HHS Public Access

Author manuscript

*Gastroenterology*. Author manuscript; available in PMC 2016 February 22.

Published in final edited form as:

*Gastroenterology*. 2015 November ; 149(6): 1361–1377. doi:10.1053/j.gastro.2015.08.034.

# Zebrafish: An Important Tool for Liver Disease Research

**Wolfram Goessling**[1,2,3,4] and **Kirsten C. Sadler**[5,6,7]

[1]Divisions of Genetics and Gastroenterology, Brigham and Women's Hospital, Boston, Massachusetts

[2]Gastrointestinal Cancer Center, Dana-Farber Cancer Institute, Boston, Massachusetts

[3]Harvard Stem Cell Institute, Harvard Medical School, Boston, Massachusetts

[4]Broad Institute of MIT and Harvard, Harvard Medical School, Boston, Massachusetts

[5]Department of Medicine, Division of Liver Diseases, Icahn School of Medicine at Mount Sinai, New York, New York

[6]Department of Developmental and Regenerative Biology, Icahn School of Medicine at Mount Sinai, New York, New York

[7]Graduate School of Biomedical Sciences, Icahn School of Medicine at Mount Sinai, New York, New York

## Abstract

As the incidence of hepatobiliary diseases increases, we must improve our understanding of the molecular, cellular, and physiological factors that contribute to the pathogenesis of liver disease. Animal models help us identify disease mechanisms that might be targeted therapeutically. Zebrafish (*Danio rerio*) have traditionally been used to study embryonic development but are also important to the study of liver disease. Zebrafish embryos develop rapidly; all of their digestive organs are mature in larvae by 5 days of age. At this stage, they can develop hepatobiliary diseases caused by developmental defects or toxin- or ethanol-induced injury and manifest premalignant changes within weeks. Zebrafish are similar to humans in hepatic cellular composition, function, signaling, and response to injury as well as the cellular processes that mediate liver diseases. Genes are highly conserved between humans and zebrafish, making them a useful system to study the basic mechanisms of liver disease. We can perform genetic screens to identify novel genes involved in specific disease processes and chemical screens to identify pathways and compounds that act on specific processes. We review how studies of zebrafish have advanced our understanding of inherited and acquired liver diseases as well as liver cancer and regeneration.

Address requests for reprints to: Kirsten C. Sadler, PhD, Biology Program, New York University, Abu Dhabi Saadiyat Campus, P.O. Box 129188, Abu, Dhabi, United Arab Emirates Office phone: +971 2 628 4569. kirsten.edepli@nyu.edu.
Kirsten C. Sadler's current affiliation is: Biology Program, New York University, Saadiyat Campus, P.O. Box 129188 Abu Dhabi, United Arab Emirates kirsten.edepli@nyu.edu

**Conflicts of interest**

The authors disclose no conflicts.

**Keywords**

Development; Technology; Liver Cancer; Toxicology; Regeneration

The function and organization of the vertebrate digestive tract is well conserved. Like humans, nonmammalian species are susceptible to liver disease caused by developmental defects, inborn errors of metabolism, infections, and environmental and dietary insults. Studies in animals such as fish can therefore elucidate common mechanisms of liver disease. Liver disease is a leading cause of morbidity and mortality worldwide,[1] so methods to effectively diagnose, treat, and prevent these diseases are urgently needed. Clinical studies have clearly defined the consequences of liver damage, fibrosis, cirrhosis, cancer, and hepatic failure and have shown both the remarkable capacity and limits of liver regeneration. Comparative studies across species can distill fundamental genetic, cellular, and physiological properties of organ function and pathology, and model organisms serve as a platform for preclinical drug testing.

Zebrafish (*Danio rerio*) have been used in research for more than 80 years (Figure 1). In the past 3 decades, discoveries on the fundamental properties of development, regeneration, cancer, and other diseases have established zebrafish as a prominent model organism in biomedical research.[2–4] We review the experimental attributes of this system and highlight exciting insights into heritable hepatobiliary disorders, fatty liver, toxin-mediated injury, liver cancer, and regeneration that have been made using zebrafish.

## History

The description of jaundice in the *Hippocratic Corpus*[5] and liver regeneration in the myth of Prometheus[6] show that hepatobiliary disease and the remarkable regenerative capacity of the liver have been appreciated for millennia (Figure 1). The metabolic contribution to fatty liver disease (FLD) was capitalized on by ancient Egyptians, who force-fed geese to produce fatty liver[7] (and produce a delicacy now known as foie gras), whereas alcoholic liver disease (ALD) was recognized in Byzantium. In the 20th century, biomedical research has increased our understanding of bile acid and bilirubin metabolism, cholesterol and lipid metabolism, genetic factors that contribute to liver disease, viral hepatitis, and drug-induced liver injury (DILI). However, mechanisms of liver cancer, fibrosis, and metabolic liver disease are not well understood and are among the most important clinical challenges in the field.

Zebrafish have a rich but relatively short history in biomedical research (Figure 1). Discovered in the Ganges River in the late 19th century,[8] zebrafish were initially used by embryologists because of their ability to produce large clutches of transparent embryos that could be reared in a laboratory setting. After the first report on zebrafish development was published in the 1930s,[9] researchers used zebrafish primarily to study the effects of toxins and alcohol on embryogenesis[10] and hepatocarcinogenesis,[11] because it is straightforward to expose the animals to toxins and chemicals by simply adding compounds to their culture water. This trait still provides a significant advantage as zebrafish are now widely used in chemical screens.[12] The popularity of zebrafish increased greatly in the 1980s, when

researchers began using molecular genetic technologies.[13] Today, thousands of investigators worldwide use zebrafish to study nearly every aspect of biology.

The rapid and external development of zebrafish embryos (Figure 2*A*) provides an advantage for studies of organ growth and differentiation. The liver and other internal organs can be visualized in zebrafish larvae using a simple microscope (Figure 2*A*). Researchers have taken advantage of the similarities between zebrafish and mammals in hepatocellular composition (Figure 2*B* and *C*) and function as well as genetics[14] to provide insight into the pathogenesis of many major liver diseases.[3] Although the small size of zebrafish can limit approaches that require serum samples, large tissue volumes, or biopsy specimens, they provide the advantage of growing 10 larvae in one well of a 96-well plate, facilitating genetic[13,15] and chemical screens[12] on a scale not feasible with other vertebrates.

## Comparative Liver Structure, Function, and Pathology

Compared with mammals, zebrafish have a unique hepatic anatomy and cellular architecture despite the high conservation of cell types within the liver. The zebrafish liver is organized in 3 contiguous lobes (2 lateral and 1 ventral) and these lobes lack the pedicle that separates the distinct lobes in the mammalian liver. Instead of a portal architecture, hepatocytes in fish livers are arranged in tubules, with bile ductules coursing between 2 rows of hepatocytes[16–19] (Figure 2*B* and *C*). The apical membranes face the inside of the tubule, and sinusoids track the basal side of hepatocytes (Figure 2*B*).[20–22] Although the cellular basis for liver diseases appears to be similar between mammals and fish, differences in the microenvironment may contribute to species-specific responses to injury.

With the exception of hepatic immune cells (ie, Kupffer cells), all other cell types of the mammalian liver have been identified in zebrafish (Figure 2*B* and *C*). Moreover, cells in zebrafish livers appear similar to mammals (see Figure 3*B* [*inset*]) and perform many of the same functions as their mammalian counterparts, including bile secretion,[23] glycogen and lipid storage,[24,25] insulin responsiveness, xenobiotic and ammonia metabolism, and secretion of serum proteins such as complement and clotting factors, transferrin, and albumin-like protein.[26] Importantly, all hepatic functions evaluated thus far are found in larvae as early as 5 days postfertilization (dpf). Fish have 2 types of biliary cells: small preductal biliary epithelial cells, which create intracellular lumens for bile transport from the hepatocytes, and larger columnar cholangiocytes, which form the full intrahepatic biliary system.[16–19] Hepatic stellate cells were recently described in zebrafish as myofibroblasts that become activated and secrete extracellular matrix on hepatic injury.[27] Endothelial cells line the hepatic vessels, with sinusoids at the basal side of hepatocytes.[28] Development of transgenic fish that express fluorescent proteins under control of cell-specific promoters has allowed each liver cell type to be visualized in live embryos and larvae (Figure 3). These provide a unique tool to visualize hepatic cell behavior and development.

## In Vivo Imaging

Zebrafish embryos and larvae are transparent, enabling in vivo imaging of developmental and pathological processes. In 1937, the first published research article on zebrafish described the use of time-lapse filming to study cell and cytoplasmic movements after

fertilization.[9] Researchers can now perform detailed studies of organ development, liver size, hepatocellular interactions, and response to insults with transgenic animals that express fluorescent proteins under specific promoters for hepatocytes (Figure 3*A*; fatty acid-binding protein 10a, *fabp10a*[29]), biliary epithelial cells (*keratin 18*[30]; notch-responsive reporter *tp1*[21] in Figure 3*B*), endothelial cells (Figure 3*C*; *flk1* and *fli1*[28]), and hepatic stellate cells (Figure 3*D*; *hand2*[27]). Fluorescent vital dyes, such as those that mark lipids,[23,31,32] are also being used to track physiological functions in live animals. These tools have been used to study morphological changes in stellate cells during development of ALD,[27,33] after hepatocyte ablation,[33] and during acute induction of hepatic endoplasmic reticulum (ER) stress.[24] Additionally, cell-specific promoters are useful for expressing functional proteins that alter liver biology. When combined with inducible or conditional genetic expression approaches, these approaches enable lineage tracing experiments to identify cells that contribute to liver regeneration[34,35] and can be applied to other liver and biliary disease models to advance our understanding of how populations of cells function, interact, and respond to pathological insults.

