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Research advances in nasal epithelial organoids and challenges in clinical disease applications

Hongjie Du[1,3], Fanyu Yuan[1,3], Wenwen Qi[1], Fangyuan Zhu[1,3], Jian Liu[1,3], Chengzhilin Li[1,3], Zishuo Guo[1,3], Zhaoze Yang[3,5], Xu Guo[1,3], Chengcheng Liu[2,3,5*,§] and Ming Xia[1,3,4*,§]

Organoids, as a novel type of 3D spherical model, possess characteristics such as tissue heterogeneity, functional simulation capability, scalability, personalization, and strong compatibility. These features endow them with significant advantages in drug testing, including the ability to highly simulate human physiological and pathological characteristics, strong clinical relevance, high-throughput and rapid screening capabilities, avoidance of the limitations of animal models, and potential for personalized treatment. As a result, organoids have become an essential tool in drug development and precision medicine.In recent years, nasal organoids have been preliminarily established. These models have been utilized to elucidate the pathogenesis of chronic and acute sinusitis through nasal organoid inflammation models, as well as to screen allergens in allergic rhinitis. Additionally, olfactory epithelial organoid models have been employed to study the mechanisms of olfactory neuron damage and regeneration. This article reviews the recent advances in the fundamental research of nasal organoids and innovatively outlines a composite culture medium formulation developed by our laboratory, providing a new technical approach for cost-effective and efficient organoid research.

Organoids are three-dimensional cellular structures formed in vitro from stem cells or tissue-derived cells, which can be broadly categorized into two types based on their origin: tissue-derived and stem cell-derived[1–3]. The nasal mucosa, serving as the first physiological defense and immune barrier between the human body and the external environment, plays a critical role in clearing pollutants, pathogenic microorganisms (such as viruses and bacteria), and allergens[4,5]. Based on histological and physiological functions, the nasal mucosa can be divided into respiratory epithelium and olfactory epithelium[6]. Currently, research on nasal organoids primarily relies on olfactory epithelium and respiratory epithelium derived from patients, providing an ideal preclinical model for studying the mechanisms of nasal inflammatory diseases, host-pathogen interactions, and the development of novel therapeutic approaches[7,8]. Furthermore, the cultivation and differentiation of olfactory epithelial organoids offer a valuable platform for exploring mechanisms of olfactory epithelial injury and regeneration, olfactory neuron maturation, and olfactory bulb signal transduction. This model not only provides new insights into the physiological and pathological mechanisms of the olfactory system but also establishes a solid scientific foundation for the development of treatment strategies for related diseases[9,10]. This article systematically reviews the latest advances in the fundamental research of nasal organoids, details the components of the culture medium and the cultivation process for olfactory epithelial organoids, and discusses the application prospects of organoid technology. It aims to provide new technical insights and methodological support for the advancement of nasal organoid cultivation techniques.

Research and application of organoids in the medical field

The selection of experimental models plays a crucial role in the study of human disease mechanisms and the development of therapeutic drugs. In recent years, with the advancements in genetic engineering and medical technology, biologics developed based on animal models have shown great potential in disease treatment. For example, drugs such as Targeted PD-1, CTLA4, and CD3 have achieved significant success in cancer therapy[11–13]. Organoids, as a novel experimental model, have gradually become a research hotspot as an alternative to animal models. In addition to drug screening, organoids also play an important role in fields such as cell

[1] *Department of Otolaryngology, Shandong Provincial Hospital Affiliated to Shandong First Medical University, No. 324, Jingwu Weiqi Road, 250033, China*

[2] *Department of Central Laboratory, Shandong Provincial Hospital Affiliated to Shandong First Medical University,544 Jingsi Road, 250033, China*

[3] *Medical Science and Technology Innovation Center, Shandong First Medical University & Shandong Academy of Medical Sciences, Jinan 250117, China*

[4] *NHC Key Laboratory of Otorhinolaryngology,107 Wenhua West Road, China*

[5] *Shandong Second Medical University, Baotong West Street, Weifang, Shandong, 261053, China*

* Corresponding author, E-mail: LCcheng211314@126.com; xiamingsdu@sohu.com

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§ These authors have contributed equally to this work.

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therapy, organ transplantation, immune research, and tissue regeneration[14,15]. Currently, scientists have successfully developed various organoid models, including those for the intestine, blood vessels, liver, heart, lungs, uterus, kidneys, mammary glands, brain, skin, and bone tissue. As organoid technology continues to advance and clinical applications expand, medical research is gradually moving towards industrialization, promoting the standardization and safety of organoid clinical applications.

At the forefront of biomedical research, scientists are utilizing organoid technology to explore new horizons in disease study and treatment. The team led by Nikolche Gjorevski developed a novel human intestinal organoid model for studying the mechanisms of intestinal inflammation and developing therapeutic approaches[16]. The Wang Kai team constructed a visual and quantitative in vitro vascular organoid model for high-throughput drug screening related to angiogenesis[17]. The Leo A. van Grunsven team created a liver organoid model to screen drug treatment strategies for liver fibrosis using a drug-induced liver injury model[18]. The Miriana Dardano team developed hematopoietic heart organoids, opening new avenues for cardiac and blood therapies[19]. The Man Chun Chiu team established a long-term expandable human lung organoid system, providing unique research tools for studying respiratory infections and drug screening[20]. Meiyan Wang and colleagues investigated a novel human brain organoid model to explore the relationship between aging and Alzheimer's disease in depth[21]. Professor Hans Clevers' team has successfully developed a novel pancreatic organoid - human fetal pancreatic organoids (hfPOs), which incorporate all three key pancreatic cell types (acinar cells, ductal cells, and endocrine cells), providing a valuable platform for developing regenerative therapies and novel drugs for pancreatic diseases[22]. Giorgia Quadrato and colleagues have established an innovative organoid model that offers a new research platform for studying human cerebellar development, homeostasis, and related disorders[23]. In a groundbreaking achievement, Sergiu P. Pasca's research team constructed the first human brain sensory organoid model capable of observing neural signal transmission from peripheral neurons to the central nervous system[24]. Professor Eri Hashino's team generated cochlear organoids from human pluripotent stem cells, demonstrating electrophysiological properties remarkably similar to native cochlear hair cells, representing a significant advancement in auditory research[25]. Researcher Lei Lanjie comprehensively summarized and elucidated the reconstruction and clinical potential of various organoids including brain, skin, bone, liver, endocrine, and intestinal organoids, while analyzing current progress in fundamental research and their clinical application value[26].

