

Leveraging Zebrafish and Medaka Differences for Enhanced Biological Research: A Complementary Perspective

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Abstract

The comparative study of complementary species has become a promising strategy for advancing our understanding of disease models. Zebrafish and medaka stand as two of the leading fish models in biomedical research, with their increased prominence over the past three decades. Although zebrafish is more widely studied than medaka, both species offer complementary advantages. Despite their similarities, there are subtle anatomical and transcriptomic differences between the two. The completion of genome sequencing for both medaka and zebrafish has shown that their genetic compositions closely resemble that of higher animals. For example, medaka shares approximately 20,000 genes with humans, exhibiting an 80% orthologous correlation, while zebrafish contains 26,000 genes, 71.4% of which are human orthologs. Both zebrafish and medaka are valuable for investigating human disorders due to their cost-effectiveness, small size, short lifespan, and high fecundity. Their transparent embryos also provide enhanced visualization during embryogenesis. This review aims to explore the anatomical and transcriptomic differences between these two species and highlight the successful application of zebrafish and medaka in complementary research areas, such as genetic manipulation, due to their evolutionary divergence.

Keywords: Zebrafish, Medaka, Biomedical Research, Models, Anatomy, Transcriptomes, Genetic Manipulation

Introduction

Over the past three decades, the mouse has established itself as the preferred laboratory animal for modeling human diseases in preclinical research [1]. However, despite its strengths, the murine model presents certain experimental limitations, particularly in large-scale

studies and complex chemical or genetic screening [2]. As alternative models, zebrafish (*Danio rerio*) and medaka (*Oryzias* sp.) provide distinct advantages when it comes to managing large sample sizes within limited timeframes [3]. These fish can be raised in small tanks, with a pair of fish capable of producing hundreds of embryos weekly. Furthermore, their embryos develop independently from the parent fish, allowing clear visualization of internal tissues and enabling various experimental manipulations [2].

One of the most notable outcomes of the zebrafish genome-sequencing project is the discovery of a 70% similarity between human protein-coding genes and zebrafish genes, including those involved in human diseases. This suggests that zebrafish are an excellent

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model for studying human physiological and pathological processes [1, 4-6]. The development of technologies for gene manipulation, live imaging, high-throughput sequencing, and genome editing further solidifies zebrafish as a leading model for biomedical research in the 21st century [2].

Despite these advancements, medaka, though a small freshwater fish frequently used in aquatic toxicology studies, has not been widely adopted for human behavioral research or for modeling human diseases, despite its significant genetic and physiological similarities with zebrafish [7]. While medaka has proven to be an important model for developmental biology and genomics [8, 9], its use in modeling human diseases

remains underexplored. Complementing zebrafish with medaka offers promising potential for the development of more effective human disease models [10-20].

This review aims to highlight the achievements made thus far in utilizing zebrafish and medaka in biological research. It focuses on examining the anatomical and transcriptomic differences between the two species and explores how these differences can be leveraged in a complementary approach to research, particularly in genetic manipulation. Additionally, we discuss how either zebrafish or medaka may be preferred for specific studies, based on their respective strengths and limitations (**Figure 1**).