## Gene and Drug Discovery Screens

The small size of zebrafish embryos and larvae allows researchers to screen thousands of animals for responses to mutations or compounds. These screens have identified processes of liver biology and disease development. Forward genetic screens involve sorting through a series of zebrafish that carry a range of randomly induced mutations for those with a phenotype of interest, such as altered liver size or structure. The spectrum of human liver diseases is reflected in the repository of zebrafish mutants, including those with hereditary liver disease,[36–39] biliary defects that can be studied as models of biliary atresia,[40–42] and susceptibility to physiological or environmentally induced FLD.[43] Conveniently, many of these are available publicly through the Zebrafish International Resource Center (zirc.org). Because 69% of all zebrafish genes have a clear human orthologue,[14] mutations that are found to cause liver-related phenotypes in zebrafish can be investigated for their relevance to human disorders.

Chemical genetic screens are a powerful technology for identifying regulators of liver development[44] and for chemicals that are protective or enhance recovery after organ injury.[45] Identifying robust and reproducible phenotypes that are easy to score is an important consideration for performing a screen in zebrafish. Phenotypes that involve subtle changes that can only be scored by examination of each embryo are feasible, but resource intensive. Phenotypes that can be scored by an automated process, or identified by reporters that can be quickly scanned and measured, allow for high-throughput screens. Once the relevant phenotype or reporter is optimized, embryos can be dispersed into multi-well plates, and several hundred compounds can be screened each week. In general, libraries of known bioactive compounds with annotated mechanisms of action provide the most useful biological insights that can be complemented by genetic approaches to target the same processes.

An excellent example of this type of phenotype is the expression of a fluorescent protein under control of a glucose-responsive promoter that is activated in the liver in response to

fasting. This reporter enabled researchers to screen 2400 small molecules for compounds that affected glucose homeostasis in zebrafish larvae, and the lead compounds were then effectively used to improve glucose sensitivity and reduce steatosis in obese mice.[46] Similar tractable and relevant approaches can be used to test compounds for their efficacy in established zebrafish models of disease as a preclinical model for identifying new therapies for patients with liver diseases attributed to the same pathophysiological mechanisms.

### Population Studies

Genetic variation is an important determinant of disease susceptibility across species and is exemplified by the wide diversity in penetrance of disease severity in patients with advanced liver disease. The same underlying principles that govern variations in phenotype and responses to environmental factors in humans, including genetic polymorphisms and epigenetic factors, alter disease susceptibility in zebrafish, which are distinctly different from most laboratory rodent strains in that most zebrafish strains are not inbred. Moreover, as each zebrafish mating generates hundreds of embryos, the range of responses can be observed in a single experiment. For example, variations in susceptibility of zebrafish to alcohol-induced steatosis or acetaminophen-mediated toxic injury are similar to those of humans.[47,48] The advances in the assembly and annotation of the zebrafish genome will allow researchers to map quantitative trait loci that affect disease susceptibility.

## Heritable and Developmental Liver Diseases

Genetic and chemical screens have identified zebrafish mutants and signaling pathways that are relevant to inherited human liver diseases. We provide a few examples; others have been described in a more extensive review of models of inborn errors of metabolism in zebrafish.[49]

Porphyrin, an important precursor for heme, can accumulate in the liver and cause severe liver disease when a gene encoding a heme biosynthetic enzyme is mutated in porphyria. Zebrafish embryos carrying mutations in heme biosynthesis genes are easily identifiable because of the absence of red blood cells in circulation or accumulation of autofluorescent heme products. Hepatoerythropoietic porphyria is caused by deficiency in uroporphyrinogen decarboxylase (UROD)[50]; zebrafish with mutations in *urod* were identified in a forward genetic screen and are characterized by erythrocytes that lyse on exposure to light.[36,37] Similarly, *ferrochelatase* mutations cause erythropoietic protoporphyria, and zebrafish with mutations in *ferrochelatase* have erythrocytes that are extremely photosensitive. Although these mutants accumulate porphyrin in the liver,[38] the extent of liver damage has not yet been examined. Genetic screens for abnormal erythrocyte formation have provided further insight into hemochromatosis type 4, which is caused by a mutation in *SLC40A1* (ferroportin).[51] Iron loading of adult zebrafish with mutations in *slc40A1* increased iron levels in hepatocytes,[39] but the effects on hepatic function and signs of liver disease were not examined. These models can be used to understand the environmental triggers that promote acute and devastating sequelae of this disease.

Although diseases caused by failed hepatocyte development are rare, pediatric biliary diseases are relatively common. Elegant developmental biology approaches have elucidated

conserved mechanisms of biliary tract formation and biliary atresia. Notch signaling is required for bile duct development, as exemplified by mutations in genes regulating this pathway causing biliary disease in humans[52] and zebrafish.[21] DNA methylation is a surprising player in biliary disease, which was uncovered by a screen for zebrafish mutants with biliary defects. Mutation in *S*-adenosyl-L-homocysteine hydrolase (*ahcy*) reduces levels of *S*-adenylmethionine,[53] which serves as the donor for DNA methylation, and zebrafish *ahcy* mutants develop steatosis and biliary defects.[53] Interestingly, the biliary phenotype was attributed to DNA hypomethylation. Importantly, administration of an inhibitor of DNA methylation, 5-azacytidine, recapitulated the biliary atresia phenotype[53] and DNA hypomethylation was reported in bile duct cells from patients with biliary atresia.[53] Moreover, DNA hypomethylation in zebrafish induced the interferon gamma signaling pathway, and injecting zebrafish larvae with interferon gamma recapitulated the biliary defects mimicking biliary atresia.[54] This provides a potential pathogenic mechanism for biliary atresia which has been proposed in some pediatric cases to be caused by a viral agent that may trigger an antiviral immune response that destroys the developing biliary tract.[55,56] The hypothesis that an antiviral immune response might cause biliary atresia is supported by the finding that corticosteroids prevent the biliary defect caused by DNA hypomethylation in zebrafish,[53] suggesting that these drugs might be used to treat patients with biliary atresia. A clinical trial of corticosteroids for treatment of infants with biliary atresia after hepatoportoenterostomy showed no significant improvement in bilirubin levels,[57] yet it is possible that a subset of patients who develop this disease after infection, or via DNA hypomethylation, might benefit from corticosteroid therapy.

An exciting recent study found a novel environmental cause of biliary atresia. The observation that Australian lambs develop biliary atresia after eating an endogenous plant inspired researchers to investigate the cause of this phenomenon.[58] By testing extracts of this plant on zebrafish, researchers identified a specific flavonoid that caused severe biliary atresia.[58] Flavonoid exposure during specific developmental periods might therefore increase susceptibility to biliary disease. This elegant study shows that zebrafish can help elucidate toxic environmental effects on the biliary system.

Sorting through data from genome-wide association studies for genes with functional relevance has been a major challenge that zebrafish researchers have applied to identify new candidate genes for biliary atresia. One study identified variants in glypican 1 (*gpc1*), a protein that binds extracellular sugar molecules, that are associated with biliary atresia and reported that levels of GPC1 are reduced in patients with biliary atresia.[59] The functional relevance and a mechanism underlying this finding was shown using zebrafish; *gpc1* knockdown caused structural biliary defects and decreased bile secretion, assessed with the fluorescent lipid reporter PED-6.[59] Activating hedgehog using an agonist or injecting recombinant protein into zebrafish produces a biliary phenotype similar to that of embryos with disruption of *gpc1*, suggesting that Gpc1-hedgehog binding might modulate this signaling pathway to direct formation of the embryonic bile duct network. Collectively, these studies highlight how zebrafish provide functional insight to data from genome-wide surveys. They can be used to identify distinct molecular processes and environmental factors that contribute to biliary disease.

## Metabolic and Fatty Liver Disease

Obesity and type 2 diabetes mellitus are the major causes of nonalcoholic FLD, which accounts for ~75% of all cases of chronic liver disease in the United States.[60] Insulin resistance and lipid overload, which accompany metabolic syndrome, create metabolic imbalances that affect liver health. In addition, oxidative stress and adipokine overload promote inflammation to exacerbate hepatocyte damage, vascular changes, and fibrosis.[60]

The same metabolic perturbations cause FLD in humans and zebrafish[43,61]: a diet high in fat[62] or fructose,[63] fasting,[64–66] toxin exposure,[25,47,66,67] methionine depletion,[68] and genetic factors.[68–71] In rare cases, fatty liver can be caused by inborn errors of metabolism and other genetic disorders,[72] several of which have been modeled in zebrafish and reviewed elsewhere.[49]

In humans and zebrafish, a number of similar features characterize FLD, including hepatocyte enlargement and ballooning, triglyceride accumulation, secretory pathway dysfunction with activation of the unfolded protein response (UPR), and increases in reactive oxygen species (ROS) (Figure 4). Although zebrafish have been useful to study the molecular and cellular factors that cause FLD, the interaction between hepatocytes and other cell types, and the progression to more severe steatohepatitis that involves inflammation and fibrosis, remain to be explored in this model. It will be important to determine whether features of steatohepatitis appear in zebrafish with FLD, and future studies may thus identify the modifying factors that influence the progression from steatosis to steatohepatitis. An interesting recent study showed that the gut microbiome influences fatty acid metabolism,[73] and it would be interesting to further evaluate how the microbiome impacts liver disease using this model.

### Imaging Fatty Liver

Lipophilic dyes, such as oil red O, can be used to stain whole larvae in screens of large populations of larvae for steatosis. Additionally, BODIPY-labeled fatty acids, which can be visualized in hepatocytes of live animals using confocal microscopy,[32] have identified steatosis in zebrafish.[43,74] Development of other vital dyes for live imaging will enable high-throughput screens of zebrafish for FLD, longitudinal studies, and identification of animals with disease susceptibility for further study.

