EXPERIMENTAL MODEL

New insights into bile salt–Fxr signaling in zebrafish

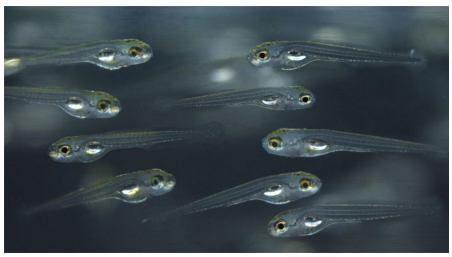
Wen, J. et al. *Sci. Adv.* **7**, eabg1371 (2021)

A new study shows that key components of bile salt–FXR signaling, including microbiota–bile salt–Fxr relationships, are conserved in zebrafish. The findings, published in *Science Advances*, establish zebrafish as a new nonmammalian model to study bile salt–FXR signaling.

Bile salts are produced in the liver from cholesterol and secreted in the intestine, where they facilitate digestion and absorption of dietary lipids. Bile salts are also important signaling molecules that regulate bile salt, lipid, and glucose metabolism by activating G-protein coupled receptors and nuclear receptors, including farnesoid X receptor (FXR/NR1H4). Although bile salts are present in several vertebrate species and FXR is conserved from teleost fish to human, mice are the most commonly used animal model for studying bile salt–FXR signaling. Mouse models have been instrumental in elucidating FXR functions in homeostasis and bile-acid related diseases, but species differences in bile acid metabolism have been documented between mice and humans.

"Additional vertebrate models are needed to provide complementary perspectives into the mechanistic relationships between microbiota, bile salts, and FXR signaling across vertebrates and to potentially reveal previously unidentified functions of FXR," write Jia Wen and colleagues from Duke University School of Medicine and University of Illinois at Urbana Champaign in their report.

Previous work has shown that zebrafish express several genes involved in bile salt homeostasis including genes encoding bile salt synthesis enzymes, bile salt transporters and FXR; but few studies have examined FXR signaling in zebrafish. Here, Wen et al. combined the use of a *fxr* mutant line (*fxr*$^{-/-}$ zebrafish harbouring a deletion in the Fxr DNA binding domain), a new reporter line Tg(-1.7*fabp6:GFP*) and qRT-PCR to decipher Fxr signaling in 7-day post-fertilization (dpf) zebrafish larvae.



Zebrafish, *Danio rerio*. Credit: Pally / Alamy Stock Photo

The results of their analysis revealed that the expression of several predicted Fxr target genes involved in bile salt homeostasis (including *fabp6* and *cyp7a1*) was reduced in *fxr*$^{-/-}$ zebrafish, as seen in *Fxr* knockout mice. These findings indicate that Fxr regulates bile salt metabolism genes in zebrafish larvae and that the signaling pathways downstream of Fxr are conserved between zebrafish and mammals.

Previous studies have already established that the major bile salt in zebrafish is 5α-cyprinol-sulfate (5αCS), a C27 bile alcohol sulfate commonly found in fish and amphibians, that differs markedly from the C24 bile acids, the common mammalian bile salts. Using liquid chromatography-mass spectrometry (LC-MS) of bile salts extracted from adult wild-type zebrafish gallbladders, Wen and colleagues confirmed that 5αCS is preponderant in zebrafish and identified several other bile salt species, including taurocholic acid (TCA), a C24 bile acid found in mammals. Despite the compositional differences in bile salt between zebrafish and mammals, Wen et al. showed that bile salts still activate Fxr signaling in zebrafish.

In mammals, intestinal microbiota shapes bile salt composition and activity by enzymatic modifications. To determine if microbial modification of bile salts is conserved in fish, Wen et al. examined bile salt composition in the intestinal content of adult zebrafish and of a closely related species, the Asian grass carp. They also performed a series of in vitro assays, in which microbiota isolated from zebrafish gut were incubated with 5αCS or TCA bile salts. Altogether, their results show that fish microbiota can modify 5αCS and TCA, which confirms that microbial modification of bile salts is a conserved feature. "To our knowledge, this is the first evidence demonstrating the ability of microbes to metabolize bile alcohols in vertebrates," write the investigators. Their findings also suggest that microbial modifications affect fxr activity in zebrafish, as seen in mammals.

Finally, the team performed single-cell RNA sequencing on intestinal epithelial cells (IEC) sorted from 6-dpf *fxr*$^{+/+}$ or *fxr*$^{-/-}$ zebrafish larvae to investigate Fxr signaling in IEC. Analysis of the sequencing dataset identified 27 distinct expression clusters corresponding to different cell types (including absorptive enterocytes, goblet cells, enteroendocrine cells, secretory precursors, ionocytes and foregut epithelial cells). Loss of Fxr induced substantial transcriptional changes in nearly one-third of the clusters, highlighting the broad impact of Fxr on IEC gene expression. Notably, the results indicate that Fxr represses genes involved in lysosome-rich-enterocytes (LRE) functions in the ileum and promotes genes involved in enterocyte differentiation in the anterior intestine.

The zebrafish dataset also revealed substantial differences with an existing dataset of mouse genes differentially regulated in response to Fxr agonist. "This raises the question of functional diversification of Fxr signaling during the course of evolution and further highlights the critical need of using multiple animal models to decode bile salt–Fxr–microbiome interactions," conclude the investigators in the discussion.

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