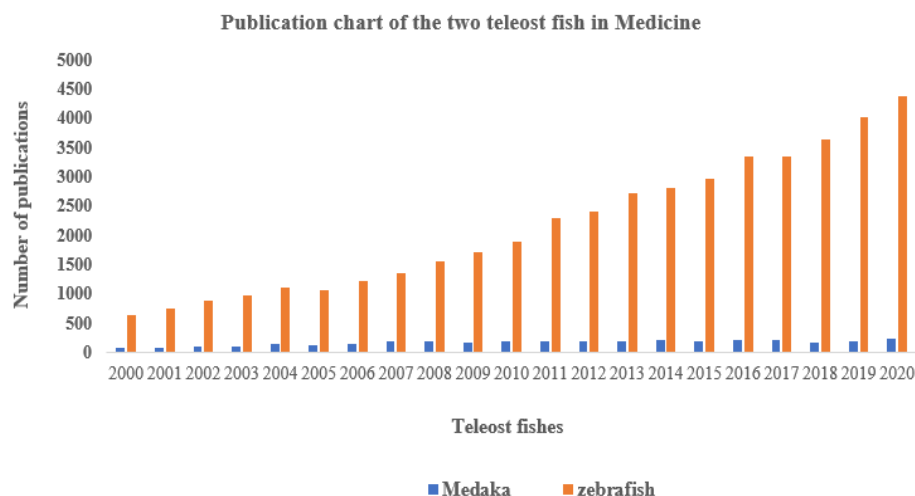


Figure 1. The number of publications related to medaka and zebrafish in medicine between the year 2000 and year 2020 (searched in PubMed only); the number of articles in which zebrafish was mentioned totaled 45,273 while that of medaka was 3,658 during the period; within twenty years, publications that featured zebrafish increased from 657 to 4,396 (669%) but for medaka, a paltry increase from 80 to 244 was recorded (305%).

Results and Discussion

Distinguishing Features Between Zebrafish and Medaka

Although zebrafish and medaka share many anatomical similarities, minor differences exist due to the significant phylogenetic distance between the two species. As research continues to explore both fish models, understanding the distinguishing features between them becomes increasingly relevant for the complementary use of these species, an area that has not yet been fully explored. Some of these differences include variations in the skeleton, dentition, pigment cell patterns, thymus,

glomerulus, eyes, heart asymmetry, and parapineal organs.

One of the key differences between the vertebral bones of zebrafish and medaka is the presence of an osteocytic network in zebrafish (**Figure 2**), a feature completely absent in medaka. Medaka, being a more evolutionarily advanced teleost, has anosteocytic bones [21]. This significant difference may suggest that the bony structures, particularly the vertebrae, which are heavily stressed, could differ in both structural and mechanical aspects. Such distinctions are likely tied to the contrasting processes of osteogenesis between anosteocytic and osteocytic bone formation [22].

In osteocytic bone formation, osteoblasts become trapped in the osteoid they secrete, undergoing several morphological and physiological changes that ultimately form a complex network of living osteocytes within the bone matrix. On the other hand, the process of forming anosteocytic bone involves osteoblasts remaining on the outer surface of the bone, without becoming embedded in the osteoid. This difference in bone formation

mechanisms indicates distinct physiological functions for osteoblasts in both types of skeletons.

Further analysis of mineral density distribution along the vertebrae of both species revealed differences, with medaka exhibiting lower mineral density at the edges of the vertebral cones compared to zebrafish. A previous study also showed that medaka vertebrae generally have higher mineral density along their entire length compared to zebrafish vertebrae [23, 24].



Figure 2. Image of zebrafish skeleton [25]

Dentition

A unique feature of teleosts is their ability to replace teeth throughout their lifespan, from the embryonic stage to adulthood. This characteristic, absent in humans and most mammals, presents a significant research opportunity. However, due to their evolutionary divergence, medaka and zebrafish exhibit notable differences in their dentition. Medaka has a higher and more variable number of teeth compared to zebrafish, which maintain a smaller and more consistent number. While zebrafish only have pharyngeal teeth, medaka possesses both oral and pharyngeal teeth. In terms of tooth shape and type, both species display mild heterodonty, showing some variation in tooth structure [25, 26]. The development of replacement teeth in both species begins with epithelial budding and involves reciprocal interactions with mesenchyme. In medaka, replacement teeth develop from the oral epithelial buds, while in zebrafish, epithelial budding starts in the outer dental epithelium during the embryonic stage and continues in a distinct successional dental lamina in adults [27].

Thymus

Like mammals, adult teleosts have a thymus with distinct medullary and cortical regions, which may consist of one or more lobules. Both medaka and zebrafish possess a single thymic lobule on each side of their body. Medaka shows early spatial organization of thymocytes, with

visible thymic structures already present at the larval stage. In contrast, zebrafish develop their thymic compartmentalization later, around 2 to 3 weeks post-fertilization (wpf) [28]. The transparency of zebrafish and medaka, crucial for tracking molecules and lymphoid progenitors, is aided by the proximity of the thymus to the skin. Zebrafish larvae typically contain between 20 and 50 thymocytes, while medaka larvae have more than 1,000 thymocytes [29]. Both species experience thymus growth to its peak output during adolescence, followed by regression due to aging. However, medaka's thymus structure remains intact throughout life, unlike zebrafish, whose thymus starts to regress as early as 15 wpf. This difference makes a comparative study of the thymus in zebrafish and medaka valuable for understanding the molecular mechanisms behind age-related thymic involution [30].