PED-6 is a fluorophore-linked phospholipid derivative that becomes fluorescent only when cleaved by intestinal lipases. It then translocates to the liver and is secreted in bile, highlighting the gallbladder.[23] A genetic screen for mutants that failed to convert PED-6 to its fluorescent form identified a loss of function in the product of *vacuolar protein sorting 51* (*vps51*) in fat-free mutant zebrafish.[23] Vps51 functions in retrograde transport from the Golgi complex,[75,76] and fat-free mutants fail to transport lipid from the gut through the enterocytes The discovery that *vps51* is required for lipid transport in enterocytes highlights the complex cellular pathways required for lipid absorption and processing and the opportunities to study these process using zebrafish.

## Screens for Regulators of FLD

Several forward genetic screens have identified regulators of hepatic lipid metabolism and point to new therapeutic targets for FLD. Zebrafish embryos subsist on yolk for the first 5 days of development; larvae that are not fed within 2 days of yolk consumption can develop fasting-induced steatosis.[47,65,66,77] Although the incidence of FLD varies by strain[77] and the assay used to measure it, researchers who found a low incidence of fasting-induced steatosis in wild-type larvae used these to screen for mutations that increased this incidence. One mutant, *red moon* (*rmn*), carried a homozygous mutation in the *solute carrier family 16, member 6a* gene (*slc16a6a*).[65] Although these mutants have no overt phenotype and are viable as adults, they have increased levels of hepatic fatty acid and triglycerides when placed on a ketogenic diet. Surprisingly, however, they do not develop high levels of cholesterol,[78] which is characteristic of most other models of FLD. This observation led to the finding that polyunsaturated fatty acids accumulate when these mutants are fed a ketogenic diet and function as endogenous inhibitors of HMG-CoA reductase (Hmgcra),[78] the rate-limiting step in cholesterol biogenesis. This effect of polyunsaturated fatty acids on cholesterol metabolism was also observed in mice.[78] Findings from zebrafish have therefore provided important insights into cholesterol metabolism, a topic with significant clinical implications.

Loss of another solute carrier, *slc7a3a*, also promotes fasting-induced steatosis, partially attributed to a decrease in nitric oxide level.[79] In studies of zebrafish, mice, and human liver cancer cells that were deficient in *slc7a3a*, NO was shown to act through AMP-activated protein kinase to signal to the lipid regulators, PGC1 and PPARA, to regulate lipid metabolism.[79] These studies identified mechanisms by which fasting regulates lipid accumulation in hepatocytes and identified an unanticipated outcome, inhibition of Hmgcr, by polyunsaturated fatty acids.

Another unexpected discovery in the mechanism of FLD was also provided by a screen for mutants that promote fasting-induced steatosis. Mutants in the guanine monophosphate synthase (*gmps*) gene increased homeostatic levels of ROS, and this reduced FLD.[64] Combining elegant imaging of fluorescently labeled lipid droplets with chemical inhibitors and genetic tools, researchers showed that mutations in *gmps* downregulated Rac1 activity, reducing homeostatic levels of ROS, which then reduced a triglyceride hydrolase (*ces3*), leading to lipid droplet accumulated in hepatocytes[64] (Figure 4). This observation was novel because ROS is considered to be a perpetrator of hepatic injury but was here shown to prevent lipid accumulation, a sign of disease. This provides the basis for the intriguing possibility that use of antioxidants to treat FLD might exacerbate the disease.

To identify genes underlying hepatic disease, mutants were screened for hepatomegaly, which is commonly observed in patients with FLD. We identified a mutant we named *foie gras* (*foigr*)[69] because it spontaneously develops steatosis, even without fasting, due to a mutation in *trappc11*. This gene functions in vesicle trafficking[80] and has been implicated in human disease.[81] Although gastroenterological defects were not reported in the few patients with *TRAPPC11* mutations,[81] it will be interesting to determine whether these patients develop FLD.

Livers of *foigr* mutants have an activated UPR, which has been observed in patients with FLD.[77] Disruption of *atf6*, which encodes a mediator of the UPR, reduced fatty liver in *foigr* mutants[66] as well as in steatosis due to prolonged ER stress[66,82] or alcohol use.[82] *Atf6* overexpression was sufficient to cause fatty liver in zebrafish under stress-free conditions.[82] This finding is significant because UPR induction and ER stress are possible mechanisms of FLD of many etiologies[83] and have been proposed as therapeutic targets, but until this study, the direct cause of FLD by a UPR player had not been clearly shown. Further studies are required before these findings can be tested in patients, because *atf6* disruption only reduced steatosis caused by prolonged stress; in contrast, Atf6 loss increased steatosis in acute stress models of FLD.[66,84–86] The dichotomous nature of the UPR in the pathology of FLD indicates that reducing activation of the UPR may not be effective for FLD of all etiologies.

## Modeling ALD

Liver disease is a leading cause of morbidity and mortality in alcoholic patients. As binge drinking increases,[87] the acute effects of alcohol on the liver are of particular concern. We developed a straightforward approach to induce acute ethanol-induced liver damage in zebrafish.[47] Larvae are bathed in ethanol after the liver has developed and hepatocytes express alcohol dehydrogenase and cytochrome P450 (CYP) family members.[48,88] Within the first 12 hours, zebrafish manifest the effects of ethanol in behavioral changes, abnormal body curvature, and hepatomegaly (Figure 5). These effects worsen over time and are reversible if ethanol is removed within 24 hours (Tsedensodnom and Sadler, unpublished observations May, 2013), but the effects exacerbate with longer exposure, leading to activation of stellate cells[27] (Figure 5).

The large number of tiny embryos generated per mating allows investigators to survey hundreds of larvae in each experiment; multiple variables can be simultaneously manipulated, such as ethanol concentration and duration of exposure. Experiments can be repeated multiple times, allowing for robust statistical power and detailed experimental design. Use of adult fish allows investigators to collect serum and larger liver samples, and adult zebrafish also show signs of liver damage when exposed to low concentrations of ethanol for prolonged periods.[89–93] However, long-term exposure of adult zebrafish poses logistical challenges and limits the numbers of animals that can be feasibly manipulated. Moreover, because intoxicated larvae cannot feed adequately, long-term exposure to higher levels of ethanol is difficult. Thus, the most effective studies are focused on the acute effects of ethanol using larvae.

Triglyceride accumulation is one of the most common features of acute and chronic alcohol abuse.[94] As in nonalcoholic FLD, increased lipogenesis, decreased lipid use, and failed lipoprotein export are the major mechanisms implicated in alcoholic steatosis.[94] On average, more than 60% of zebrafish develop steatosis within 24 hours of exposure to ethanol. In part, this is due to lipogenesis mediated by the sterol regulatory element binding protein (SREBP) transcription factors,[47] as found in mice.[95,96] ROS has been implicated in nearly every aspect of ALD and oxidation of hepatocyte proteins, membranes, and DNA and is believed to be the major cause of hepatocyte dysfunction in ALD. In contrast to the homeostatic levels of ROS, which were shown to limit fasting-induced steatosis,[64] higher

levels of ROS generated by CYP-mediated ethanol metabolism cause mitochondrial dysfunction, which reduces lipid oxidation and contributes to steatosis.[94] In zebrafish, ethanol-induced liver damage is accompanied by oxidative stress (Figure 5).[48] Similar to rats,[97] blocking ethanol metabolism or administering antioxidants reduces steatosis in zebrafish and improves hepatic function.[48] Oxidative stress is therefore a conserved aspect of ethanol-induced liver damage. Although antioxidants have shown little effect in patients with ALD or alcoholic hepatitis,[98] zebrafish could provide a platform for screening treatment options or combination therapies to improve outcomes of patients with ALD.

In alcoholic patients, serum protein deficiency reflects impaired hepatocyte secretion. UPR activation (typically used interchangeably with the term “ER stress”) can be a harbinger of secretory pathway dysfunction in ALD.[99] We found strong upregulation of UPR markers[24,48,100] and a decrease in a marker of hepatocyte secretion[24] in zebrafish after ethanol exposure, indicating that the hepatic secretory pathway is dysfunctional in ethanol-treated zebrafish. In zebrafish genetic studies, *aft6* was found to be necessary and sufficient for ethanol-induced steatosis[82] (Figure 4) and UPR activation was found to cause ethanol-induced liver disease. These findings point to Atf6 as an important mediator of alcohol-induced FLD. Interestingly, transcriptome analysis identified a nearly complete overlap of the ethanol-induced UPR-ome with the genes induced by overexpression of Atf6.[82] ATF6 might therefore serve as a mechanism that senses and mediates the hepatocyte protein secretory response to alcohol, which may differentiate ALD from other etiologies of FLD.

## Fibrosis and Cirrhosis

Chronic liver injury and inflammation lead to collagen deposition and fibrosis. Fibrosis and cirrhosis (loss of liver metabolic and synthetic function) and the vascular consequences of altered hemodynamics with portal hypertension are responsible for the high levels of morbidity and mortality among patients with chronic liver disease. There are no effective treatments to prevent or reverse cirrhosis, and preclinical animal models are required for testing of any new therapy.

Deposition of the extracellular matrix can be studied in transgenic zebrafish that express green fluorescent protein (GFP) in hepatic stellate cells (*Tg(hand2:GFP)*; Figure 3). Using this tool, investigators showed that stellate cell activation occurs within 24 hours of ethanol exposure and is sustained for at least 3 days after ethanol is removed.[27] Another study used a genetic approach to deplete hepatocytes, in combination with ethanol exposure, and cause substantial and sustained hepatic injury. The researchers found that hepatic stellate cells, marked by GFP, became activated and secreted the extracellular components associated with fibrosis,[33] as in humans.

