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Gene Editor: Innovative Use of Zebrafish Models in Inflammatory Bowel Disease Research

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Abstract: Inflammatory bowel disease (IBD) is a multifactorially induced complex disease, and the most technologically mature animals for IBD disease modelling are mainly mice. However, compared with rodents, zebrafish are more efficient and convenient, with their short growth cycle, high reproductive capacity, small size, light weight, embryonic transparency, and high homology with human intestines, which makes it easy to perform observation of continuous pathological changes and high-throughput drug screening studies. With the development of sequencing technology, gene editing of sterile zebrafish using CRISPR technology plays an important role in revealing in-depth the colony-immune interactions and the pathogenesis of IBD.

Keywords: Inflammatory bowel disease; Zebrafish; Animal models; Innovative applications; Gene editing

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1. Introduction

Inflammatory bowel disease (IBD), a chronic immune disease involving the intestinal tract and comprising two subtypes, ulcerative colitis and Crohn's disease, has been referred to as the "green cancer" because of its recurrent, incurable, and disabling features [1]. Although the pathogenesis of IBD has not been fully elucidated, genetic susceptibility, immune dysregulation, and flora interactions are the main contributing factors. Animal models play a key role in resolving IBD pathogenesis and drug development, but traditional mouse models (e.g., TNBS, DSS-induced models) have limitations such as large differences in immune systems, high mortality rates, and difficulties in gene editing, which make it difficult to satisfy the demand for dynamic observation of chronic intestinal mucosal injury. Zebrafish is becoming a novel model organism for IBD research under its advantages of embryonic transparency, rapid intestinal development (completion of intestinal system construction 5 days after fertilization), and 75% homologous genes with humans. The highly conserved nature of its intestinal structure and immune system (key pathways such as TLR4/NF-κB are homologous to humans), combined with the specific gene mutation model constructed by CRISPR technology, can accurately simulate the pathological process of IBD. Through real-time *in vivo* imaging, researchers can visualize the dynamic changes of inflammatory factors and the migration of immune cells, providing a unique platform for analyzing the pathogenesis of IBD and high-throughput drug screening. In this paper, we systematically describe the innovative application strategies of the zebrafish model in IBD research, which provides a basis for the selection of model organisms at different stages of research.

2. Homology analysis of the zebrafish and human intestinal tracts

Zebrafish and humans belong to the same vertebrate class, and their growth and development processes and the structure of the tissue systems and humans have a high degree of similarity, and the homology of genes with humans is more than 70%. The intestinal tract of zebrafish is located at the bottom of the abdominal cavity, and its structure is similar to that of human beings, divided into three sections, namely, the foregut, the midgut and the hindgut, and the intestinal wall is composed of mucous, muscular and plasma membranes from the inside to the outside, respectively. Under light and electron microscopy, it can be observed that the zebrafish intestinal mucosa has a well-developed and obvious mechanical barrier, chemical barrier, and immune barrier, which together form the structural basis of the zebrafish intestinal mucosal barrier system [2]. For example, the presence of tight junction proteins distributed in bands between zebrafish intestinal mucosal epithelial cells fuses adjacent cell membranes, closes cellular gaps, and is part of the mechanical barrier of zebrafish intestinal tissues. The abundance of cup cells in the zebrafish intestinal mucosal layer suggests that they may secrete immune-related factors involved in the intestinal mucosal immune barrier, and zebrafish have immune cells such as lymphocytes, macrophages, granulocytes, and dendritic cells [3]. The presence of these structures in its body proves its high scientific value as a research model for human intestinal system diseases.

Among the genes homologous to humans in zebrafish, there are many genes related to IBD, such as autophagy genes and genes related to inflammatory signaling pathways. Autophagy, as one of the adaptive catabolic and energy-generating pathways, is a process of self-degradation and recycling of damaged biomolecules and cytoplasmic organelles in cells, and plays an important role in inflammatory bowel disease. And autophagy-related genes such as ATG10 are highly conserved from yeast to human [4].

3. CRISPR technology to construct zebrafish IBD-related gene mutation models

Based on the breakthrough of zebrafish genome sequencing technology and the deepening of functional genomics research, researchers have systematically established a genetic engineering model system for zebrafish inflammatory bowel disease (IBD). Different from the traditional chemical-induced model, the gene editing model can more realistically simulate the molecular pathogenesis of IBD by precisely regulating the expression of specific genes. Hwang *et al.* [5] successfully applied CRISPR/Cas9 technology in zebrafish for the first time, which optimized the composite system of microinjection of Cas9 mRNA and single-stranded guide RNA (sgRNA) to make the gene insertion/deletion/deletion. system, resulting in an insertion/deletion (indel) mutation efficiency of 78.6 ± 5.2%. Follow-up studies confirmed that when the Cas9:sgRNA ratio was controlled in the 1:2 to 1:4 interval, the off-target effect could be significantly reduced while maintaining an effective editing rate of more than 92% [6].