Eye

The eyes of medaka and zebrafish also show some differences. While medaka's lens remains largely unchanged throughout its life, zebrafish lenses exhibit notable increases in crystallin abundance and aggregation as they age [13]. Additionally, the optic primordium forms earlier in zebrafish, completing 12 hours post-fertilization (hpf), compared to 26 hpf in medaka. Similarly, zebrafish undergo retinotectal projection differentiation within 48 hpf, whereas in medaka, this phase takes 4 to 6 days post-fertilization (dpf). Zebrafish

also exhibit concurrent expression of rod opsin and Zpr-1 (important photoreceptor markers) in photoreceptor cell layers, which is distinct from medaka, where the expression occurs approximately 24 hours apart [31].

Pattern of Pigment Cells

Zebrafish are known for their striking stripes, formed by the autonomous patterning of skin pigment cells, with mutations in seven loci contributing to this pattern. In contrast, medaka adults lack these distinctive stripes, instead displaying a simpler, more evenly distributed pattern of pigment cells. Understanding the molecular mechanisms behind these differences in pigmentation could provide valuable insights into the complex process of pigment formation in vertebrates, a phenomenon that remains poorly understood [32].

Heart Asymmetry

In vertebrates, the left-side dominance of heart asymmetry is a well-established trait, though some species can show occasional reversals of this pattern. The frequency of such reversals has decreased throughout vertebrate evolution: about 5% in fish, 1-2% in amphibians and birds, and a mere 0.1% in mammals, indicating a trend toward stabilization of heart laterality. Medaka, however, stands out by showing no heart laterality (0%), while zebrafish maintain a slight 5% laterality. This difference is thought to be influenced by the inbreeding nature of medaka strains, which results in more stable symmetry and makes medaka less susceptible to genetic and environmental variations compared to zebrafish and other teleosts. The process of establishing heart laterality involves the generation of a leftward flow of extracellular fluid in the Kupffer's vesicle (KV) in teleosts and the ventral node in mammals. Recent research suggests that the KV of medaka shares more similarities with the mammalian node than with zebrafish, particularly in the structure of ciliated cells and the robustness of nodal flow [33].

Parapineal Organ

The left-sided position of the parapineal organ is a common feature in the brain asymmetry of teleosts. However, there are considerable differences between medaka and zebrafish regarding the size of the parapineal organ concerning the pineal and their efferent connectivity patterns. In zebrafish, the parapineal organ is relatively small, about 10% of the pineal size, with its efferent pathways spread evenly in the left habenula. On the other hand, medaka's parapineal organ is much larger, around 60% of the size of the pineal, and its efferent connections form a prominent and well-defined antero-dorsomedial neuropil within the left habenula [33].

Glomerulus

Medaka's pronephros glomerulus exhibits several structural differences from zebrafish. In medaka, the glomerular primordium takes on a C-shape, with a balloon-like capillary that later splits into smaller capillaries. Zebrafish, in contrast, form the glomerulus by fusing two pronephric glomeruli at the midline. Medaka's glomeruli, however, do not fuse due to the presence of interglomerular mesangium. The mesangial cells in medaka also contain cytoplasmic granules, which could potentially include renin protein [34]. Studying the development of the glomerulus in medaka alongside zebrafish provides valuable insights, particularly in podocyte differentiation. Medaka's podocyte development is more similar to mammals than zebrafish, with the C-shaped epithelial layer resembling the primitive podocytes of mammals, suggesting potential parallels between podocyte development in medaka and mammals. This offers a foundation for future studies on medaka mutants with glomerular defects, which could improve our understanding of glomerular function and human glomerular diseases [35]. Distinctive features in Zebrafish and Medaka are presented in **Table 1**.