One limitation of this approach is that neither advanced fibrosis nor cirrhosis has been observed in adult zebrafish. Instead, the changes in stellate cell activity that have been found in acute ethanol-induced liver disease[24,27] might prove to be a useful phenotype to screen for genes and compounds that modify stellate cell activation and develop antifibrotic therapies.

## Liver Cancer

Hepatocellular carcinoma (HCC) is one of the most aggressive and lethal cancers worldwide, leading to more than 500,000 deaths annually.[101] Zebrafish have been used for cancer studies for more than a decade, and the state of the field was recently reviewed.[4] Fish, like all vertebrates, develop cancer spontaneously. Older adult zebrafish develop HCC, hepatic adenomas, and cholangiocarcinoma; development of cancer can be accelerated with hepatotropic carcinogens,[102,103] disruption of genes encoding tumor suppressors,[104] or overexpression of oncogenes.[105–108] Importantly, liver tumors in zebrafish and humans are similar in histological appearance (Figure 6*A* and *B*) and gene expression.[109] Review of hundreds of zebrafish tumors by multiple pathologists and zebrafish investigators has generated a common criteria for diagnosis of HCC (Figure 6*C*).

Many carcinogens are predominantly metabolized by hepatocytes. Zebrafish exposed to carcinogens therefore frequently develop liver neoplasia over several months, a time frame similar to that observed in mice. When zebrafish are exposed to carcinogens at 3 weeks of age, they develop liver neoplasms as adults; exposure to dimethylbenzanthracene (DMBA) at this age led to development of hepatic neoplasia (half carcinomas) in 38% of zebrafish by 9 months of age.[103] Gene expression profiles of DMBA-induced zebrafish tumors correlate with those of human liver tumors.[109] However, it is not clear how alterations in expression of these genes and their pathways contribute to carcinogenesis.

Screens to identify genetic modifiers of carcinogenesis susceptibility uncovered the mitotic regulator *separase*. Mutations in this gene increased susceptibility to Methyl-nitronitrosoguanidine induced tumors by 9-fold.[110] Studies of chemical-induced carcinogenesis in zebrafish have validated human genes as tumor suppressors. The adenomatous polyposis coli (*APC*) gene is a tumor suppressor in humans that regulates WNT signaling via β-catenin; zebrafish with mutations in *apc* have higher rates of spontaneous and DMBA-induced liver, intestinal, and pancreatic neoplasia than fish without these mutations.[104] A recent study using zebrafish overexpressing β-catenin in zebrafish hepatocytes[108] confirmed the concept that WNT signaling via β-catenin is a central pathway to liver carcinogenesis across species. Evason et al generated β-catenin–driven liver tumors and performed a chemical screen to identify pathways involved. They showed that β-catenin overexpression causes large livers in larvae that progress to HCC in adults. Using the large liver size as a surrogate marker in a chemical screen, they identified Jnk inhibitors and selective serotonin reuptake inhibitors (currently used as antidepressants) as 2 novel classes of drugs that could block formation of HCC.[108]

Oncogene-induced HCC was first shown in transgenic zebrafish, which expressed activated k-*ras* in hepatocytes from the highly specific *fabp10* promoter (*fabp10:k-ras*). These transgenic fish developed hepatic neoplasms that progressed to HCC within 1 week.[105] In a modified approach that used an inducible transgenic system, agents that targeted MEK, PI3K, and mTOR were tested for their efficacy in reducing growth of ras-induced tumors.[105] Other oncogenes, including *myc*[111] and *src*,[112] and hepatitis B and C viral proteins[113] have been overexpressed in zebrafish hepatocytes. Coexpression of HBX and HCV core protein cause cancer in zebrafish, primarily fibrosis-associated intrahepatic cholangiocarcinoma,[113]

whereas mice typically develop HCC.[114] These differences may be attributed to the time of development when these viral proteins are overexpressed and to the plasticity of zebrafish biliary cells in their response to hepatic injury. The ability to track growth of tumors in live fish[115] and the use of promoters that respond to compounds added to the water of zebrafish have enabled long-term studies of tumor growth in which the activities of oncogenes can be temporally regulated.[105,111]

Zebrafish studies have made many important contributions to studies of cancer biology. Overexpression of UHRF1, a regulator of DNA methylation, causes HCC in zebrafish and is highly upregulated in aggressive human HCCs.[106] This highlights the importance of epigenetics as a key factor underlying HCC, as evidenced by the prominent role of DNA methylation in human liver tumors.[116,117] These studies show how zebrafish can be used to study the functions of oncogenes, establish synergy between oncogenic pathways, and test potential therapeutic agents.

## DILI

DILI can lead to fulminant liver failure due to massive hepatocyte death. Fish hepatocytes express many CYP enzymes[118] and are adept at metabolizing xenobiotics via the same pathways as mammalian hepatocytes.[119] DILI can readily be studied in larvae after hepatocytes have developed at 3 dpf. Hepatic repair in zebrafish was initially shown after exposure to 4-chloroaniline; in this study, hepatic histology and animal survival were affected in a dose-dependent manner.[120] Since then, many compounds have been assessed in zebrafish for their hepatotoxic effects. There has been much progress in our understanding of the effects of acetaminophen and environmental toxins on the liver, and the use of zebrafish to screen compounds for hepatotoxicity might also be used to identify new compounds to counteract DILI.

**Acetaminophen—**The ability of toxins and medications to injure the liver came into focus with the advent of acetaminophen in the 1950s as a leading analgesic and antipyretic (Figure 1). Acetaminophen causes acute liver failure due to the production of toxic intermediates and depletion of glutathione stores. Although *N*-acetylcysteine (NAC) has been used as an antidote for acetaminophen-induced liver injury, it must be administered immediately to be effective. Acetaminophen toxicity still contributes to nearly 50% of all cases of acute hepatic failure.[121] Acetaminophen is toxic to adult zebrafish in a dose-dependent manner,[45] increasing serum levels of aminotransferase and causing histological changes, necrosis, hemorrhage, and death, as in humans. NAC can reduce acetaminophen-induced liver damage in zebrafish, as in humans.

Acetaminophen is also toxic to zebrafish larvae, and a focused chemical screen was used to identify prostaglandin $E_2$ as a compound that limits the toxicity of acetaminophen and enhances proliferation in zebrafish larvae and adults.[45] Additionally, nitric oxide and protein *S*-nitrosylation improve outcomes after acetaminophen-induced liver injury.[122] Inhibiting *S*-nitrosoglutathione reductase (Gsnor) prevents acetaminophen-induced liver damage and mortality through sustained activation of the Nrf2–Keap1 oxidative stress response pathway. Gsnor-deficient mice are resistant to acetaminophen-induced liver injury. Inhibitors of

Gsnor and exposure to NAC increase survival of zebrafish with acetaminophen-induced liver damage.[122] It will be interesting to determine whether this combined therapy would be useful for patients with acetaminophen-induced liver injury.

**Environmental toxins—**Aquatic species have been a mainstay of toxicology research. The effects of environmental toxins on the liver range from chronic copper overload to acute liver injury. The hepatotoxic effects of organics, metals, endocrine disruptors, aflatoxin,[123] and arsenic[124] have all been examined in zebrafish.[125] Arsenic is at the top of the Agency for Toxic Substances and Disease Registry Priority List of Hazardous Substances. Prenatal or childhood exposure can increase lifetime all-cause mortality and increase the incidence of HCC.[126] The arsenic-metabolizing enzyme (encoded by *as3mt*) is expressed in livers of mammals and zebrafish and makes the liver susceptible to toxic injury.[127] Transcriptional[124,128] and proteomic[129] analyses of livers from zebrafish exposed to arsenic identified lipid metabolic and carcinogenic pathways that were upregulated. Integration of high-throughput genomic, proteomic, and metabolomic analyses with studies of transgenic zebrafish could elucidate the mechanisms of arsenic-induced hepatotoxicity and predisposition to liver cancer, a goal that has not been achieved with any other animal model of arsenic toxicity.

## Drug Testing

Many agents fail in late-stage clinical trials because they are hepatotoxic. Screens to identify potentially hepatotoxic agents are of primary importance, and zebrafish provide an excellent, affordable system to identify hepatotoxic compounds.[119,130] The advantages of using zebrafish in toxicity studies include the ability to rapidly screen for the effects of multiple agents in an entire organism during different stages of development. Indeed, biotechnology companies have capitalized on the comparative cost benefit of using zebrafish to assess toxicity (for a review, see Driessen et al[119] and McGrath and Li[131]).

In 2007, the Environmental Protection Agency initiated ToxCast, which included the use of zebrafish to determine the developmental toxicity of a large number of chemical toxicants.[132] This provided an important alternative to the expensive and laborious process of testing the toxicity of agents in rodents. A similar program initiated by the European Union (EU Reference Laboratory for Alternatives to Animal Testing) aims to characterize the toxicity of a subset of those chemicals produced and marketed in large quantities. The EU Reference Laboratory for Alternatives to Animal Testing found that the zebrafish developmental toxicity assay identified teratogenic compounds with high levels of sensitivity and specificity and recommended zebrafish as a high-throughput system for toxicity screening.[133] Therefore, in addition to use in biomedical research on mechanisms of toxin-induced liver injury, zebrafish are proving useful to identify environmental toxins, many of which are implicated in liver damage.

## Liver Regeneration

Liver function must be rapidly restored after liver transplantation,[134,135] resection, or massive cell death from toxic or immune injury. In addition, learning the basic principles of

coordinated cell proliferation during regeneration has provided insights into mechanisms of tumor growth. Liver regeneration has been studied for more than 80 years using rodents that have undergone partial hepatectomy.[136] After partial hepatectomy, hepatocyte proliferation[137] and hypertrophy[138] cause the liver to grow until it achieves the appropriate size relative to that of the organism; this is similar to the hepatic growth observed in embryos or transplanted or resected livers in adults.

