^2	p
Not used	45 (14.2 %)	61 (19.2 %)	106		
Use	65 (20.4 %)	147 (46.3 %)	212	4.343	.037
Total	110 (34.6 %)	208 (65.5 %)	318		

4.3.2. Analysis of the Impact of Cultural and Creative Product Usage on Awareness of HFMD

Focusing on the primary outcome indicator, namely the HFMD knowledge qualification rate of family caregivers, this section analyzes the correlation between the use of health cultural and creative products and qualified cognition. Secondary indicators such as healthy behaviors are not included in this univariate test.

We conducted a chi-square test to analyse whether there was a difference in awareness of HFMD among family caregivers depending on whether they had been exposed to cultural and creative products. In this context, a chi-square test (2×2) can be used for analysis, provided the following assumptions are met: Assumption 1: The observed variable is a binary variable; in this study, exposure to the HFMD virtual simulation app is a multi-category variable: non-use or use.Assumption 2: The observations are mutually independent; for example, in this study, the information for each participant is independent, with no mutual interference. As shown in Figure 2 .

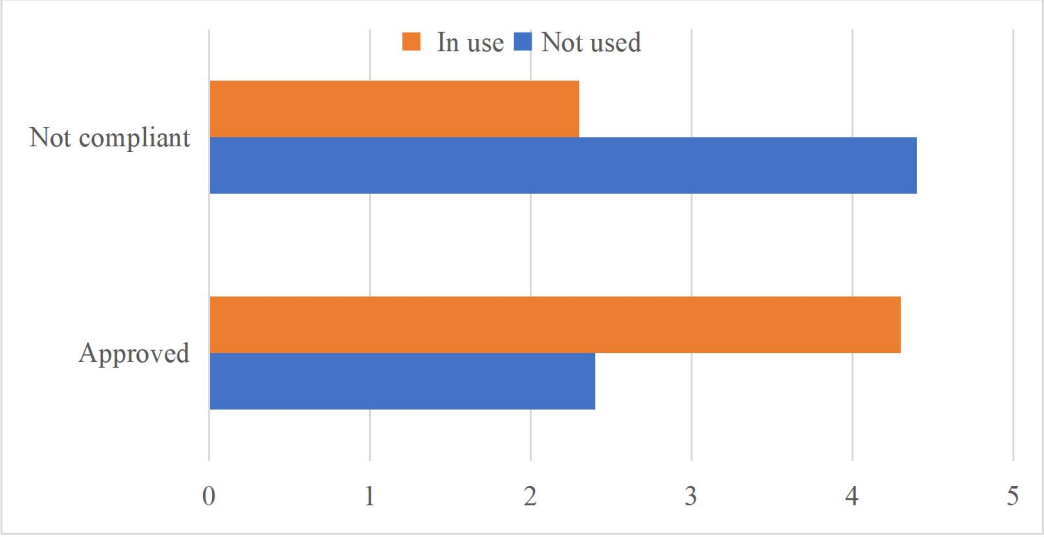


Figure 2. Distribution of knowledge levels among family caregivers before and after using cultural and creative products

Chi-square test results: $\chi^2 = 4.486$, $P = 0.034 < 0.05$; the difference is statistically significant. Exposure to health-themed cultural and creative products significantly improves the pass rate for knowledge of HFMD among family caregivers. The pass rate for those who did not use cultural and creative products was 54.6% (83/152), whilst the pass rate for those who did use them was

75.3% (125/166). The cultural and creative products feature key information on the prevention, disinfection and recognition of severe cases of HFMD printed on the surfaces of water cups, stationery and children's daily necessities. Caregivers with low digital literacy, such as grandparents, do not need to operate a mobile phone; instead, they absorb the scientific information gradually and subtly through daily use, thereby addressing the coverage gaps of purely online app-based health education and effectively enhancing disease prevention awareness among family caregivers of all age groups (Table 8).

Table 8. Chi-square test table examining the effect of whether family caregivers use cultural and creative products on their knowledge of HFMD

Grouping	Failed	Pass	Total	x^2	p
Not used	69 (21.7 %)	83 (26.1 %)	152	4.486	0.034
Used	41 (12.9 %)	125 (39.3 %)	166		
Total	110 (34.6 %)	208 (65.4 %)	318		

4.4. Univariate Analysis of the use of Educational Tools by Kindergarten Staff and Their Awareness of HFMD

This section adopts the primary outcome indicator — the HFMD knowledge qualification status of kindergarten staff as the observed variable to carry out univariate chi-square tests, with secondary outcome indicators of healthy behaviors excluded from the analysis.