Table 1. Distinctive features in Zebrafish and Medaka

Features	Zebrafish	Medaka	Reference
Skeleton	Osteocytic skeleton type	Anosteocytic skeleton type	[22]
Dentition	Pharyngeal teeth, no oral teeth, variable number, large number	Pharyngeal teeth, oral teeth present, constant number, few numbers	[25-27]
Thymus	a. One thymic lobe on each side of the body b. Compartmentalization at embryo stage c. 20-50 thymocytes d. Regression starts at 15 weeks	a. One thymic lobe on each side of the body b. Compartmentalization at the juvenile stage c. More than 1000 thymocytes d. No regression observed until adulthood	[27-30]

Glomerulus	Pronephric glomeruli fuse	Pronephric glomeruli do not fuse	[34, 35]
Eye	a. Lens shows crystallin aggregation in older fish	a. No crystallin aggregation in the lens of older medaka	[13]
	b. Apparent retinal changes with age	b. No retinal changes with age	
Pigment cells	Elegant stripes and ordered body pigmentation	No stripes or ordered body pigmentation	[32]
Heart	5% heart laterality	0% heart laterality	[33]
asymmetry			
parapineal organ	About 10% of the size of the pineal organ	About 60% of the size of the pineal organ	[33]

Gene Expression Pattern Comparison Between Zebrafish and Medaka

To expand on previous research, RNA-seq, and genomics tracks for key histone modifications (H3k4me3 and H3k27ac) were generated from 1 day 20 hours old medaka embryos, which correspond anatomically with 24-hour post-fertilization (hpf) zebrafish embryos during the phylotypic period. A comparative analysis of their transcriptomes shows that tissue-specific gene expressions correlate with shared anatomical structures and developmental timing differences (heterochrony) between the two species [36].

In addition, a comparative analysis of active regulatory regions (PARRs) demonstrates a 64% dissimilarity in sequence between zebrafish and medaka (**Figure 3**). Among the conserved regions, only 14% overlap as shared putative active regulatory regions (SPARRs), where both species are active during the phylotypic stage. The genes in these regions exhibit a more complex regulatory network, rich in transcription factors and signaling molecules responsible for tissue and organ formation [37].

Despite these differences, significant conservation is seen in the timing of developmental events, particularly during mid-embryogenesis. Both species show similar development of the lens vesicle, optic cup, brain structures, and the initiation of heartbeats. However, some heterochrony is evident. For example, zebrafish complete somitogenesis earlier than medaka. While zebrafish show trunk and tail movement at 24 hpf, medaka remains motionless. Moreover, zebrafish develop their fin buds earlier, by 22 hpf, compared to medaka's delayed formation at 2 days 10 hours. Additionally, medaka's pancreatic and hepatic buds develop faster than zebrafish's [37].

A comparison of 9,178 orthologous genes (excluding low RNA expression ones) revealed a high correlation between zebrafish and medaka transcriptomes (Pearson

correlation of 0.71), consistent with other studies on vertebrate transcriptomes during the pharyngula stage. A genetic comparison of the two species, using the ZFIN expression database, focused on tissue-specific genes [38]. In the muscles, zebrafish had 30% more genes upregulated (by over 4 times) compared to medaka, reflecting anatomical differences. However, only 11.3% of eye-specific genes showed comparable expression levels in both species [37].

Further analysis of nervous system-specific genes revealed that zebrafish exhibited significantly higher RNA expression, suggesting earlier development of the nervous system. This is supported by zebrafish embryos' ability to actively twitch their tail muscles at 24 hpf, indicating neuromuscular junction formation. No significant differences were found in other tissue-specific genes, except for a small but notable difference in gene expression in the epidermis [36].

The faster development of the nervous system and muscles in zebrafish compared to medaka may be attributed to ecological factors. Zebrafish produce large clutches of eggs (up to 300), with rapid development that leads to free-swimming larvae within about 2 days. In contrast, medaka produces fewer eggs (10-30), and their embryos develop more slowly. This suggests that, while both species share anatomical similarities at the phylotypic stage, their distinct ecological strategies and developmental timings are key factors influencing tissue-specific development [39].