The application of organoid technology is not limited to the aforementioned organs; it has also demonstrated significant potential in the study of nasal inflammation. As a crucial part of the human respiratory system, the health of the nasal cavity directly impacts respiratory function and olfactory ability. Nasal inflammation, a common disease, has similarly benefited from advancements in organoid technology[27–29].

Translating organoids from basic research to clinical trials and applications.

The core objective of organoid technology in scientific research is to support clinical applications and medical translation, playing a vital role throughout the drug development process. This includes antigen immunization, sequence construction, druggability evaluation, preclinical research, and clinical trials[30,31]. As organoid technology is gradually integrated into clinical trials, the number of related registrations has been increasing year by year, significantly accelerating the development of clinical therapeutics. For instance, in 2022, Bran Herpers' team successfully screened a bispecific antibody, MCLA-158, targeting both EGFR and LGR5 using patient-derived organoid (PDO) models, demonstrating the potential of organoid technology in drug screening[32]. Georgios Vlachogiannis and other researchers have demonstrated the use of patient-derived organoids (PDOs) in treating patients with colorectal cancer and gastroesophageal cancer[33]. Currently, metastatic, heavily pretreated patients with colorectal cancer and gastroesophageal cancer are still being recruited for phase 1/2 clinical trials. Further studies continue to validate the application value of organoid technology in personalized medicine.

According to statistical data from 2017 to the present, organoid-related clinical trials are predominantly parallel observational studies (Fig.1). Among these, the proportion of interventional studies in the United States is slightly higher than that in China. However, China has made rapid progress in the field of tumor organoid research, particularly in the treatment of common cancers such as colorectal, breast, pancreatic, and lung cancers. With the continuous maturation of organoid technology and its deepening application in medical translation, it is anticipated that more interventional clinical studies will enter the trial phase in the future, providing more precise and efficient technical means for clinical treatment. This trend not only drives the further development of organoid technology but also opens new avenues for personalized medicine and drug development[34,35].

Organoid technology has demonstrated revolutionary potential in precision medicine, drug development, and regen-

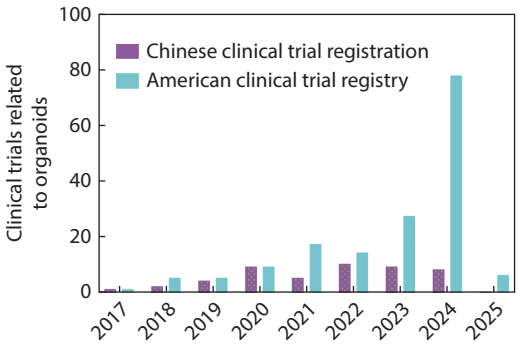

Fig. 1 Statistics of clinical trials related to organoids. Statistical analysis of clinical trial registration data from 2017 to 2025 reveals that while the United States maintains a relative advantage in the overall volume of organoid-related clinical research, China has demonstrated particularly notable growth in the field of tumor organoid studies.

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erative medicine. However, its translation from basic research to clinical application faces three major challenges: insufficient physiological relevance, limited reproducibility, and scalability bottlenecks.

In terms of physiological relevance, organoid technology has achieved significant breakthroughs in mimicking the three-dimensional architecture, cellular composition, and functional characteristics of source tissues. However, current organoid models still have important limitations: the absence of vascular networks and immune microenvironments restricts drug permeability studies and immunotherapy evaluation; it remains difficult to simulate complex biomechanical stimuli (e.g., peristalsis) and dynamic biochemical gradients in vivo; and long-term culture-induced genomic instability may compromise experimental reliability. These physiological simulation deficiencies have become key research priorities[36,37].

Regarding standardization and reproducibility, organoid technology faces significant challenges. Organoids from different donors show substantial individual variations in growth kinetics, morphological features, and drug sensitivity; batch-to-batch variations in critical culture components and matrix materials directly affect organoid quality; and the lack of unified characterization and quality control standards severely limits the reliability of organoids in clinical diagnosis and therapeutic decision-making[38].

At the scale-up application level, organoid technology encounters multiple obstacles: high culture costs primarily due to expensive growth factors and matrix materials; prolonged maturation periods that hinder rapid drug screening; and suboptimal cryopreservation techniques that compromise long-term sample storage and transportation. These scalability issues restrict the widespread application of organoid technology in drug development and clinical practice[39,40].

The application of nasal organoids in nasal inflammatory conditions.

The nasal mucosa, serving as the first physiological line of defense and immune barrier between the human body and the external environment, plays a crucial role in clearing pollutants, pathogenic microorganisms (such as viruses, bacteria, allergens, etc.)[41], and allergens. Based on histological and physiological functions, the nasal mucosa can be divided into respiratory epithelium and olfactory epithelium. The respiratory epithelium consists of ciliated cells, columnar cells, goblet cells, and basal cells, primarily responsible for filtering, humidifying, and cleaning inhaled air. When the respiratory mucosa is damaged, its physiological functions are disrupted, potentially leading to nasal inflammatory diseases such as sinusitis. The olfactory epithelium, located at the top of the nasal cavity, is composed of olfactory cells, supporting cells, and basal cells, mainly responsible for olfactory perception[42–44]. Damage to the olfactory epithelium can result in olfactory dysfunction, and in severe cases, complete loss of smell[45]. In recent years, stem cell therapy has shown great potential in the field of nasal mucosa repair. Stem cells, with their self-renewal and differentiation capabilities, can promote the repair of nasal mucosa and exert immunomodulatory effects[46]. For example, ongoing clinical research on human umbilical cord mesenchymal stem cell injections for the treatment of moderate-to-severe persistent allergic rhinitis offers a new direction for nasal mucosa injury treatment. This therapy holds promise for providing more effective treatment options for patients with nasal diseases[47].