The valid sample of kindergarten staff totalled 216. Using a passing mark of 60 points as the benchmark for knowledge, the impact of the use of the two types of educational tools—the app and cultural and creative materials—on the cognitive levels of kindergarten teachers and nursery assistants was analysed separately.

4.4.1. Impact of the use of the HFMD Virtual Simulation APP on the Level of Knowledge Among Kindergarten Staff

The 216 kindergarten staff members were divided into two groups: those who did not use the app and those who did. The distribution of knowledge proficiency was analysed and a chi-squared test was conducted; the results are shown in Table 9.

The sample met the requirements for a chi-square test involving independent, dichotomous variables. The test yielded $\chi^2 = 4.329$, $P = 0.038 < 0.05$; thus, the use of the app had a significant effect on the proportion of kindergarten staff achieving the required level of knowledge regarding HFMD. The pass rate for knowledge among staff who did not use the app was 66.0% (68/103), whilst the pass rate for those who did use the app was 83.2% (94/113).As kindergarten teachers and nursery assistants are responsible for the day-to-day hygiene management of all children in the nursery, the app's built-in simulations of childcare scenarios (such as the collective disinfection of toys, the management of suspected cluster cases, and guidance on home-school communication) specifically address gaps in staff knowledge regarding the identification of severe cases and the management of outbreaks. The online self-directed learning model is well-

suited to the fragmented work schedules typical of nursery settings, resulting in a marked improvement in health education outcomes.

Table 9. Chi-square test table for the relationship between kindergarten staff's use of the app and their proficiency in HFMD knowledge

Grouping	Failed	Pass	Total	x^2	p
Not used	35(16.2%)	68 (31.5 %)	103	4.329	0.038
Used	19 (8.8 %)	94 (43.5 %)	113		
Total	54 (25.0 %)	162 (75.0 %)	216		

4.4.2. The Impact of the Use of Health-Related Cultural and Creative Products on the Level of Awareness Among Kindergarten Staff

The sample of 216 staff members was categorised according to their exposure to cultural and creative products; the results of the chi-square test are available at Table 10.

The test yielded $\chi^2 = 4.375$, $P = 0.036 < 0.05$, indicating a statistically significant difference. The pass rate for knowledge among staff who did not use cultural and creative products was 66.7% (66/99), whilst the pass rate for those who did use them was 82.1% (96/117). Kindergartens have commissioned bulk orders of science-themed dinner plates, educational tote bags and class-based cultural and creative teaching aids. As a result, staff are continuously exposed to science-related text and images during their daily teaching and childcare activities. At the same time, they can utilise these cultural and creative teaching aids to conduct further science outreach to children and parents. Under the combined influence of these two factors, staff members' own understanding of disease prevention has been further enhanced.

Table 10. Chi-square Test Table for the Relationship between the Use of Cultural and Creative Products by Kindergarten Staff and Their Knowledge of HFMD

Grouping	Failed	Pass	Total	x^2	p
Not used	33 (15.3 %)	66 (30.6 %)	99	4.375	0.036
Used	21 (9.7 %)	96 (44.4 %)	117		
Total	54 (25.0 %)	162 (75.0%)	216		

4.5. Comprehensive Comparison of Univariate Results Between the Two Groups

Statistical consistency: In both the family caregivers and kindergarten staff samples, the use of virtual simulation apps and health-themed cultural and creative products was positively associated with passing the HFMD knowledge test, with P-values in both cases less than 0.05. Both online and offline educational platforms significantly improved disease prevention awareness among those involved in early childhood care and education;

Population differences: Among family caregivers, the difference in pass rates before and after using the app (21.9 %) was greater than that for cultural and creative products (20.7 %), with middle-aged and young parents showing a greater preference for online, digital, interactive learning; among kindergarten staff, the increase in pass rates achieved through cultural and creative products (15.4 %) was similar to that achieved through the app (17.2 %), indicating that both online and offline models are equally effective in enhancing knowledge among kindergarten staff.