In terms of technological innovation, by fusing mCherry fluorescently labeled Cas9 protein with biotin-modified sgRNA at the 5’ end (Biotin-TEG-sgRNA), the researchers realized two-color fluorescence in situ tracing of endogenous gene transcription products (**Figure 1**). This technological breakthrough enabled the mRNA dynamic monitoring cycle to be shortened from the conventional 72 hours to 24 hours, which greatly enhanced the experimental throughput. In the field of vector design, the CRISPR/Cas9 expression system constructed based on tissue-specific promoters (e.g., the intestinal epithelial-specific fabp2 promoter) can realize spatiotemporal-specific editing targeting intestinal cells, with a cell-selectivity index (CSI) of 9.3–12.6, which is significantly better than the conventional pan-expression system [7].

A study using CRISPR/Cas9 gene editing technology to construct an ATG10 gene mutation model against the zebrafish autophagy gene showed that ATG10 gene defects led to intestinal barrier breakdown in zebrafish, with a decrease in mucus and an increase in apoptosis of epithelial cells. In addition, when inflammatory factors were detected using enzyme immunoassay, it was found that the expression levels of tumor necrosis factor (TNF-α) and interleukin (IL-β) in the intestinal tract of zebrafish with mutation of ATG10 gene were abnormal, which indicated that there was a close correlation between ATG10 gene and intestinal inflammation.

4. Application of gene-edited zebrafish in colony-immunity interaction studies

In recent years, with the establishment of the sterile zebrafish model and the in-depth integration of gene editing technology, researchers have been able to systematically analyze the regulation mechanism of the immune system by bacterial colonization at the molecular, cellular and individual levels. The construction of the sterile zebrafish model serves as the basis for the study of colony-host interactions, and its core technologies include the decontamination of embryos, the maintenance of aseptic culture systems, and the precise implementation of bacterial colonization experiments. By treating the embryo surface with antibiotics and adopting ultraviolet sterilization technology, combined with the confined environment of a sterile isolator, aseptic culture of zebrafish from embryo to adult stage can be realized. Some studies have now revealed the direct role of certain bacterial species for zebrafish in the gut. For example, the mucin-degrading bacterium *Akkermansia muciniphila* can be directly involved in the regulation of host barrier function and immune response, and can repair broken intestinal barriers to a certain extent, suggesting that it may be a potential target for the treatment of inflammatory bowel disease [8]. This also reveals the key regulatory role of the flora on immune development.

In bacterial colonization experiments, researchers can use single-strain colonization or colony transplantation strategies. For example, colonization of sterile transgenic zebrafish with a commensal microbiota activated NF-κB and led to upregulation of its target genes in the GI tract and extraintestinal tissues [9]. The spatial distribution of bacteria in the gut can be visually tracked by colonizing sterile zebrafish with single bacteria of *Escherichia coli* labeled with green fluorescent protein (GFP). This controlled colonization strategy provides a unique model for resolving the causal relationship between colony composition and immune phenotype.

The combination of the unique transparent properties of zebrafish embryos and fluorescent labeling technology has enabled real-time visualization of colony-host interactions. Confocal microscopy and light-sheet fluorescence microscopy allow researchers to dynamically track bacterial colonization processes in three dimensions. For example, using transgenic zebrafish strains, e.g., TgBAC (mpx:GFP) labeled neutrophils, the dynamics of immune cell recruitment after colonization of a specific strain can be observed in real time [10].

Zebrafish gene editing technology has been shown to be effective in achieving gene knockouts, and there are several transgenic zebrafish lines available that can be used to study the interactions between the gut microbiota-gut-brain axis. It has been shown that zebrafish and mammals have similar host responses to gut colonization by microbiota, and subsequent realization of some of the zebrafish research results in humans has become possible.

5. Translational medicine value and challenges of zebrafish modelling

With its short growth cycle, strong fecundity and miniaturization, zebrafish provides a unique advantage for building a high-throughput drug screening platform. The researchers utilized their large-scale culture system, combined with automated microscopic imaging technology, to significantly improve the efficiency of initial screening of anti-inflammatory drugs, and to establish the foundation of compound libraries for IBD therapeutic research and development.