Since the successful cultivation of mouse intestinal epithelial organoids by Hans Clevers' team in 2009, organoid technology based on adult tissue-specific stem cells has been widely applied to functional studies of various organs[48]. In recent years, this technology has also been successfully utilized in research on nasal tissues. For example, Ramezanpour et al. successfully constructed nasal organoids with self-renewal and passaging capabilities from patients with chronic rhinosinusitis (CRS). They compared the expression differences of the Lgr5 gene between nasal organoids and monolayer cultures and systematically characterized the morphology, cellular composition, and functional parameters of the nasal organoids[49]. Dobzanski et al. found that epithelial organoids cultured in the sinus fluid environment of patients with CRSwNP (chronic rhinosinusitis with nasal polyps) exhibited enhanced proliferative capacity[27]. They also confirmed that the Wnt signaling pathway is involved in regulating the differentiation and colony-forming efficiency of epithelial organoids[28]. Carolina Nunes Franca et al. used cells derived from nasal polyps for 3D organoid culture and discovered that these cells could maintain a differentiated state for a longer period and form ciliated structures, providing an important tool for evaluating the remodeling process of nasal polyp tissue. Additionally, Li Cun et al. established a respiratory tract organoid culture system using nasal epithelial cells to study the effects of continuous passaging of human rhinovirus C (HRV-C) in nasal organoids on viral replication and explored its antiviral inhibitory effects[50]. These studies have not only advanced the understanding of the mechanisms underlying nasal diseases but also laid a solid foundation for the application of organoid technology in the research and treatment of nasal diseases.

By optimizing the construction of nasal organoids and the air-liquid interface (ALI) culture system, a research platform that highly simulates the human tissue microenvironment has been successfully established[51–53]. This model provides an ideal experimental system for studying the pathological mechanisms of diseases such as chronic sinusitis and nasal polyps, demonstrating significant advantages, particularly in elucidating the interaction mechanisms of upper respiratory tract diseases. Through this platform, researchers can delve into the associations between rhinitis and asthma, rhinitis and bronchopneumonia, and achieve breakthrough progress in key mechanisms such as viral replication and transmission, antibody blockade effects, immune cell infiltration, and immune regulation[54,55]. Furthermore, the model's high-throughput drug screening capability offers crucial technical support for the development of innovative treatment strategies for related diseases, holding broad prospects for clinical application.

Functional research on the construction of olfactory organs and olfactory diseases

The olfactory system, as a crucial component of nasal function, plays a key role in detecting and discriminating odors.

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Olfactory receptor neurons in the olfactory epithelium are responsible for recognizing odor molecules and transmitting signals to the olfactory bulb, which then forms olfactory perception through the cerebral cortex[56,57].

In recent years, significant progress has been made in the study of olfactory epithelial organoids. Several research teams have explored the complex regulatory network of the olfactory system through diverse experimental designs and molecular mechanisms. For instance, Li Wang et al. utilized an olfactory epithelial organoid model to investigate the association between Ym2 protein and inflammatory responses, discovering that the downregulation of Ym2 significantly affects the proliferation and differentiation capabilities of olfactory epithelial organoids. This finding provides new insights into the function of Ym2 in the olfactory system[58]. Li Weihao et al. focused on the impact of aging on the olfactory epithelium, revealing differential gene expression profiles related to olfactory degeneration during aging through single-cell sequencing technology.They further explored the interactions between HBC cells and immune cells and elucidated the critical role of Egr1 in the development of mature olfactory neurons by regulating Egr1 expression in olfactory epithelial organoids[59]. Wenwen Ren et al. achieved a significant breakthrough in optimizing the culture conditions for olfactory epithelial organoids. Using a Matrigel-based 3D culture system, they found that the chemicals VPA and CHIR99021 could significantly increase the proportion of Lgr5-egfp+ cells in Lgr5+ cell-derived organoids, thereby activating the proliferative potential of stem cells. This discovery provides a new strategy for the efficient cultivation of olfactory epithelial organoids[60]. Chen Mengfei et al. investigated the effects of RelA deficiency on immune cell infiltration through TNF stimulation experiments, uncovering the potential role of HBC cells in amplifying inflammatory signals[61]. This study offers important clues for understanding the molecular mechanisms of the olfactory epithelium under inflammatory conditions.Lastly, Li Xuewen et al. examined the effects of Notch activators and inhibitors on aging-induced differentiation in olfactory epithelial organoids by modulating the Notch signaling pathway[62]. They found that the activation of Notch signaling could promote the proliferation and differentiation capabilities of Lgr5+ cells into immature and mature neurons, providing a novel therapeutic approach for repairing aging-induced olfactory epithelial damage.Collectively, these studies have advanced the field of olfactory epithelial organoids, offering profound insights into the physiological and pathological mechanisms of the olfactory system and laying a solid foundation for the development of treatment strategies for related diseases.

The olfactory system is a highly complex organizational structure that is closely connected to the olfactory bulb and the central nervous system of the brain. Its function relies on the precise transmission and recognition of signaling molecules by olfactory sensory neurons[63,64]. Notably, olfactory sensory neurons possess the ability to continuously renew themselves throughout their lifespan, a characteristic primarily attributed to the presence of stem cells in the olfactory epithelium. Sofia Haglin and colleagues, by using methimazole to construct a model of olfactory epithelium injury, observed that four weeks post-injury, olfactory sensory neurons exhibited a certain degree of recovery, and the olfactory epithelium demonstrated regenerative capacity[65]. This finding not only reveals the self-repair potential of the olfactory system but also provides important experimental evidence for studying the mechanisms of olfactory disorders and neuronal damage.

Additionally, the cultivation technology of olfactory epithelium organoids and their differentiation process into mature neurons lays a solid foundation for further exploration of the molecular mechanisms underlying olfactory disorders and neuronal damage. At the same time, this model serves as an important research platform for investigating the developmental mechanisms of olfactory bulb neurons, further advancing research on the interactions between the olfactory system and the central nervous system. These research findings open new directions for understanding the physiological and pathological processes of the olfactory system and provide theoretical support for the development of treatment strategies for related diseases.

Research and development of olfactory epithelial organ culture system

In the cultivation of olfactory epithelial organoids, cytokines serve as core regulatory factors, playing an indispensable role in the formation and maturation of organoids[66]. They provide the essential molecular foundation for organoid construction by precisely regulating cell growth, differentiation, proliferation, and the maintenance of stem cell states. For example, Wnt-3a has demonstrated significant functionality in various organoid culture systems, promoting the self-renewal and directed differentiation of stem cells, thereby ensuring a stable cell source for organoid formation[67,68]. Noggin, on the other hand, maintains the undifferentiated state of stem cells and promotes their proliferation by inhibiting differentiation, creating the necessary conditions for the sustained growth of organoids[69,70]. Additionally, R-spondin 1 plays a crucial role in organ development, epithelial stem cell regulation, and cancer initiation and suppression by activating the Wnt-β-catenin signaling pathway, further highlighting its central importance in organoid culture[71,72]. The synergistic effects of these cytokines not only provide precise regulatory strategies for the cultivation of olfactory epithelial organoids but also establish a critical experimental and theoretical foundation for research on the development, regeneration, and disease mechanisms of the olfactory system.