Partial hepatectomy by surgery in zebrafish involves removing one-third (the small ventral lobe) of the liver[139] ; removing more than this amount of liver kills the fish.[140] This is analogous to the response of patients who undergo resection of a liver mass. The response to partial hepatectomy in zebrafish is true regeneration (the resected lobe regrows), in contrast to mammals, in which the remaining lobes become enlarged. Analyses of liver regeneration, using in vivo ultrasonographic measurements of liver volume and the length of the regenerating lobe in adult fish, revealed similar kinetics of liver regrowth between zebrafish and mice. Livers took approximately 1 week to fully regrow after surgical resection.[115]

The cellular mechanism of repair through hepatocyte proliferation appears to be conserved, because most hepatocytes in the zebrafish resected lobe proliferate within 2 to 3 days of partial hepatectomy[139,140] Studies in zebrafish have uncovered several mechanisms that regulate liver regeneration, including epigenetics and multiple signaling pathways. The epigenetic regulator encoded by *uhrf1* was found to regulate embryonic liver growth and regeneration, based on the finding that the liver of *uhrf1*$^{+/-}$ adults did not fully regrow after resection.[139] This gene is also required for hepatocyte proliferation in embryos[139,141] and, as discussed previously, overexpression of UHRF1 in zebrafish hepatocytes led to development of HCC.[106] UHRF1 therefore appears to regulate hepatocyte proliferation in response to physiological (development and regeneration) and carcinogenic stimuli.

Studies of zebrafish and mice with mutations in *apc* showed the conserved importance of WNT signaling during embryonic and adult liver growth, and subsequent investigations showed regulatory interactions between prostaglandin $E_2$ and the WNT signaling pathways in vertebrate regeneration.[142] In particular, prostaglandin $E_2$ acts through a specific receptor, Pger4, to mediate growth and proliferation of differentiated hepatocytes after partial hepatectomy.[143] Chemical inhibition of the regulatory enzyme S-nitrosoglutathione reductase was found to increase hepatic recovery, whereas inhibition of NO synthesis limited regeneration.[122] Topoisomerase 2a[144] and the FGF and BMP signaling pathways are required for optimal liver regeneration.[140] These findings indicate that the zebrafish model of partial hepatectomy could facilitate screens for chemical and genetic modifiers of liver regeneration. Although liver regeneration is typically studied in adult animals, zebrafish embryos provide a useful system for studying hepatocyte regeneration. An interesting mutant was identified in a forward genetic screen for mutants that failed in hepatic outgrowth. Zebrafish with mutations in mitochondrial import gene *tomm22* formed normal livers as embryos, but then hepatocytes quickly died, leaving behind a liver skeleton comprising the biliary network.[145] Zebrafish with mutations in *tomm22*, or transient knockdown of expression, might be used as a model to study acute hepatocyte death and regeneration.

Liver regeneration can also be studied in zebrafish embryos using a genetic approach to ablate hepatocytes that is easier and produces a more uniform effect than surgical approaches. Nitroreductase converts metronidazole to a potent toxin; exposing transgenic zebrafish with hepatocyte-specific expression of nitroreductase to metronidazole results in hepatocyte death[146] and total loss of hepatocytes within 6 hours.[34,35,146] Investigators directly visualized the emergence of new hepatocytes, marked by a fluorescent protein, to observe the process of liver regeneration in larvae, which takes place within 2 days of hepatocyte ablation. Two recent studies used this approach to show that biliary cells contribute to hepatocyte regeneration after complete hepatocyte ablation.[33–35] Notch and WNT signaling were found to be important in biliary differentiation to hepatocytes, and WNT agonists promote regeneration by increasing the differentiation of biliary-derived progenitors to hepatocytes.[33] This process is similar to findings using mice in which bile ducts were found to function as a reservoir for cells that could differentiate into hepatocytes after injury.[147] The origin of hepatic progenitors is controversial, and this may be clarified by comparative studies that can identify common themes that drive liver regeneration.

## Future Directions

Zebrafish have contributed to our understanding of liver development, regeneration, and pathophysiology. The number of investigators using zebrafish to study liver pathologies is growing,[2] evidenced by the establishment of the Zebrafish Disease Models Society in 2014. The number of hepatobiliary diseases studied in zebrafish is growing in step. New tools and approaches are being developed, including gene editing technologies to mutate any gene of interest, large-scale chemical screens, and advanced imaging approaches to observe, mark, and track hepatic cells. Zebrafish researchers are set to not only increase our understanding of the mechanisms of liver disease but also identify new therapeutic targets and test candidate compounds for efficacy and safety before they are tested in patients.

## Acknowledgments

The authors thank Jaime Chu for critical reading of the manuscript, Jason Hornick for images of normal human liver and HCC histology, and Chunyue Yin for the image of *Tg(hand2:GFP)* larvae.

**Funding**

Supported by grants from the National Institutes of Health (5R01DK080789 and 5R01AA018886 to K.C.S and R01DK090311 to W.G.). W.G. is a Pew Scholar in the Biomedical Sciences.

## Abbreviations used in this paper

| | |
|---|---|
| **ALD** | alcoholic liver disease |
| **CYP** | cytochrome P450 |
| **DILI** | drug-induced liver injury |
| **DMBA** | dimethylbenzanthracene |
| **dpf** | days postfertilization |
| **ER** | endoplasmic reticulum |

| | |
|---|---|
| **FLD** | fatty liver disease |
| **GFP** | green fluorescent protein |
| **HCC** | hepatocellular carcinoma |
| **NAC** | *N*-acetylcysteine |
| **ROS** | reactive oxygen species |
| **UPR** | unfolded protein response |

## References


1. Lozano R, Naghavi M, Foreman K, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet. 2012; 380:2095–2128. [PubMed: 23245604]
2. Phillips JB, Westerfield M. Zebrafish models in translational research: tipping the scales toward advancements in human health. Dis Model Mech. 2014; 7:739–743. [PubMed: 24973743]
3. Wilkins BJ, Pack M. Zebrafish models of human liver development and disease. Compr Physiol. 2013; 3:1213–1230. [PubMed: 23897685]
4. White R, Rose K, Zon L. Zebrafish cancer: the state of the art and the path forward. Nat Rev Cancer. 2013; 13:624–636. [PubMed: 23969693]
5. Papavramidou N, Fee E, Christopoulou-Aletra H. Jaundice in the Hippocratic Corpus. J Gastrointest Surg. 2007; 11:1728–1731. [PubMed: 17896166]
6. Power C, Rasko JE. Whither prometheus' liver? Greek myth and the science of regeneration. Ann Intern Med. 2008; 149:421–426. [PubMed: 18794562]
7. Guemene D, Guy G. The past, present and future of force-feeding and "foie gras" production. World Poultry Sci J. 2004; 60:210–222.
8. Engeszer RE, Patterson LB, Rao AA, et al. Zebrafish in the wild: a review of natural history and new notes from the field. Zebrafish. 2007; 4:21–40. [PubMed: 18041940]
9. Roosen-Runge E. Observations of the early development of the zebra fish, Brachydanio rerio. Anat Rec. 1937; 70:103.
10. Laale HW. Ethanol induced notochord and spinal cord duplications in the embryo of the zebrafish, Brachydanio rerio. J Exp Zool. 1971; 177:51–64. [PubMed: 4998733]
11. Stanton MF. Diethylnitrosamine-induced hepatic degeneration and neoplasia in the aquarium fish, Brachydanio rerio. J Natl Cancer Inst. 1965; 34:117–130. [PubMed: 14287275]
12. Rennekamp AJ, Peterson RT. 15 years of zebrafish chemical screening. Curr Opin Chem Biol. 2014; 24C:58–70. [PubMed: 25461724]
13. Streisinger G, Walker C, Dower N, et al. Production of clones of homozygous diploid zebra fish (Brachydanio rerio). Nature. 1981; 291:293–296. [PubMed: 7248006]
14. Howe K, Clark MD, Torroja CF, et al. The zebrafish reference genome sequence and its relationship to the human genome. Nature. 2013; 496:498–503. [PubMed: 23594743]
15. Stuart GW, McMurray JV, Westerfield M. Replication, integration and stable germ-line transmission of foreign sequences injected into early zebrafish embryos. Development. 1988; 103:403–412. [PubMed: 2852096]
16. Hinton, D.; Couch, J. Architectural pattern, tissue and cellular morphology in livers of fishes: relationship to experimentally-induced neoplastic responses. In: Braunbeck, T.; Hinton, D.; Streit, B., editors. Fish ecotoxicology. Basel: Birkhauser Verlag; 1998. p. 141-164.
17. Hampton JA, Lantz RC, Hinton DE. Functional units in rainbow trout (Salmo gairdneri, Richardson) liver: III. Morphometric analysis of parenchyma, stroma, and component cell types. Am J Anat. 1989; 185:58–73. [PubMed: 2782277]