To further explore the transcriptomic differences between zebrafish and medaka, a research team utilized the edgeR package to analyze differentially expressed genes [40]. After applying a false discovery rate (FDR) threshold of 5% and selecting genes with a fold change greater than fourfold, a total of 1,085 genes (representing 15.2% of the orthologous gene list) were found to have high expression in zebrafish, while 600 genes (8.4% of the orthologous genes) were upregulated in medaka [36].

Interestingly, when the DAVID gene ontology (GO) analysis was applied to these differentially expressed genes, zebrafish showed significant upregulation in genes related to neurological processes and muscle tissue development. Furthermore, differences between the species were observed in biological processes not easily detected through morphological analysis, such as cardiac muscle tissue development, protein localization, and signaling cascades. In contrast, upregulated genes in medaka were notably enriched in pathways associated with oxidation-reduction and cofactor metabolism processes [36].

The bioinformatics tool PANTHER was also used to compare the enrichment of GO terms in zebrafish and medaka. It revealed that zebrafish had significantly enriched GO terms related to muscle development and synaptic transmission, such as synaptic transmission, neurological system processes, mesoderm development, nerve impulse transmission, and muscle organ development. In contrast, medaka showed fewer enriched GO terms, primarily related to metabolic processes like lipid metabolism, carbohydrate metabolism, and cellular amino acid metabolism. These findings align with the DAVID analysis [37].

Additionally, in situ hybridization was employed to investigate the gene expression patterns in the brains of zebrafish and medaka, focusing on 14 specific genes. It was found that the upregulation of certain genes in the telencephalic region of medaka embryos varied from zebrafish at corresponding stages (48-54 hours post-fertilization (hpf) for medaka and 24-28 hpf for

zebrafish). For instance, the gene *pax6* was expressed in the dorsal telencephalon of medaka, while in zebrafish, *pax6* and another gene, *pax6.1*, were expressed in the caudal telencephalon at 24 hpf [41]. Similarly, differences in the expression of *otx1* and *emx2* were observed between the species at 24 hpf. However, the expression patterns of *bf1* and *dlx2* in the telencephalon showed minimal differences. Outside the telencephalon, gene expression patterns in other regions of the brain were similar between zebrafish and medaka [42].

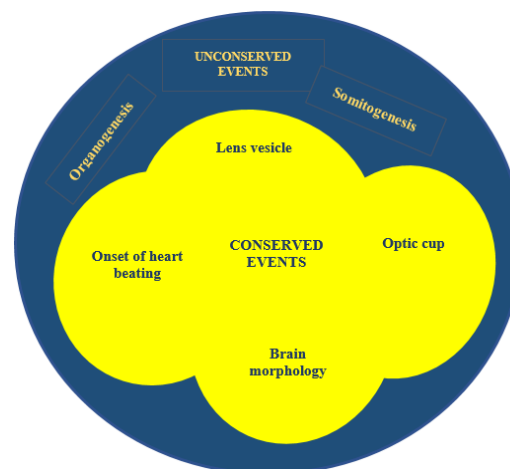


Figure 3. The conserved and unconserved events between zebrafish and medaka during embryogenesis

A summary of comparative transcriptomic and epigenetic data between Zebrafish and Medaka is presented in **Table 2**.

Table 2. Summary of comparative transcriptomic and epigenetic data between Zebrafish and Medaka

Events/analytical packages	Zebrafish	Medaka	References
RNA-seq and genomics tracks			[37]
a. Somitogenesis	The trunk and tail show instantaneous vibrations	Remain immobile at 24hpf	
b. Organogenesis	Formation of hepatic and pancreatic bud is slower	Formation of the bud is faster	
Analysis using edgeR	1,085 genes identified with high expression	600 genes upregulated	[37, 40]
Analysis using DAVID	Genes related to the neurological system and muscle development upregulated	Genes linked to oxidation-reduction and cofactor metabolic processes overrepresented	[37]
Analysis using PANTHER	Enrichment in GO terms related to muscle development and synaptic transmission	Enrichment in GO terms related to lipid metabolism, carbohydrate metabolism, and cellular metabolism	[37]