In addition to cytokines, small molecule compounds are also indispensable nutrients in organoid culture, playing a crucial role in regulating stem cell fate and organoid development. For instance, Y27632, a specific inhibitor of the Rho-associated serine-threonine protein kinase (ROCK) family, can effectively inhibit stem cell apoptosis and enhance cell survival rates[73,74]. CHIR99021, an inhibitor of glycogen synthase kinase 3β (GSK-3β), promotes the differentiation of human embryonic stem cells into endoderm and significantly enhances cell proliferation and differentiation capabilities in olfactory epithelial organoid cultures[75,76]. Furthermore, LY-411575, a potent and selective γ-secretase inhibitor, induces stem cell proliferation by inhibiting the Notch signaling path-

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way, providing essential support for the growth of organoids[77,78].

In the cultivation of olfactory epithelial organoids, cellular supplements are essential nutrients. Gibco™ GlutaMAX™ Supplement, as a substitute for L-glutamine, offers superior stability and effectively reduces the accumulation of toxic ammonia, thereby significantly enhancing cell viability and growth, and further improving cell health[79,80]. Additionally, B-27 and N2 supplements are optimized serum-free formulations widely used to support the growth of embryonic, postnatal, and adult hippocampal and other central nervous system neurons. They promote both short-term and long-term cell survival, whether in low-density or high-density cultures[81,82]. Sodium pyruvate is often used as a carbon source in cell culture media. Although it is an intermediate metabolite in the glycolytic pathway, it is not required for all cell cultures[83,84].

Currently, the cultivation technology for olfactory epithelium organoids has matured, typically employing a combination of commercial culture media along with cytokines, small molecules,cell supplements and antibiotics for cultivation. However, the long production cycles and high costs associated with commercial media and cytokines undoubtedly increase research expenses and laboratory burdens. Nevertheless, optimizing the growth and differentiation media for olfactory epithelium organoids remains crucial for studying the physiological functions of the nasal mucosa. The vitality of these organoids directly determines their ability to mimic the natural physiological state of the nasal mucosa, thereby laying a solid foundation for research on nasal mucosa-related diseases. To gain a more comprehensive understanding of the application of nasal cavity organoid culture media, Table1 provides a systematic summary of the media used in previous studies, offering important references for future research. These advancements not only promote the development of olfactory epithelium organoid culture technology but also provide powerful tools for in-depth studies of the physiological and pathological mechanisms of the nasal mucosa.

Our laboratory is dedicated to investigating the mechanisms of olfactory dysfunction and optimizing and innovating the culture system for olfactory epithelial organoids. We have successfully established an efficient organoid culture, passage, and cryopreservation system (Fig. 2). The nasal organoid culture medium in our study includes conditioned medium containing R-Spondin, Noggin, and Wnt3a, supplemented with additional components: Y27632 (15 μM), EGF (80 ng ml^{-1}), N2 (1%), B27 (2%), HEPES (1 mM), and Primocin (150 μg/ml). This formulation is based on the WENR protocol (Wnt3a, EGF, Noggin, and R-Spondin) developed by Professor Hans Clevers' team at the Hubrecht Institute in the Nether-

Table 1. Summary of reported medium for Nasal organoid culture

Time	Source	Components of nasal organoid culture medium	Researcher
2018	mice	DMEM,10% FBS, 1% Glutamax, 1% Pen/strep, 1% NEAA	Alex Dobzanski, MS
2019	mice	NeuroCult medium, 10% Proliferation Supplement (Stem Cell Technology), 20 ng/mL EGF,and 10 ng/mL bFGF	Chen Mengfei
2021	human	DMEM/F12, R-spondin-1 (200ng ml^{-1};), Noggin (100ng ml^{-1}), Wnt3a (50 ng ml^{-1}), Y27632 (10 mM), EGF (50ng ml^{-1}), N2 (1%), B27 (2%), HEPES (1 mM), GlutaMAX (1%),100 μg mL^{-1} PrimocinTM, 2 μM 616452, 500 nM A83-01 and10 μM SB431542	Ren Wenwen
2022	human	PneumaCult™ Airway Organoid Basal Medium (STEMCELL Technologies CAT:05061)	Mahnaz Ramezanpour
2024	mice	DMEM/F12, 1% GlutaMAX, R-Spondin (200 ng ml^{-1}), Noggin (100 ng ml^{-1}), Wnt3a (50 ng ml^{-1}), Y27632 (10 mm), EGF(50 ng ml^{-1}), N2 (1%), B27 (2%), HEPES (1 mM;), Primocin (100 mg ml^{-1})	Li Weihao