18. Hampton JA, Lantz RC, Goldblatt PJ, et al. Functional units in rainbow trout (Salmo gairdneri, Richardson) liver: II. The biliary system. Anat Rec. 1988; 221:619–634. [PubMed: 2970812]
19. Weis P. Hepatic ultrastructure in two species of normal, fasted and gravid teleost fishes. Am J Anat. 1972; 133:317–331. [PubMed: 4112714]
20. Hardman RC, Volz DC, Kullman SW, et al. An in vivo look at vertebrate liver architecture: three-dimensional reconstructions from medaka (Oryzias latipes). Anat Rec (Hoboken). 2007; 290:770–782. [PubMed: 17516461]
21. Lorent K, Moore JC, Siekmann AF, et al. Reiterative use of the notch signal during zebrafish intrahepatic biliary development. Dev Dyn. 2010; 239:855–864. [PubMed: 20108354]
22. Matthews RP, Lorent K, Russo P, et al. The zebrafish onecut gene hnf-6 functions in an evolutionarily conserved genetic pathway that regulates vertebrate biliary development. Dev Biol. 2004; 274:245–259. [PubMed: 15385156]
23. Farber SA, Pack M, Ho SY, et al. Genetic analysis of digestive physiology using fluorescent phospholipid reporters. Science. 2001; 292:1385–1388. [PubMed: 11359013]
24. Howarth DL, Yin C, Yeh K, et al. Defining hepatic dysfunction parameters in two models of fatty liver disease in zebrafish larvae. Zebrafish. 2013; 10:199–210. [PubMed: 23697887]
25. Braunbeck T, Gorge G, Storch V, et al. Hepatic steatosis in zebra fish (Brachydanio rerio) induced by long-term exposure to gamma-hexachlorocyclohexane. Ecotoxicol Environ Saf. 1990; 19:355–374. [PubMed: 1694756]
26. Noel ES, Reis MD, Arain Z, et al. Analysis of the Albumin/alpha-Fetoprotein/Afamin/Group specific component gene family in the context of zebrafish liver differentiation. Gene Expr Patterns. 2010; 10:237–243. [PubMed: 20471496]
27. Yin C, Evason KJ, Maher JJ, et al. The basic helix-loop-helix transcription factor, heart and neural crest derivatives expressed transcript 2, marks hepatic stellate cells in zebrafish: analysis of stellate cell entry into the developing liver. Hepatology. 2012; 56:1958–1970. [PubMed: 22488653]
28. Sakaguchi TF, Sadler KC, Crosnier C, et al. Endothelial signals modulate hepatocyte apicobasal polarization in zebrafish. Curr Biol. 2008; 18:1565–1571. [PubMed: 18951027]
29. Her GM, Chiang CC, Chen WY, et al. In vivo studies of liver-type fatty acid binding protein (L-FABP) gene expression in liver of transgenic zebrafish (Danio rerio). FEBS Lett. 2003; 538:125–133. [PubMed: 12633865]
30. Wilkins BJ, Gong W, Pack M. A novel keratin18 promoter that drives reporter gene expression in the intrahepatic and extrahepatic biliary system allows isolation of cell-type specific transcripts from zebrafish liver. Gene Expr Patterns. 2014; 14:62–68. [PubMed: 24394404]
31. Flynn EJ III, Trent CM, Rawls JF. Ontogeny and nutritional control of adipogenesis in zebrafish (Danio rerio). J Lipid Res. 2009; 50:1641–1652. [PubMed: 19366995]
32. Carten JD, Bradford MK, Farber SA. Visualizing digestive organ morphology and function using differential fatty acid metabolism in live zebrafish. Dev Biol. 2011; 360:276–285. [PubMed: 21968100]
33. Huang M, Chang A, Choi M, et al. Antagonistic interaction between Wnt and Notch activity modulates the regenerative capacity of a zebrafish fibrotic liver model. Hepatology. 2014; 60:1753–1766. [PubMed: 24995814]
34. Choi TY, Ninov N, Stainier DY, et al. Extensive conversion of hepatic biliary epithelial cells to hepatocytes after near total loss of hepatocytes in zebrafish. Gastroenterology. 2014; 146:776–788. [PubMed: 24148620]
35. He J, Lu H, Zou Q, et al. Regeneration of liver after extreme hepatocyte loss occurs mainly via biliary transdifferentiation in zebrafish. Gastroenterology. 2014; 146:789–800. e8. [PubMed: 24315993]
36. Ransom DG, Haffter P, Odenthal J, et al. Characterization of zebrafish mutants with defects in embryonic hematopoiesis. Development. 1996; 123:311–319. [PubMed: 9007251]
37. Wang H, Long Q, Marty SD, et al. A zebrafish model for hepatoerythropoietic porphyria. Nat Genet. 1998; 20:239–243. [PubMed: 9806541]
38. Childs S, Weinstein BM, Mohideen MA, et al. Zebrafish dracula encodes ferrochelatase and its mutation provides a model for erythropoietic protoporphyria. Curr Biol. 2000; 10:1001–1004. [PubMed: 10985389]

39. Fraenkel PG, Traver D, Donovan A, et al. Ferroportin1 is required for normal iron cycling in zebrafish. J Clin Invest. 2005; 115:1532–1541. [PubMed: 15902304]
40. Dong PD, Munson CA, Norton W, et al. Fgf10 regulates hepatopancreatic ductal system patterning and differentiation. Nat Genet. 2007; 39:397–402. [PubMed: 17259985]
41. Schaub M, Nussbaum J, Verkade H, et al. Mutation of zebrafish Snapc4 is associated with loss of the intrahepatic biliary network. Dev Biol. 2012; 363:128–137. [PubMed: 22222761]
42. EauClaire SF, Cui S, Ma L, et al. Mutations in vacuolar H+ -ATPase subunits lead to biliary developmental defects in zebrafish. Dev Biol. 2012; 365:434–444. [PubMed: 22465374]
43. Asaoka Y, Terai S, Sakaida I, et al. The expanding role of fish models in understanding non-alcoholic fatty liver disease. Dis Model Mech. 2013; 6:905–914. [PubMed: 23720231]
44. Garnaas MK, Cutting CC, Meyers A, et al. Rargb regulates organ laterality in a zebrafish model of right atrial isomerism. Dev Biol. 2012; 372:178–189. [PubMed: 22982668]
45. North TE, Babu IR, Vedder LM, et al. PGE2-regulated wnt signaling and *N*-acetylcysteine are synergistically hepatoprotective in zebrafish acetaminophen injury. Proc Natl Acad Sci U S A. 2010; 107:17315–17320. [PubMed: 20855591]
46. Gut P, Baeza-Raja B, Andersson O, et al. Whole-organism screening for gluconeogenesis identifies activators of fasting metabolism. Nat Chem Biol. 2013; 9:97–104. [PubMed: 23201900]
47. Passeri MJ, Cinaroglu A, Gao C, et al. Hepatic steatosis in response to acute alcohol exposure in zebrafish requires sterol regulatory element binding protein activation. Hepatology. 2009; 49:443–452. [PubMed: 19127516]
48. Tsedensodnom O, Vacaru AM, Howarth DL, et al. Ethanol metabolism and oxidative stress are required for unfolded protein response activation and steatosis in alcoholic liver disease. Dis Model Mech. 2013; 6:1213–1226. [PubMed: 23798569]
49. Wager K, Mahmood F, Russell C. Modelling inborn errors of metabolism in zebrafish. J Inherit Metab Dis. 2014; 37:483–495. [PubMed: 24797558]
50. de Verneuil H, Grandchamp B, Beaumont C, et al. Uroporphyrinogen decarboxylase structural mutant (Gly281—Glu) in a case of porphyria. Science. 1986; 234:732–734. [PubMed: 3775362]
51. Njajou OT, Vaessen N, Joosse M, et al. A mutation in SLC11A3 is associated with autosomal dominant hemochromatosis. Nat Genet. 2001; 28:213–214. [PubMed: 11431687]
52. Oda T, Elkahloun AG, Pike BL, et al. Mutations in the human Jagged1 gene are responsible for Alagille syndrome. Nat Genet. 1997; 16:235–242. [PubMed: 9207787]
53. Matthews RP, Eauclaire SF, Mugnier M, et al. DNA hypomethylation causes bile duct defects in zebrafish and is a distinguishing feature of infantile biliary atresia. Hepatology. 2011; 53:905–914. [PubMed: 21319190]
54. Cui S, Eauclaire SF, Matthews RP. Interferon-gamma directly mediates developmental biliary defects. Zebrafish. 2013; 10:177–183. [PubMed: 23448251]
55. Richardson SC, Bishop RF, Smith AL. Reovirus serotype 3 infection in infants with extrahepatic biliary atresia or neonatal hepatitis. J Gastroenterol Hepatol. 1994; 9:264–268. [PubMed: 8054525]
56. Morecki R, Glaser JH, Cho S, et al. Biliary atresia and reovirus type 3 infection. N Engl J Med. 1982; 307:481–484. [PubMed: 6285193]
57. Bezerra JA, Spino C, Magee JC, et al. Use of corticosteroids after hepatoportoenterostomy for bile drainage in infants with biliary atresia: the START randomized clinical trial. JAMA. 2014; 311:1750–1759. [PubMed: 24794368]
58. Lorent K, Gong W, Koo KA, et al. Identification of a plant isoflavonoid that causes biliary atresia. Sci Transl Med. 2015; 7:286ra67.
59. Cui S, Leyva-Vega M, Tsai EA, et al. Evidence from human and zebrafish that GPC1 is a biliary atresia susceptibility gene. Gastroenterology. 2013; 144:1107–1115. e3. [PubMed: 23336978]
60. Cohen JC, Horton JD, Hobbs HH. Human fatty liver disease: old questions and new insights. Science. 2011; 332:1519–1523. [PubMed: 21700865]
61. Schlegel A. Studying non-alcoholic fatty liver disease with zebrafish: a confluence of optics, genetics, and physiology. Cell Mol Life Sci. 2012; 69:3953–3961. [PubMed: 22678663]