Application Orientation: A single educational medium has limitations in reaching all target groups; digital apps are well-suited to middle-aged and young adults, whilst cultural and creative products cater to older carers with lower digital literacy. A blended model combining online apps with offline cultural and creative products can achieve equitable science communication coverage across the entire population, consistent with the overall conclusions of this study.

This study surveyed 318 + 216 participants, dividing them into two groups based on whether or not they used cultural and creative products. Analysis of the pass-to-fail ratios among those who did and did not use such products revealed that the pass-to-fail ratio for users was significantly higher than that for non-users. It is possible that school carers, whilst using cultural and creative products, tend to pay closer attention to the information regarding HFMD printed on the products. Through conscious, self-directed learning, they have gained a better grasp of key points and precautions regarding disease prevention and care; consequently, school carers who use cultural and creative products demonstrate a higher level of awareness of HFMD compared to those who do not.

In summary, when conducting health education on HFMD for school carers, cultural and creative products can be utilised as an intervention to teach the general public about childhood infectious diseases in a way that is relevant to everyday life.

4.6. Multivariate Logistic Regression Analysis

This regression model takes the study's primary outcome (whether participants achieve qualified HFMD knowledge) as the binary dependent variable and conducts multivariate analyses after adjusting for demographic confounding variables. The pass rate of healthy behaviors, a secondary outcome indicator, is not used as the dependent variable in regression analyses.

4.6.1. Binary Logistic Regression Analysis for Family Caregivers

The omnibus test of model coefficients ($P = 0.028 < 0.05$) indicates that at least one of the included independent variables has a significant effect on the 'knowledge proficiency' outcome, and the model as a whole is statistically significant. The Hosmer-Lemeshow goodness-of-fit test ($P = 0.283 > 0.05$) indicates no model fit issues, and the current combination of independent variables adequately explains the sample data. The overall predictive accuracy of the model is 65.4%, indicating that this model possesses moderate discriminatory power regarding whether family caregivers meet the knowledge criteria for HFMD.

As shown in Table 11, after controlling for confounding variables such as age, educational attainment and caregiving status, the use of a virtual simulation app and the use of health-related

cultural and creative products were both independent positive protective factors for family caregivers achieving the required level of knowledge regarding HFMD; specifically, the regression coefficient for app use was $\beta = 0.776$, $P = 0.012 < 0.05$, with an odds ratio (OR) of 2.174,with the 95% confidence interval not containing 1. All other factors being equal, family caregivers who used the virtual simulation app were 2.174 times more likely to demonstrate adequate knowledge than those who did not. The virtual simulation learning model, based on scenario-based interactive design, can systematically address gaps in the public's disease prevention knowledge and effectively increase the probability of achieving the required level of understanding;For the use of health-themed cultural and creative products, the corresponding $\beta = 0.737$, $P = 0.018 < 0.05$,OR = 2.091, 95% CI = (1.136, 3.849). The probability of knowledge compliance among carers exposed to health-themed cultural and creative products is 2.091 times that of those not exposed. Integrating disease prevention knowledge into everyday items through a lifestyle-oriented science communication approach can reach middle-aged and elderly grandparents with limited digital literacy, effectively addressing the coverage gaps of purely online health education;With regard to age, using young parents aged 21–30 as the reference group, the OR values for all age groups over 45 were less than 1 and $P > 0.05$, indicating that the overall probability of older carers achieving the required knowledge level is relatively lower; however, age was present only as a confounding variable and had no independent statistical effect;In terms of educational attainment, the P-values for the groups with college diplomas, bachelor's degrees, master's degrees and above were all less than 0.05. The higher the educational level, the higher the odds ratio for achieving the required level of knowledge. Highly educated groups demonstrate a stronger willingness to accept digital online science communication and science communication via cultural and creative media, and are able to more proactively absorb health knowledge related to HFMD.