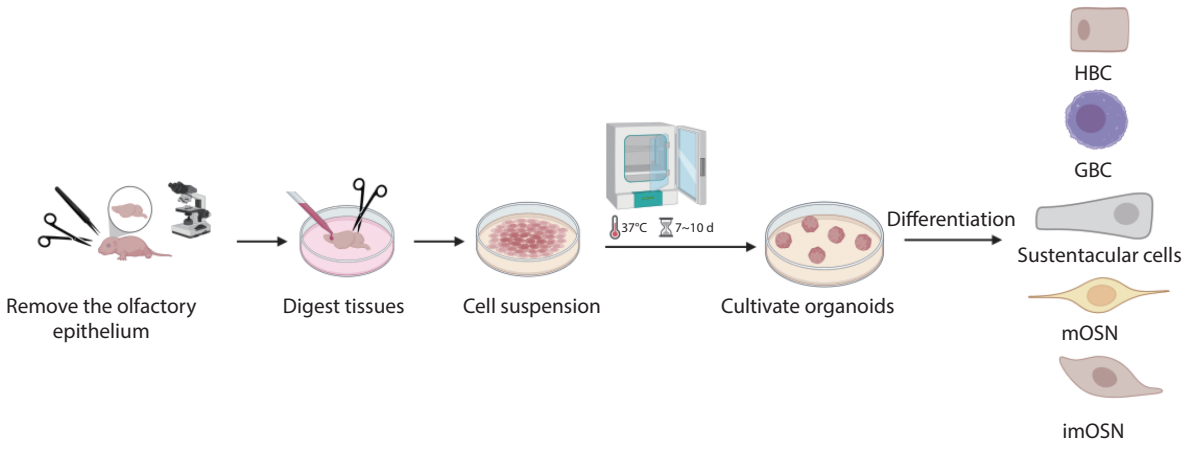

Fig. 2 The culture process of mice olfactory epithelial organoids. First, the mouse is anesthetized, and the head is removed. The olfactory epithelial tissue is dissected and isolated under a stereo microscope. Subsequently, the tissue is minced into small pieces under cold conditions and digested using trypsin. The digested tissue is then centrifuged and filtered to obtain a single-cell suspension. The suspension is mixed with Matrigel matrix and allowed to solidify, after which growth medium is added for cultivation. After 7 days of culture, organoids of varying sizes are formed. Finally, by adding specific cytokines and small molecule compounds, the organoids are induced to differentiate into various cell types.HBC:Horizontal basal cells, GBC:globe basal basal cell, mOSN:mature olfactory sensory neurons, imOSM:immature olfactory sensory neurons.

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lands, which has been widely applied in culturing various organoids including gastric, small intestinal, colonic, pancreatic, and hepatic organoids.Wnt3a serves as an activator of the Wnt pathway by stabilizing β-catenin protein, thereby initiating downstream target genes and participating in cell development, proliferation, and differentiation processes. EGF binds to its receptors, activating the MAPK/ERK pathway to promote cell proliferation and survival. Noggin, an endogenous inhibitor of bone morphogenetic proteins (BMP), blocks BMP-receptor binding to enhance stem cell proliferation, thereby providing sufficient cells for organoid formation. R-spondin functions as a Wnt signaling enhancer by binding to Frizzled and LRP5/6 receptors, amplifying Wnt signaling across various tissues to promote stem cell proliferation and organoid structural development. These three factors are expressed by the commercially available L-WRN cell line (ATCC CRL-2647).Compared to direct addition of recombinant proteins, the cytokines in L-WRN conditioned medium more closely resemble the natural in vivo state and exhibit higher biological activity. However, several critical factors must be carefully controlled to ensure the efficacy and consistency of the conditioned medium, including: passage stability of the producer cells, cryopreservation status, batch-to-batch consistency, and cell culture conditions[85]. Beyond the WENR components, Y-27632, a potent ATP-competitive ROCK inhibitor, prevents stem cell apoptosis while improving cloning efficiency and extending passage capability[86]. HEPES, B27, and N2 supplements serve as essential basal medium components, providing necessary nutrients and maintaining optimal pH for organoid culture. Compared to directly adding recombinant proteins, the cytokines in L-WRN conditioned medium are closer to their natural in vivo state, exhibiting higher biological activity.

However, the stability of cell passages, the state of cryopreserved cells, batch consistency, and cell culture conditions are all critical factors in ensuring the efficiency and stability of the conditioned medium. Studies have shown that Eloise Mussard et al. successfully cultured rabbit cecal organoids using L-WRN conditioned medium, observing significantly enhanced proliferation and differentiation capabilities[87]. Additionally, Robin H. Powell and Michael S. Behnke demonstrated that L-WRN conditioned medium supports long-term expansion and high-density growth of intestinal organoids from large farm animals and companion animals. These studies further validate the broad application potential of L-WRN conditioned medium in organoid culture[88].

Our research team has systematically established a complete technical system, ranging from tissue dissection and digestion to Matrigel embedding and in vitro culture, successfully achieving efficient cultivation of olfactory epithelial organoids (Fig. 3). Experimental results demonstrate that this culture system can stably form organoid colonies with uniform morphology and moderate diameter within 7 to 10 days. The results demonstrate that as the number of passages increases, the organoids exhibit excellent stability in both morphological integrity and proliferative capacity. Based on the current findings, we plan to further conduct systematic analysis of the active components in the conditioned medium to elucidate their molecular mechanisms, thereby providing a theoretical foundation for the precise optimization of the culture system. The success of this study not only advances the standardization of olfactory epithelial organoid culture techniques but also offers important references for methodological innovation in related fields.

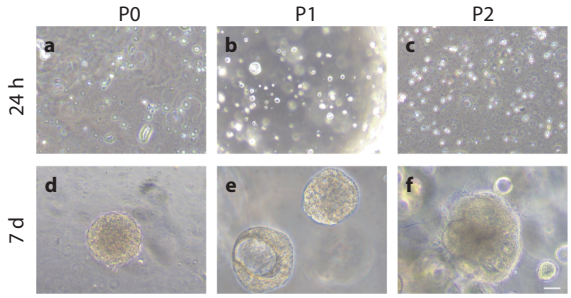

Fig. 3 The morphological characteristics and proliferative status of olfactory epithelial organoids. **a**-**c** show microscopic images (10×) of primary P0, first-generation P1, and second-generation P2 organoids 24 hours after culture, respectively; **d**-**f** display microscopic images (10×) of P0, P1, and P2 organoids on day 7 of culture, respectively. All images were captured using an inverted microscope, with a scale bar of 50 μm.

Study on differentiation induction of different functional cells in nasal organoids

Basal cells, including horizontal basal cells (HBCs) and globose basal cells (GBCs), are stem cells in the nasal mucosa responsible for differentiating into cells with various functions. When GBCs transition from a quiescent state to an active state, they continuously differentiate into immature olfactory neurons, which eventually develop into mature olfactory neurons[89,90]. If GBCs in the olfactory epithelium are damaged or functionally impaired, dormant HBCs are activated and transition into an active state, subsequently differentiating into new GBCs to enhance their stem cell properties, thereby maintaining the regenerative and repair capacity of the olfactory epithelium[89,91].