62. Walters JW, Anderson JL, Bittman R, et al. Visualization of lipid metabolism in the zebrafish intestine reveals a relationship between NPC1L1-mediated cholesterol uptake and dietary fatty acid. Chem Biol. 2012; 19:913–925. [PubMed: 22749558]
63. Sapp V, Gaffney L, EauClaire SF, et al. Fructose leads to hepatic steatosis in zebrafish that is reversed by mechanistic target of rapamycin (mTOR) inhibition. Hepatology. 2014; 60:1581–1592. [PubMed: 25043405]
64. Nussbaum JM, Liu LJ, Hasan SA, et al. Homeostatic generation of reactive oxygen species protects the zebrafish liver from steatosis. Hepatology. 2013; 58:1326–1338. [PubMed: 23744565]
65. Hugo SE, Cruz-Garcia L, Karanth S, et al. A monocarboxylate transporter required for hepatocyte secretion of ketone bodies during fasting. Genes Dev. 2012; 26:282–293. [PubMed: 22302940]
66. Cinaroglu A, Gao C, Imrie D, et al. Activating transcription factor 6 plays protective and pathological roles in steatosis due to endoplasmic reticulum stress in zebrafish. Hepatology. 2011; 54:495–508. [PubMed: 21538441]
67. Amali AA, Rekha RD, Lin CJ, et al. Thioacetamide induced liver damage in zebrafish embryo as a disease model for steatohepatitis. J Biomed Sci. 2006; 13:225–232. [PubMed: 16456712]
68. Matthews RP, Lorent K, Manoral-Mobias R, et al. TNF {alpha}-dependent hepatic steatosis and liver degeneration caused by mutation of zebrafish s-adenosylhomocysteine hydrolase. Development. 2009; 136:865–875. [PubMed: 19201949]
69. Sadler KC, Amsterdam A, Soroka C, et al. A genetic screen in zebrafish identifies the mutants vps18, nf2 and foie gras as models of liver disease. Development. 2005; 132:3561–3572. [PubMed: 16000385]
70. Her GM, Hsu CC, Hong JR, et al. Overexpression of gankyrin induces liver steatosis in zebrafish (Danio rerio). Biochim Biophys Acta. 2011; 1811:536–548. [PubMed: 21722753]
71. Shieh YS, Chang YS, Hong JR, et al. Increase of hepatic fat accumulation by liver specific expression of Hepatitis B virus X protein in zebrafish. Biochim Biophys Acta. 2010; 1801:721–730. [PubMed: 20416398]
72. Ishak KG. Inherited metabolic diseases of the liver. Clin Liver Dis. 2002; 6:455–479. [PubMed: 12122865]
73. Semova I, Carten JD, Stombaugh J, et al. Microbiota regulate intestinal absorption and metabolism of fatty acids in the zebrafish. Cell Host Microbe. 2012; 12:277–288. [PubMed: 22980325]
74. Holtta-Vuori M, Salo VT, Nyberg L, et al. Zebrafish: gaining popularity in lipid research. Biochem J. 2010; 429:235–242. [PubMed: 20578994]
75. Ho SY, Lorent K, Pack M, et al. Zebrafish fat-free is required for intestinal lipid absorption and Golgi apparatus structure. Cell Metab. 2006; 3:289–300. [PubMed: 16581006]
76. Perez-Victoria FJ, Schindler C, Magadan JG, et al. Ang2/fat-free is a conserved subunit of the Golgi-associated retrograde protein complex. Mol Biol Cell. 2010; 21:3386–3395. [PubMed: 20685960]
77. Vacaru AM, Di Narzo AF, Howarth DL, et al. Molecularly defined unfolded protein response subclasses have distinct correlations with fatty liver disease in zebrafish. Dis Model Mech. 2014; 7:823–835. [PubMed: 24973751]
78. Karanth S, Tran VM, Kuberan B, et al. Polyunsaturated fatty acyl-coenzyme As are inhibitors of cholesterol biosynthesis in zebrafish and mice. Dis Model Mech. 2013; 6:1365–1377. [PubMed: 24057001]
79. Gu Q, Yang X, Lin L, et al. Genetic ablation of solute carrier family 7a3a leads to hepatic steatosis in zebrafish during fasting. Hepatology. 2014; 60:1929–1941. [PubMed: 25130427]
80. Scrivens PJ, Noueihed B, Shahrzad N, et al. C4orf41 and TTC-15 are mammalian TRAPP components with a role at an early stage in ER-to-Golgi trafficking. Mol Biol Cell. 2011; 22:2083–2093. [PubMed: 21525244]
81. Bogershausen N, Shahrzad N, Chong JX, et al. Recessive TRAPPC11 mutations cause a disease spectrum of limb girdle muscular dystrophy and myopathy with movement disorder and intellectual disability. Am J Hum Genet. 2013; 93:181–190. [PubMed: 23830518]
82. Howarth DL, Lindtner C, Vacaru AM, et al. Activating transcription factor 6 is necessary and sufficient for alcoholic fatty liver disease in zebrafish. PLoS Genet. 2014; 10:e1004335. [PubMed: 24874946]

83. Imrie D, Sadler KC. Stress management: how the unfolded protein response impacts fatty liver disease. J Hepatol. 2012; 57:1147–1151. [PubMed: 22732510]
84. Wu J, Rutkowski DT, Dubois M, et al. ATF6alpha optimizes long-term endoplasmic reticulum function to protect cells from chronic stress. Dev Cell. 2007; 13:351–364. [PubMed: 17765679]
85. Rutkowski DT, Wu J, Back SH, et al. UPR pathways combine to prevent hepatic steatosis caused by ER stress-mediated suppression of transcriptional master regulators. Dev Cell. 2008; 15:829–840. [PubMed: 19081072]
86. Yamamoto K, Takahara K, Oyadomari S, et al. Induction of liver steatosis and lipid droplet formation in ATF6alpha-knockout mice burdened with pharmacological endoplasmic reticulum stress. Mol Biol Cell. 2010; 21:2975–2986. [PubMed: 20631254]
87. Naimi TS, Brewer RD, Mokdad A, et al. Binge drinking among US adults. JAMA. 2003; 289:70–75. [PubMed: 12503979]
88. Reimers MJ, Hahn ME, Tanguay RL. Two zebrafish alcohol dehydrogenases share common ancestry with mammalian class I, II, IV, and V alcohol dehydrogenase genes but have distinct functional characteristics. J Biol Chem. 2004; 279:38303–38312. [PubMed: 15231826]
89. Gerlai R, Lee V, Blaser R. Effects of acute and chronic ethanol exposure on the behavior of adult zebrafish (Danio rerio). Pharmacol Biochem Behav. 2006; 85:752–761. [PubMed: 17196640]
90. Schneider AC, Machado AB, de Assis AM, et al. Effects of Lactobacillus rhamnosus GG on hepatic and serum lipid profiles in zebrafish exposed to ethanol. Zebrafish. 2014; 11:371–378. [PubMed: 24987799]
91. Tran S, Nowicki M, Chatterjee D, et al. Acute and chronic ethanol exposure differentially alters alcohol dehydrogenase and aldehyde dehydrogenase activity in the zebrafish liver. Prog Neuropsychopharmacol Biol Psychiatry. 2015; 56:221–226. [PubMed: 25290637]
92. Dlugos CA, Rabin RA. Ethanol effects on three strains of zebrafish: model system for genetic investigations. Pharmacol Biochem Behav. 2003; 74:471–480. [PubMed: 12479969]
93. Jang ZH, Chung HC, Ahn YG, et al. Metabolic profiling of an alcoholic fatty liver in zebrafish (Danio rerio). Mol Biosyst. 2012; 8:2001–2009. [PubMed: 22532405]
94. Gao B, Bataller R. Alcoholic liver disease: pathogenesis and new therapeutic targets. Gastroenterology. 2011; 141:1572–1585. [PubMed: 21920463]
95. You M, Fischer M, Deeg MA, et al. Ethanol induces fatty acid synthesis pathways by activation of sterol regulatory element-binding protein (SREBP). J Biol Chem. 2002; 277:29342–29347. [PubMed: 12036955]
96. Ji C, Chan C, Kaplowitz N. Predominant role of sterol response element binding proteins (SREBP) lipogenic pathways in hepatic steatosis in the murine intragastric ethanol feeding model. J Hepatol. 2006; 45:717–724. [PubMed: 16879892]
97. Ozaras R, Tahan V, Aydin S, et al. *N*-acetylcysteine attenuates alcohol-induced oxidative stress in the rat. World J Gastroenterol. 2003; 9:125–128. [PubMed: 12508366]
98. Nguyen-Khac E, Thevenot T, Piquet MA, et al. Glucocorticoids plus *N*-acetylcysteine in severe alcoholic hepatitis. N Engl J Med. 2011; 365:1781–1789. [PubMed: 22070475]
99. Dara L, Ji C, Kaplowitz N. The contribution of endoplasmic reticulum stress to liver diseases. Hepatology. 2011; 53:1752–1763. [PubMed: 21384408]
100. Howarth DL, Vacaru AM, Tsedensodnom O, et al. Alcohol disrupts endoplasmic reticulum function and protein secretion in hepatocytes. Alcohol Clin Exp Res. 2012; 36:14–23. [PubMed: 21790674]
101. Altekruse SF, McGlynn KA, Reichman ME. Hepatocellular carcinoma incidence, mortality, and survival trends in the United States from 1975 to 2005. J Clin Oncol. 2009; 27:1485–1491. [PubMed: 19224838]
102. Spitsbergen JM, Tsai HW, Reddy A, et al. Neoplasia in zebrafish (Danio rerio) treated with N-methyl-N'-nitro-N-nitrosoguanidine by three exposure routes at different developmental stages. Toxicol Pathol. 2000; 28:716–725. [PubMed: 11026608]
103. Spitsbergen JM, Tsai HW, Reddy A, et al. Neoplasia in zebrafish (Danio rerio) treated with 7,12-dimethylbenz[a] anthracene by two exposure routes at different developmental stages. Toxicol Pathol. 2000; 28:705–715. [PubMed: 11026607]