In situ hybridization (14 gene probes in the brain)	Pax6 and Pax6.1 expressed in the caudal region of the telencephalon	Pax6 expressed in the dorsal region of the telencephalon	[38]
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The combination of zebrafish and medaka has proven highly successful in various research fields. For instance, zebrafish's biological features, which Medaka shares, have greatly facilitated large-scale mutagenesis studies. These features include fecundity, short generation times, rapid development, and ease of husbandry [32]. Both species have transparent, externally fertilized embryos that develop quickly, making them ideal for cellular-level developmental studies [43]. While there are minor differences between zebrafish and medaka, especially regarding the timing of organ development during embryogenesis, they remain complementary in many research contexts.

Medaka, in particular, offers several unique qualities that make it an invaluable complement to zebrafish. One key advantage is its usefulness in developing low-temperature models to study temperature-sensitive alleles [32]. Medaka is also well-suited for studying inbred strains, which have minimal phenotypic variation and allow for advanced research such as cell transplantation in adults. For example, it has been instrumental in validating carcinoma cells. Moreover, medaka was one of the first fish species in which successful long-term maintenance of mutant strains was achieved, thanks to the reliable storage of frozen sperm [44].

While zebrafish remain an indispensable model for developmental biology, recent advances in large-scale genomics have revealed that zebrafish do not exhibit the same level of synteny as medaka [45]. Nevertheless, the importance of zebrafish in scientific research cannot be overstated. Incorporating complementary models like medaka can help validate zebrafish-based findings and provide additional insights, especially in the context of human diseases [46].

The comparative approach of studying evolutionary relationships between biomedical models benefits the scientific community significantly. If two or more models yield similar results in side-by-side analyses, the findings are strengthened. On the other hand, discrepancies between models reveal alternative mechanisms, furthering our understanding. Hence, using multiple models to study identical variables enhances the research process. This comparative strategy offers an incredibly powerful experimental tool to address

complex issues like the etiology and progression of human diseases [46].

One area where the two species complement each other is in genetic manipulation. The development of concurrent technologies in medaka and zebrafish highlights the reciprocal exchange of methods and tools between these two systems, advancing research. Medaka was the first model to demonstrate stable transgenesis [47]. Later, researchers identified the Tol2 transposon system in medaka, which was subsequently adapted for transgenesis in zebrafish [48]. This breakthrough revolutionized genetic manipulation in model organisms. The use of Tol2 transposase to insert BAC constructs has been widely adopted in zebrafish to create reporter lines that effectively mirror endogenous gene expression patterns. Although medaka was the original model for Tol2 transgenesis, zebrafish exhibit greater efficiency with this system. Additionally, transgenesis in medaka has been facilitated by using I-SceI meganuclease for insertional transgenesis [49].

Emerging evidence suggests that medaka could soon become the preferred model for studying diabetic nephropathy, a common complication of diabetes in humans. The Animal Model of Diabetic Complication Consortium (AMDCC) has indicated that no rodent model is entirely suitable for mimicking diabetic nephropathy. However, medaka has shown promise as a potential model for this condition. In studies where both medaka and zebrafish were given a high-fat diet, only medaka developed symptoms of diabetic nephropathy, including enlarged glomeruli, elevated blood glucose levels, and glomerular capillary dilation [10]. Additionally, medaka might be better suited than zebrafish for investigating other diseases, including chronic mycobacteriosis, xenobiotic-induced hepatic fibrosis, hypohidrotic ectodermal dysplasia, osteoporosis, alcohol-related conditions, and human neurotoxicology [10, 50].

Conclusion

In conclusion, while there are some minor differences between zebrafish and medaka, the similarities between these species are significant. The comparison between these two closely related species is essential for advancing research, as it highlights medaka as a valuable

alternative to zebrafish. Despite zebrafish being more widely used in research, understanding how and when to use medaka can enrich the research community's knowledge base. Both species are relatively easy to house and maintain at a similar cost, which makes them practical for use in various studies. This work guides how best to utilize both species for research purposes, considering their unique advantages and the possibility of combining them for comparative studies. By analyzing the differences and similarities between zebrafish and medaka, researchers can deepen their understanding of human diseases and improve the development of treatment strategies.

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