In this process, the fate of HBCs and GBCs differentiating into various cell types in the olfactory epithelium is precisely regulated by multiple signaling pathways, such as the Wnt and Notch pathways. The Wnt/β-Catenin signaling pathway plays a critical role in embryonic development and stem cell proliferation, and the intensity of its signaling can determine whether GBCs tend to maintain their self-renewal capacity or differentiate into specific cell types[92–94]. Lgr5, an important regulator of the Wnt signaling pathway, has been shown to promote the proliferation of Lgr5-positive stem cells and drive their differentiation into olfactory sensory neurons when activated[95–97]. Additionally, the BMP signaling pathway is involved in the proliferation and differentiation of stem cells and plays a significant role in determining cell fate[98,99]. On the other hand, the Notch signaling pathway promotes dedifferentiation and self-renewal, maintaining the stemness of progenitor cells and the stability of the stem cell pool, while also participating in cell fate decisions and guiding progenitor cells toward specific differentiation paths[100,101]. ΔNp63, a key transcription factor in horizontal basal cells, can activate the Notch pathway, regulate the proliferation of Lgr5-positive precursor cells in the olfactory epithelium, and

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promote the regeneration of mature olfactory sensory neurons. The synergistic actions of these signaling pathways collectively ensure the dynamic balance and functional integrity of the olfactory epithelium[102].

In the organoids derived from olfactory epithelium primary stem cell cultures, the proliferation and differentiation processes of the cells can only generate a limited number of specific functional cell types, such as supporting cells, horizontal basal cells (HBCs), and globose basal cells (GBCs). However, mature olfactory sensory neurons cannot be spontaneously generated and require further induced differentiation to achieve this[103,104]. Research by Wang Han et al. found that although immature olfactory sensory neurons exist in the cultured organoids, mature olfactory sensory neurons are lacking[105]. Scholars such as Wenwen Ren have activated the Wnt3a signaling pathway using small molecules VPA and CHIR99021 to promote the proliferation of Lgr5 stem cells and their specific expression[105]. At the same time, they successfully induced the generation of more mature olfactory sensory neurons in mouse organoids by inhibiting the Notch signaling pathway with LY411575. Additionally, they further promoted the maturation and development of olfactory sensory neurons by employing specific combinations of culture media and small molecules at different time points.

However, the signaling pathway networks governing the maintenance and cellular fate transitions of various cell types in the olfactory epithelium remain unclear. Further elucidation of how these signaling pathways regulate the self-renewal and differentiation of olfactory epithelial stem cells is necessary. This research not only helps to reveal the mechanisms of repair and regeneration in the olfactory epithelium but also provides an important theoretical foundation and practical guidance for exploring therapeutic strategies to promote the repair of olfactory epithelium damage.

The future development direction and prospects of nasal organoids

Respiration and olfaction are essential functions in mammals that play an irreplaceable role in maintaining vital activities and health. Dysfunction in either of these functions not only significantly reduces quality of life but is also closely related to diseases such as asthma, pneumonia, Alzheimer's disease, and Parkinson's disease[106,107]. In recent years, the loss of smell caused by COVID-19 infections has prompted scientists to focus on the mechanisms of olfactory development and central nervous system signaling, making it a hot topic of research[108,109]. Olfactory organoids, as a dynamic model, can highly simulate the morphogenesis of the olfactory system and the maturation process of olfactory neurons, providing an important tool for related studies. Several studies have confirmed a significant association between olfaction and Alzheimer's disease. For example, the Yi Dong team revealed the correlation between olfactory recognition function and cognitive impairment through a large sample cohort study, suggesting that neurodegenerative diseases may share a common pathological mechanism with loss of smell and recognition impairment[110]. Research by Chen Ming and colleagues further indicated that olfactory decline is not only a marker of structural changes in specific brain regions but may also serve as an early warning signal for cognitive impairment[111]. In this context, the process of differentiating olfactory epithelial organoids into mature olfactory neurons, the development of neurons in olfactory bulb organoids, and their interaction with the hippocampus provide an ideal experimental platform for in-depth research on the mechanisms of olfactory disorders and neurodegenerative diseases[112,113].

Despite significant progress in nasal organoid models over the past few decades, substantial challenges remain in simulating the physiological state of real human disease environments. Currently, the construction of nasal cavity organoid disease models is still a key bottleneck in technical research. The associations between allergic rhinitis, chronic sinusitis, rhinitis, and asthma, as well as the interactions between rhinitis and olfactory disorders, and the intrinsic links between olfactory disorders and neurodegenerative diseases, are core directions for future model construction and mechanism studies[114,115]. These challenges also represent major obstacles limiting the widespread application of nasal organoid technology in research related to nasal clinical diseases, and they constitute key areas of focus in our research. By overcoming these technical bottlenecks, we hope to provide a more precise research platform for elucidating the pathological mechanisms and clinical treatments of nasal-related diseases (Fig 4).

Current research on olfactory organoids faces several key challenges, primarily in system integration and functional simulation. In terms of system integration, existing models can only replicate the peripheral olfactory epithelial structure and lack functional connections with central nervous system components such as the olfactory bulb and cortex. This limitation prevents the complete reconstruction of the "olfactory receptor-olfactory bulb-higher cortex" signaling pathway, while culture conditions also restrict synaptic plasticity and the stability of electrical activity. Regarding immune-neural interactions, the recreation of the nasal cavity's unique immune microenvironment remains challenging, with key neuroinflammatory mediators like microglia and astrocytes not yet effectively incorporated. To address these limitations, our laboratory is actively developing a co-culture system combining olfactory bulb organoids and olfactory epithelial organoids. Future research must focus on achieving coordinated simulation of the neural-immune-epithelial tripartite microenvironment by integrating organ-on-a-chip technology with mechanical sensing modules, combining multidisciplinary approaches from neurobiology, tissue engineering, and computational modeling to realize a qualitative leap from "structural simulation" to "functional recapitulation."

Organoid technology at the crossroads: prospects, obstacles and road ahead

In recent years, significant advancements have been made in drug screening research based on organoid models, demonstrating broad application prospects across various disease models. The team led by Li Xiaoxing established a biobank of gastric cancer organoids and, through RNA sequencing, successfully identified differential gene expression profiles between 5-fluorouracil and oxaliplatin-sensitive and