Table 11. Three-line table of binary logistic regression analysis for HFMD knowledge proficiency among family caregivers

Independent variables	β	S.E.	Wald χ^2	P	OR	95% CI (OR)
App usage (Yes = 1, No = 0)	0.776	0.308	6.342	0.012	2.174	1.187–3.976
Whether cultural and creative products are used (Yes = 1, No = 0)	0.737	0.311	5.621	0.018	2.091	1.136–3.849
Age groups (reference group: 21–30 years)						
31–44 years	0.224	0.267	0.703	0.402	1.251	0.741–2.111
45–59 years	-0.461	0.325	2.011	0.156	0.631	0.334–1.192
60 years and over	-0.815	0.472	2.982	0.084	0.442	0.175–1.116

Independent variables	β	S.E.	Wald χ^2	P	OR	95% CI (OR)
Educational attainment (reference group: lower secondary education or below)						
Upper secondary / Vocational college	0.345	0.293	1.389	0.239	1.412	0.796–2.501
College / Undergraduate	0.683	0.281	5.910	0.015	1.980	1.142–3.433
Master's degree or higher	0.926	0.414	5.003	0.025	2.524	1.119–5.689
Status (reference group: grandparents acting as carers)						
Parental carers	0.412	0.278	2.203	0.138	1.510	0.875–2.604
Other carers	-0.187	0.304	0.379	0.538	0.829	0.457–1.503

4.6.2. Binary Logistic Regression Analysis of Kindergarten Staff

The omnibus test of model coefficients ($P = 0.016 < 0.05$) indicated that the model was valid as a whole; the Hosmer-Lemeshow goodness-of-fit test ($P = 0.140 > 0.05$) showed good fit with no significant deviation; the overall classification accuracy of the model was 68.5%, which effectively distinguished between staff members with and without adequate knowledge of HFMD.

Table 12 shows that, after adjusting for potential confounders, including age, educational attainment, and job role, the use of virtual simulation applications and health-related cultural and creative products was independently associated with a greater likelihood of achieving a passing level of knowledge about HFMD. Specifically, virtual simulation application use was a significant positive predictor ($\beta = 0.674$, OR = 1.963, $P = 0.025$), indicating that staff who used such applications had 1.963 times the odds of achieving a passing knowledge score compared with non-users. The platform's built-in simulation modules—such as those for managing cluster outbreaks in childcare settings and collective disinfection of young children—are tailored to frontline work and effectively address knowledge gaps in areas such as identifying severe cases and providing guidance to families and at home. The regression coefficient for the use of cultural and creative products was $\beta = 0.651$, $P = 0.029 < 0.05$,OR = 1.917; the probability of staff meeting cognitive standards increased by 91.7% for those exposed to cultural and creative products. The nursery's centralised procurement of science-based cultural and creative products as routine teaching aids enables staff to continuously consolidate their disease prevention knowledge through regular use, whilst also utilising these products to conduct secondary science outreach to children and parents, thereby reinforcing their own knowledge base in both directions. Results from age stratification show that, compared with young kindergarten staff aged 21–30, the OR values for middle-aged and older staff were all less than 1 and $P > 0.05$; age does not have an

independent significant effect on staff knowledge acquisition, and the kindergarten's routine hygiene management system is able to mitigate the cognitive gaps arising from differences in learning ability across age groups. With regard to educational attainment, the P-values for the junior college, undergraduate and master's degree groups were all <0.05, indicating that staff with higher educational qualifications demonstrated greater acceptance of and more efficient absorption of science communication through digital and cultural and creative formats. However, for different roles such as kindergarten staff and childcare workers, the P-values were all >0.05, suggesting that the kindergarten's standardised hygiene regulations have levelled out differences in cognitive levels across roles.

Table 12. Contingency Table for Binary Logistic Regression Analysis of HFMD Knowledge Proficiency Among Kindergarten Staff

Independent variables	β	S.E.	Wald χ^2	P	OR	95% CI (OR)
App usage (Yes = 1, No = 0)	0.674	0.301	5.012	0.025	1.963	1.088–3.541
Use of cultural and creative products (Yes = 1, No = 0)	0.651	0.297	4.817	0.029	1.917	1.073–3.425
Age groups (reference group: 21–30 years)						
31–44 years	0.195	0.282	0.477	0.490	1.215	0.699–2.109
Aged 45–59	-0.388	0.341	1.295	0.255	0.678	0.348–1.319
60 years and over	-0.742	0.495	2.248	0.134	0.476	0.180–1.259
Educational attainment (reference group: lower secondary education or below)						
Upper secondary / Vocational college	0.311	0.304	1.047	0.306	1.365	0.752–2.477
College / Undergraduate	0.642	0.290	4.904	0.027	1.900	1.076–3.353
Master's degree or higher	0.873	0.431	4.102	0.043	2.394	1.028–5.575
Post (Reference Group: Nursery/Health Teachers)						
Full-time kindergarten staff	0.356	0.289	1.515	0.218	1.428	0.811–2.513