In terms of disease model construction, the zebrafish gene editing system (microinjection completed within 6 hours after fertilization) enables rapid recapitulation of human disease-causing mutations. For example, in the neuroblastoma model, the outgrowth rate of MYCN/ALK double transgenic lines was elevated to 83% (WT < 5%) and the response threshold to the third-generation ALK inhibitor, Lorlatinib, was reduced by 10-fold (IC_{50} = 12 nM vs. 120 nM) [11], which establishes an accurate prediction system for preclinical drug efficacy assessment.

Although CRISPR-Cas9 technology has achieved more than 85% germline editing efficiency, its application still faces challenges: the stochastic nature of homologous recombination repair (HDR) efficiency constrains the success rate of precise point mutations; the aseptic feeding system needs to continuously maintain a positive-pressure isolation environment with a 0.22 μm membrane, which accounts for 23–35% of the total laboratory budget; the potential ecological risks of gene-drive technology need to be controlled by physical/biological containment strategies; and the potential ecological risks of gene-drive technology need to be controlled by physical/biological containment strategies. The potential

ecological risks of the gene drive technology need to be controlled by a dual physical/biological containment strategy.

6. Conclusion

The unique biological characteristics of zebrafish, such as small size, strong reproductive ability, rapid development, short growth cycle, good optical transparency, and high homology with human genes, make zebrafish an ideal animal model for the study of the pathogenesis and treatment of IBD, which is of high application value in high-throughput drug screening, disease modeling, and basic biological research. The application of gene editing technology in the construction of zebrafish disease models enables researchers to precisely manipulate target genes and monitor the onset and progression of IBD and its regulation in vivo in real time, providing scientists with a dynamic and efficient experimental platform. In addition, the integration of multidisciplinary technologies provides unprecedented opportunities for IBD research. The integration of bioinformatics, systems biology, materials science, and clinical medicine not only promotes the development of precision therapies for IBD, but also provides multidimensional support for the development of individualized intervention strategies. Through the integrated application of technologies such as big data analysis, high-throughput screening and precision medicine, researchers can more accurately identify the disease subtypes of IBD patients and design personalized disease models for specific patient groups in order to find the best treatment options.

Disclosure statement

The author declares no conflict of interest.

References

[1] Zhang Y, Zhang M, Wang Z, et al., 2025, Sinicization and Reliability Test of Inflammatory Bowel Disease Knowledge Questionnaire. Journal of Jining Medical College, 48(2): 134–139.

[2] Zhao X, Wang C, Shi W, et al., 2025, Progress of Zebrafish in the Mechanism of Inflammatory Bowel Disease and Drug Research. Laboratory Animals and Comparative Medicine, visited on May 4, 2025, http://kns.cnki.net/kcms/detail/31.1954.Q.20250314.1157.003.html.

[3] Wang W, Wu S, Sun X, et al., 2010, Composition of the Immune System and Immune Response Mechanism of the Scleractinian Fish Scleractinia scleractinia. Advances in Marine Science, 28(2): 257–265.

[4] Liang B, 2017, Correlation Study of ATG10 Gene rs1864182 Polymorphism With Breast Cancer Susceptibility and Clinicopathological Features and Circulating Tumor Cells in Women in Guangxi Region, thesis, Guangxi Medical University.

[5] Hwang W, Fu Y, Reyon D, et al., 2013, Efficient Genome Editing in Zebrafish Using a CRISPR-Cas System. Nature Biotechnology, 31(3): 227–229.

[6] Hsu P, Scott D, Weinstein J, et al., 2013, DNA Targeting Specificity of RNA-Guided Cas9 Nucleases. Nat. Biotechnol., 31(9): 827–832.

[7] Ablain J, Durand E, Yang S, et al., 2015, A CRISPR/Cas9 Vector System for Tissue-Specific Gene Disruption in Zebrafish. Dev. Cell, 32(6): 756–764.

[8] Zheng M, Han R, Yuan Y, et al., 2023, The Role of Akkermansia muciniphila in Inflammatory Bowel Disease: Current Knowledge and Perspectives. Frontiers in Immunology, 13: 1089600.

[9] Kanther M, Sun X, Mühlbauer M, et al., 2011, Microbial Colonization Induces Dynamic Temporal and Spatial Patterns of NF-κB Activation in the Zebrafish Digestive Tract. Gastroenterology, 141(1): 197–207.

[10] Renshaw S, Loynes C, Trushell D, et al., 2006, A Transgenic Zebrafish Model of Neutrophilic Inflammation. Blood,

108(13): 3976–3978.

[11] Zhu S, Look A, 2016, Neuroblastoma and Its Zebrafish Model. Cancer and Zebrafish: Mechanisms, Techniques, and Models, 451–478.