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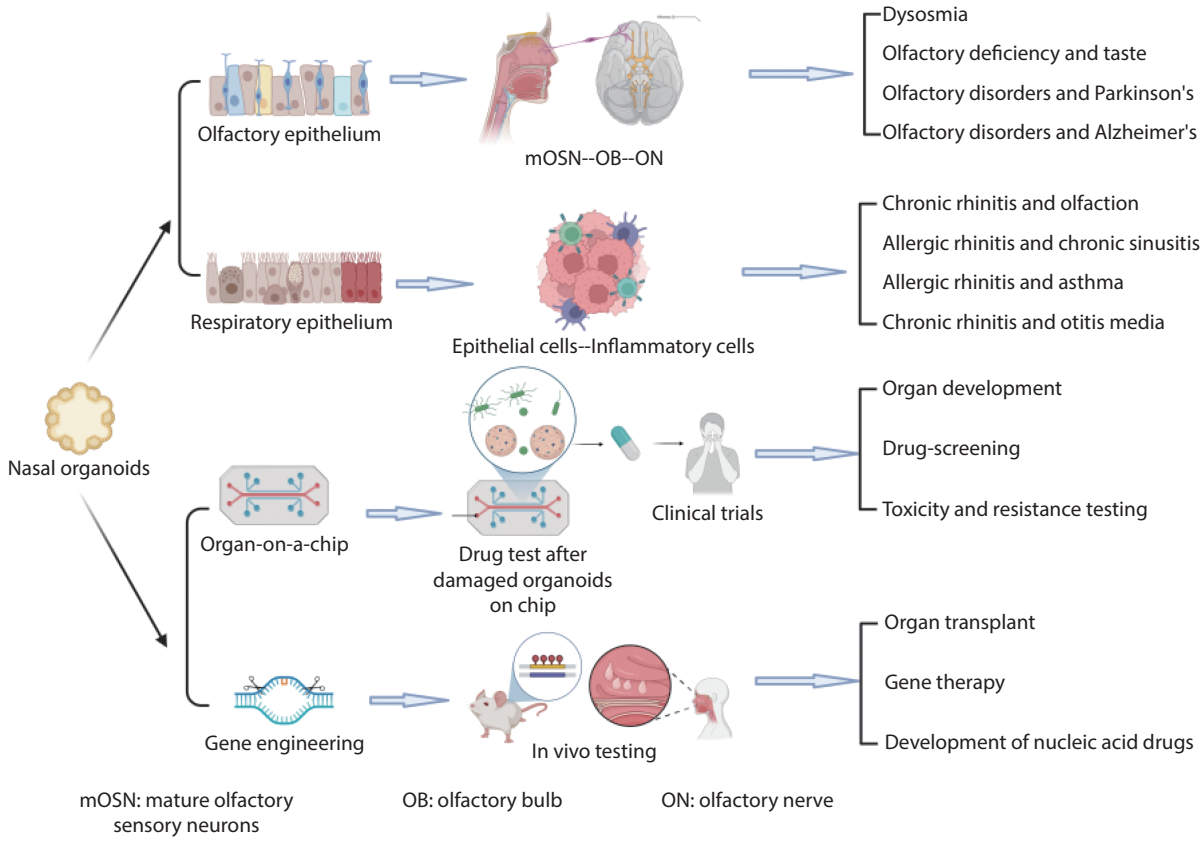

Fig. 4 Application prospects of nasal cavity organoids in the medical field. As a novel in vitro research model, nasal cavity organoids provide an ideal platform for investigating the pathogenesis of nasal-related diseases, drug screening, and therapeutic efficacy evaluation. By integrating organoid-on-a-chip technology and gene editing tools, this model can be further extended to personalized medicine, regenerative medicine, and precision drug development, offering comprehensive technical support and innovative solutions for the diagnosis and treatment of respiratory diseases.

resistant organoids, providing new molecular biomarkers for predicting chemotherapy sensitivity[116]. In the field of cartilage regeneration, Bai Xiaochun research team developed a cartilage organoid system that allows for real-time dynamic monitoring of cartilage formation and hypertrophy. Their high-throughput screening revealed that the α-AR antagonist phenylephrine promotes cartilage differentiation while inhibiting hypertrophy, showcasing a dual effect[117]. The ovarian cancer organoid model constructed by Lai Hungcheng displayed significant individual variability in drug response during in vitro drug testing, revealing marked differences in sensitivity to chemotherapeutic agents such as cisplatin, carboplatin, and paclitaxel among organoids derived from different patients[118]. Fang Jingyuan team utilized a colorectal cancer organoid model to confirm the antitumor effects of statins and innovatively discovered that a combination treatment with chloroquine significantly enhances therapeutic efficacy[119]. Yang Jinjie team established a lung cancer organoid library comprising 160 samples, providing an important platform for drug screening. Their findings indicated that combination therapies with osimertinib and either savolitinib or cabozantinib result in superior tumor control[120]. Organoid engineering is undergoing a critical transition from basic research to clinical applications, a process that urgently requires the synergistic support of novel biomaterials and cutting-edge technologies to construct fully functional organoid composite systems. Dr. Liu Liangle systematically investigated the current applications of bio-assisted materials in organoid modeling and drug screening[121]. Based on a comprehensive analysis of existing technological limitations, he highlighted the latest breakthroughs in novel biomaterials for optimizing organoid models, providing innovative solutions for clinical translation. Meanwhile, the research team led by Lei Lanjie adopted a multidisciplinary technological integration approach to thoroughly examine engineering strategies—including microporous arrays, bioreactor systems, microfluidics, bioprinting, and hydrogel matrices—for promoting organoid maturation[122]. Their work has yielded forward-looking technical pathways for clinical applications in tissue repair and functional reconstruction. Collectively, these studies are driving the leapfrog development of organoid engineering from laboratory research to clinical practice.

Breakthroughs in organoid technology are driving revolutionary advances in the field of regenerative medicine. In organ-on-a-chip and vascularization technologies, researchers have successfully constructed brain, liver, and kidney organoid models with functional vascular networks through innovative endothelial cell co-culture systems[123,124]. These vascularized structures precisely simulate in vivo hemodynamic characteristics, significantly enhancing nutrient delivery and metabolic waste clearance efficiency. The establishment of immune-organoid co-culture systems represents another ma-

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jor breakthrough. By integrating immune components such as T cells and macrophages, researchers have developed disease models with complete immune microenvironments, providing a novel platform for studying inflammatory disease mechanisms, autoimmune disease treatment, and cancer immunotherapy development[125,126]. Significant achievements have been made in the application of gene editing technologies. Using the CRISPR-Cas9 system, researchers have successfully repaired disease-causing gene mutations in patient-derived organoids, providing a reliable tool for validating precision gene therapy strategies[127]. With these key technological breakthroughs, organoid engineering is rapidly transitioning from basic research to clinical regenerative medicine applications. Notably, the deep integration of artificial intelligence is injecting new momentum into organoid research. The research team led by Li Shisheng proposed the concept of "digital organoids," constructing a high-throughput, multi-dimensional organoid characterization and evaluation system by integrating bioinformatics, high-resolution microscopic imaging, AI algorithms, and multi-omics analysis[128]. This provides a revolutionary research paradigm for drug screening optimization and precision medical decision-making. These synergistically innovative technological systems are reshaping the developmental landscape of regenerative medicine. In summary, the deep integration of organoid technology with cutting-edge technologies such as artificial intelligence, high-throughput screening, gene editing, 3D bioprinting and novel biomaterials is progressively demonstrating its tremendous medical potential and value[129,130]. With broad application prospects in disease diagnosis, personalized medicine, drug screening and development, organ transplantation, tumor profiling, drug sensitivity assessment, genetic research and pathogen infection studies, this convergence promises to bring revolutionary breakthroughs to modern medical research and clinical practice[131](Fig 5).