A comparison of the two sets of logistic regression models yields two key conclusions. The first concerns a pattern of consistency:In both the family caregivers and kindergarten staff samples, the odds ratios (ORs) for the virtual simulation app and health-themed cultural and creative products were greater than 1 and $P < 0.05$. After adjusting for demographic confounding variables, both online and offline educational platforms were found to independently enhance the population's awareness of HFMD prevention and control; the blended online-offline educational model demonstrates stable and reliable intervention value. Second, the pattern of differences across populations and confounding variables: the OR values for the apps and cultural and creative products were higher among the family caregivers, indicating that these educational methods are more effective at enhancing knowledge among parents; for kindergarten staff, who have already received basic health training, the scope for further improvement through education is relatively limited;Furthermore, both sample groups demonstrated that the probability of achieving the required level of knowledge increases with higher educational attainment, whilst older adults tended to have lower levels of knowledge. Consequently, future public health outreach initiatives could adopt a stratified strategy: prioritising the distribution of offline health-themed cultural and creative products to groups with lower educational attainment and older adults, whilst prioritising the promotion of virtual simulation apps among middle-aged and young adults with higher educational attainment, thereby achieving targeted and differentiated health education.

5. Discussion

This study set the 3-month intervention as the unified experimental endpoint and hierarchically defined primary outcomes (qualified rate of HFMD prevention knowledge) and secondary outcomes (pass rate of healthy behaviors). Statistical results verified that integrated health education combining the online virtual simulation APP and offline health cultural and creative products could significantly improve the primary outcome indicators of both populations. Baseline data revealed that family caregivers had a low baseline level of primary outcomes. Although kindergarten staff possessed more basic disease prevention knowledge than caregivers, they still had cognitive gaps in core knowledge points including severe case identification and transmission routes. In terms of secondary outcomes, kindergarten staff already maintained a high standard of healthy behaviors at baseline; thus, the integrated intervention exerted no statistically significant improvement on their secondary outcomes, while family caregivers achieved a prominent increase in secondary outcome indicators.

Both univariate chi-square tests and binary Logistic regression models were constructed based on primary outcome indicators. The consistent results confirmed that both the APP and cultural and creative products served as independent positive protective factors for increasing the population's knowledge qualification rate, and the two carriers exhibited differentiated adaptability to different groups. Educational attainment was an important confounding factor affecting the level of primary outcome indicators; participants with college education or above had a significantly higher probability of reaching the standard for primary outcomes compared with those with lower educational backgrounds.

In contrast to existing foreign studies, overseas digital health education only focuses on online channels without supporting offline cultural and creative carriers to bridge the digital divide. This study innovatively adopted daily-use cultural and creative carriers to specifically elevate the primary outcome indicators of middle-aged and elderly groups, filling the gaps in existing research.The highly educated group is more willing to actively seek health information and has a higher acceptance of innovative science popularisation media (Li, 2008).

This study nevertheless has limitations:

(1) This study adopted a before-and-after self-controlled quasi-experimental design, which carries inherent methodological limitations. No parallel control groups were established in this research, including a blank control group receiving neither the online APP nor offline cultural and creative product intervention, a control group with only the online intervention, and a control group with only the offline cultural and creative product intervention. As a result, external confounding factors such as temporal effects, seasonal hand-foot-mouth disease (HFMD) science popularization campaigns, and concurrent community public health education cannot be fully ruled out as influences on outcome indicators. It is difficult to completely isolate the independent effects of the combined intervention adopted in this study and other external factors on improvements in participants' HFMD-related health knowledge and health behaviours, which limits the causal attribution of the intervention effects.

(2) Stratified sampling was conducted exclusively in Wenzhou, introducing regional selection bias. Wenzhou features region-specific characteristics in residents' health literacy, coverage of childcare institutions, family caregiving models, and penetration of digital devices. The socioeconomic, educational and caregiving composition of the sample cannot directly represent preschool caregiver populations in urban and rural areas of other provinces, central and western China, or economically underdeveloped regions, restricting the generalisability of the study's conclusions.