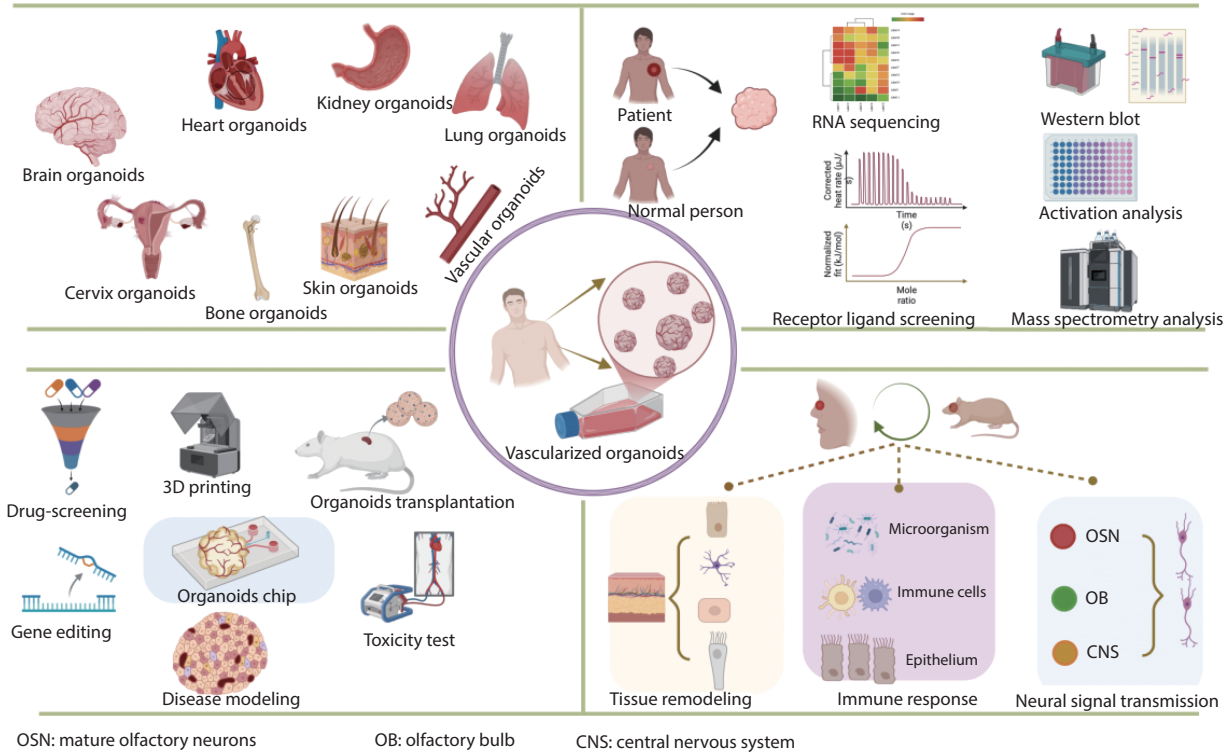

Fig. 5 Clinical application prospects of organoid technology sketch map:Current clinical developments:systematically presents organoids derived from diverse tissues with clinical translation potential.Technology Integration:Highlights cutting-edge convergence with gene editing, 3D bioprinting and organ-on-a-chip systems.Research Methodologies:Illustrates key technical approaches including single-cell sequencing.Nasal Organoid Specialization:Details specific applications and research directions for nasal organoids.

■ AUTHOR CONTRIBUTIONS

MX and CL provided guidance and direction throughout the entire manuscript preparation process. HD conducted a literature search and wrote the original manuscript. FY, CL, and ZG provided constructive suggestions and made significant revisions to the manuscript. ZY conducted a detailed analysis of the content expression in the article. XG has provided suggestions for modifying the images. All authors have read and approved the final manuscript.

■ DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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■ ETHICS STATEMENT

All animal experiments were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC) of Shandong Provincial Hospital Affiliated to Shandong First Medical University. The study protocol was reviewed and approved by the Ethics Committee (Approval No.: NSFC(Preliminary)2023-265). All procedures were performed in compliance with the ARRIVE guidelines and the National Research Council's Guide for the Care and Use of Laboratory Animals.

■ DATA AVAILABILITY

Data will be made available on request.

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Biographies

Du Hongjie has been pursuing his Ph.D. at Shandong First Medical University since 2024, under the supervision of Professor Xia Ming. His research focuses on investigating the pathogenesis of allergic rhinitis and chronic rhinosinusitis.

Liu Chengcheng is an Associate Researcher and Chief Technologist at the Provincial Hospital affiliated with Shandong First Medical University, as well as a Doctoral Supervisor and Professor at Shandong First Medical University. He obtained his Ph.D. from Shandong University in 2017 and conducted postdoctoral research there in 2018. In 2020, he joined the Provincial Hospital affiliated with Shandong First Medical University. In 2023, he was honored with the title of Outstanding Young Innovation Team Leader. He serves as an editorial board member for journals such as Sensory Neuroscience. His primary research focuses on olfactory disorders and the pathogenic mechanisms of allergic rhinitis.

Xia Ming is a Chief Physician at the Provincial Hospital affiliated with Shandong First Medical University, a doctoral supervisor, and a researcher. He obtained his Ph.D. from Shandong University in 2008. In 2018, he was honored as a Shandong Taishan Scholar Young Expert. He serves as an editorial board member for journals such as Flavour and Fragrance Journal and the Chinese Journal of Medical Science Research Management. His primary research focuses on the pathogenic mechanisms of chronic rhinosinusitis, allergic rhinitis, and age-related hearing loss.