(3) This study only set the 3-month intervention endpoint as the sole data collection time point, with no long-term follow-up surveys conducted at 6 and 12 months after intervention completion. The long-term retention and attenuation patterns of intervention-related health knowledge and behaviours could not be observed. Short-term improvements do not confirm that the intervention delivers sustained and stable health promotion effects, leading to short-term measurement bias.

6. Conclusions

This study adopted the 3-month intervention as the standardized experimental endpoint, conducted hierarchical evaluations by distinguishing primary outcomes (qualified rate of hand-foot-mouth disease knowledge) and secondary outcomes (pass rate of healthy behaviors), and drew the following conclusions based on empirical data collected from preschool-related populations in Wenzhou:In terms of baseline characteristics of the Wenzhou samples in this study, family caregivers demonstrated an overall low baseline awareness of hand-foot-mouth disease (HFMD) knowledge; kindergarten staff possessed basic HFMD prevention knowledge but had prominent cognitive deficiencies in key contents including severe case identification and viral

transmission routes. While kindergarten staff maintained a high level of healthy behavior compliance before the intervention, the integrated online-offline intervention produced no statistically significant improvement in their healthy behaviors, whereas family caregivers saw a dramatic rise in the qualified rate of healthy behaviors after intervention. The 3-month integrated intervention combining the virtual simulation APP and health cultural and creative products significantly elevated the overall HFMD knowledge awareness rate of both family caregivers and kindergarten staff with statistically significant differences ($P < 0.05$). After adjusting for confounding variables such as age, educational attainment, and caretaking/occupational identity, the use of the virtual simulation APP and exposure to health cultural and creative products both served as independent positive protective factors for reaching the standard of HFMD knowledge among the two groups. The online virtual simulation APP better fits the learning preferences of young and middle-aged caregivers and serving kindergarten teachers, and offline health cultural and creative products can effectively reach elderly family caregivers with poor digital operation ability; the combination of the two eliminates population coverage gaps caused by a single education carrier and realizes science popularization for all target groups. The data of this study also reveal a positive correlation between educational level and the probability of reaching the HFMD knowledge standard, as participants with college education or above were more likely to achieve qualified cognition, while elderly caregivers and staff relatively mastered less disease prevention knowledge. The integrated intervention model constructed in this study, which integrates digital online interaction and offline popularization through daily cultural and creative products, adapts to two major health education scenarios including community families and kindergartens, features low implementation costs and strong hierarchical adaptability, and can be applied as a standardized intervention scheme for routine HFMD health education targeting populations related to preschool childcare.

Author Contributions:

Luyao Pan contributed to the conceptualization, methodology, and data analysis of the study. Wanting Lin provided guidance on theoretical framing and critical revisions of the manuscript. Yue Qian supervised the overall project and coordinated the research process. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement:

This study's research protocol underwent ethical review. Prior to any questionnaire distribution and intervention implementation, written informed consent was collected from all participating family primary caregivers and kindergarten staff. Every participant was fully informed of the research objectives, intervention procedures, data usage rules, and their right to voluntarily quit the study at any time without penalty.

In full compliance with the Declaration of Helsinki and international journal publishing standards for human subject research, strict data anonymization measures were enforced throughout the whole research cycle to protect participant privacy. All personally identifiable information, including names, contact details, residential addresses, affiliated kindergarten information, and children's private identity data, was permanently deleted immediately after questionnaires were recovered. Each valid sample was assigned an exclusive random digital code as the only identification marker for subsequent statistical analysis, and no individual-level raw records involving personal information entered the dataset for SPSS analysis.

For privacy protection and data security management, the original completed paper questionnaires and unprocessed electronic raw data will not be provided to any third party, including journal reviewers and readers. Only aggregated statistical indicators such as composition ratios, mean scores, chi-square and regression analysis outputs are presented in this manuscript; no complete individual original data will be disclosed in any form in published articles, supplementary materials or post-publication data sharing. All original research materials are stored in password-encrypted local storage devices accessible solely to core research team members, and all raw data archives will be securely incinerated and deleted five years after the completion of relevant follow-up studies to eliminate the risk of personal privacy leakage. No identifiable personal information appears in all tables, figures and text of the present paper.

Data Availability Statement:

Not applicable.

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Conflict of Interest:

The authors declare no conflict of interest.

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