104. Haramis AP, Hurlstone A, van der Velden Y, et al. Adenomatous polyposis coli-deficient zebrafish are susceptible to digestive tract neoplasia. EMBO Rep. 2006; 7:444–449. [PubMed: 16439994]
105. Nguyen AT, Emelyanov A, Koh CH, et al. An inducible krasV12 transgenic zebrafish model for liver tumorigenesis and chemical drug screening. Dis Model Mech. 2012; 5:63–72. [PubMed: 21903676]
106. Mudbhary R, Hoshida Y, Chernyavskaya Y, et al. Overexpression of UHRF1 drives DNA hypomethylation and hepatocellular carcinoma. Cancer Cell. 2014; 25:1–14. [PubMed: 24654260]
107. Lu JW, Liao CY, Yang WY, et al. Overexpression of endothelin 1 triggers hepatocarcinogenesis in zebrafish and promotes cell proliferation and migration through the AKT pathway. PLoS One. 2014; 9:e85318. [PubMed: 24416389]
108. Evason KJ, Francisco MT, Juric V, et al. Identification of chemical inhibitors of beta-catenin-driven liver tumorigenesis in zebrafish. PLoS Genet. 2015; 11:e1005305. [PubMed: 26134322]
109. Lam SH, Wu YL, Vega VB, et al. Conservation of gene expression signatures between zebrafish and human liver tumors and tumor progression. Nat Biotechnol. 2006; 24:73–75. [PubMed: 16327811]
110. Shepard JL, Amatruda JF, Finkelstein D, et al. A mutation in separase causes genome instability and increased susceptibility to epithelial cancer. Genes Dev. 2007; 21:55–59. [PubMed: 17210788]
111. Li Z, Zheng W, Wang Z, et al. A transgenic zebrafish liver tumor model with inducible Myc expression reveals conserved Myc signatures with mammalian liver tumors. Dis Model Mech. 2013; 6:414–423. [PubMed: 23038063]
112. Lu JW, Yang WY, Tsai SM, et al. Liver-specific expressions of HBx and src in the p53 mutant trigger hepatocarcinogenesis in zebrafish. PLoS One. 2013; 8:e76951. [PubMed: 24130815]
113. Liu W, Chen JR, Hsu CH, et al. A zebrafish model of intrahepatic cholangiocarcinoma by dual expression of hepatitis B virus X and hepatitis C virus core protein in liver. Hepatology. 2012; 56:2268–2276. [PubMed: 22729936]
114. Yu DY, Moon HB, Son JK, et al. Incidence of hepatocellular carcinoma in transgenic mice expressing the hepatitis B virus X-protein. J Hepatol. 1999; 31:123–132. [PubMed: 10424292]
115. Goessling W, North TE, Zon LI. Ultrasound biomicroscopy permits in vivo characterization of zebrafish liver tumors. Nat Methods. 2007; 4:551–553. [PubMed: 17572681]
116. Revill K, Wang T, Lachenmayer A, et al. Genome-wide methylation analysis and epigenetic unmasking identify tumor suppressor genes in hepatocellular carcinoma. Gastroenterology. 2013; 145:1424–1435. e1–e25. [PubMed: 24012984]
117. Villanueva A, Portela A, Sayols S, et al. DNA methylation-based prognosis and epidrivers in hepatocellular carcinoma. Hepatology. 2015; 61:1945–1956. [PubMed: 25645722]
118. Goldstone JV, McArthur AG, Kubota A, et al. Identification and developmental expression of the full complement of cytochrome P450 genes in Zebrafish. BMC Genomics. 2010; 11:643. [PubMed: 21087487]
119. Driessen M, Kienhuis AS, Vitins AP, et al. Gene expression markers in the zebrafish embryo reflect a hepatotoxic response in animal models and humans. Toxicol Lett. 2014; 230:48–56. [PubMed: 25064622]
120. Burkhardt-Holm P, Oulmi Y, Schroeder A, et al. Toxicity of 4-chloroaniline in early life stages of zebrafish (Danio rerio): II. Cytopathology and regeneration of liver and gills after prolonged exposure to waterborne 4-chloroaniline. Arch Environ Contam Toxicol. 1999; 37:85–102. [PubMed: 10341046]
121. Norris W, Paredes AH, Lewis JH. Drug-induced liver injury in 2007. Curr Opin Gastroenterol. 2008; 24:287–297. [PubMed: 18408456]
122. Cox AG, Saunders DC, Kelsey PB Jr, et al. S-nitrosothiol signaling regulates liver development and improves outcome following toxic liver injury. Cell Rep. 2014; 6:56–69. [PubMed: 24388745]

123. Troxel CM, Reddy AP, O'Neal PE, et al. In vivo aflatoxin B1 metabolism and hepatic DNA adduction in zebrafish (Danio rerio). Toxicol Appl Pharmacol. 1997; 143:213–220. [PubMed: 9073610]

124. Xu H, Lam SH, Shen Y, et al. Genome-wide identification of molecular pathways and biomarkers in response to arsenic exposure in zebrafish liver. PLoS One. 2013; 8:e68737. [PubMed: 23922661]

125. Dai YJ, Jia YF, Chen N, et al. Zebrafish as a model system to study toxicology. Environ Toxicol Chem. 2014; 33:11–17. [PubMed: 24307630]

126. Wang W, Cheng S, Zhang D. Association of inorganic arsenic exposure with liver cancer mortality: a metaanalysis. Environ Res. 2014; 135C:120–125. [PubMed: 25262084]

127. Hamdi M, Yoshinaga M, Packianathan C, et al. Identification of an *S*-adenosylmethionine (SAM) dependent arsenic methyltransferase in Danio rerio. Toxicol Appl Pharmacol. 2012; 262:185–193. [PubMed: 22575231]

128. Carlson P, Van Beneden RJ. Arsenic exposure alters expression of cell cycle and lipid metabolism genes in the liver of adult zebrafish (Danio rerio). Aquat Toxicol. 2014; 153:66–72. [PubMed: 24176670]

129. Carlson P, Smalley DM, Van Beneden RJ. Proteomic analysis of arsenic-exposed zebrafish (Danio rerio) identifies altered expression in proteins involved in fibrosis and lipid uptake in a gender-specific manner. Toxicol Sci. 2013; 134:83–91. [PubMed: 23570992]

130. Zhang X, Li C, Gong Z. Development of a convenient in vivo hepatotoxin assay using a transgenic zebrafish line with liver-specific DsRed expression. PLoS One. 2014; 9:e91874. [PubMed: 24626481]

131. McGrath P, Li CQ. Zebrafish: a predictive model for assessing drug-induced toxicity. Drug Discov Today. 2008; 13:394–401. [PubMed: 18468556]

132. Padilla S, Corum D, Padnos B, et al. Zebrafish developmental screening of the ToxCast Phase I chemical library. Reprod Toxicol. 2012; 33:174–187. [PubMed: 22182468]

133. Selderslaghs IW, Blust R, Witters HE. Feasibility study of the zebrafish assay as an alternative method to screen for developmental toxicity and embryotoxicity using a training set of 27 compounds. Reprod Toxicol. 2012; 33:142–154. [PubMed: 21871558]

134. Florman S, Miller CM. Live donor liver transplantation. Liver Transpl. 2006; 12:499–510. [PubMed: 16555328]

135. Francavilla A, Zeng Q, Polimeno L, et al. Small-for-size liver transplanted into larger recipient: a model of hepatic regeneration. Hepatology. 1994; 19:210–216. [PubMed: 8276357]

136. Higgins GM, Anderson RM. Experimental pathology of the liver—restoration of the liver of the white rat following partial surgical removal. Arch Pathol. 1931:12.

137. Taub R. Liver regeneration: from myth to mechanism. Nat Rev Mol Cell Biol. 2004; 5:836–847. [PubMed: 15459664]

138. Miyaoka Y, Ebato K, Kato H, et al. Hypertrophy and unconventional cell division of hepatocytes underlie liver regeneration. Curr Biol. 2012; 22:1166–1175. [PubMed: 22658593]

139. Sadler KC, Krahn KN, Gaur NA, et al. Liver growth in the embryo and during liver regeneration in zebrafish requires the cell cycle regulator, uhrf1. Proc Natl Acad Sci U S A. 2007; 104:1570–1575. [PubMed: 17242348]

140. Kan NG, Junghans D, Izpisua Belmonte JC. Compensatory growth mechanisms regulated by BMP and FGF signaling mediate liver regeneration in zebrafish after partial hepatectomy. FASEB J. 2009; 23:3516–3525. [PubMed: 19546304]

141. Jacob V, Chernyavskaya Y, Chen X, et al. DNA hypomethylation induces a DNA replication-associated cell cycle arrest to block hepatic outgrowth in uhrf1 mutant zebrafish embryos. Development. 2015; 142:510–521. [PubMed: 25564650]

142. Goessling W, North TE, Loewer S, et al. Genetic interaction of PGE2 and Wnt signaling regulates developmental specification of stem cells and regeneration. Cell. 2009; 136:1136–1147. [PubMed: 19303855]

143. Nissim S, Sherwood RI, Wucherpfennig J, et al. Prostaglandin E2 regulates liver versus pancreas cell-fate decisions and endodermal outgrowth. Dev Cell. 2014; 28:423–437. [PubMed: 24530296]

144. Dovey M, Patton EE, Bowman T, et al. Topoisomerase II alpha is required for embryonic development and liver regeneration in zebrafish. Mol Cell Biol. 2009; 29:3746–3753. [PubMed: 19380487]

145. Curado S, Ober EA, Walsh S, et al. The mitochondrial import gene tomm22 is specifically required for hepatocyte survival and provides a liver regeneration model. Dis Model Mech. 2010; 3:486–495. [PubMed: 20483998]

146. Curado S, Anderson RM, Jungblut B, et al. Conditional targeted cell ablation in zebrafish: A new tool for regeneration studies. Dev Dyn. 2007; 236:1025–1035. [PubMed: 17326133]

147. Tarlow BD, Pelz C, Naugler WE, et al. Bipotential adult liver progenitors are derived from chronically injured mature hepatocytes. Cell Stem Cell. 2014; 15:605–618. [PubMed: 25312494]

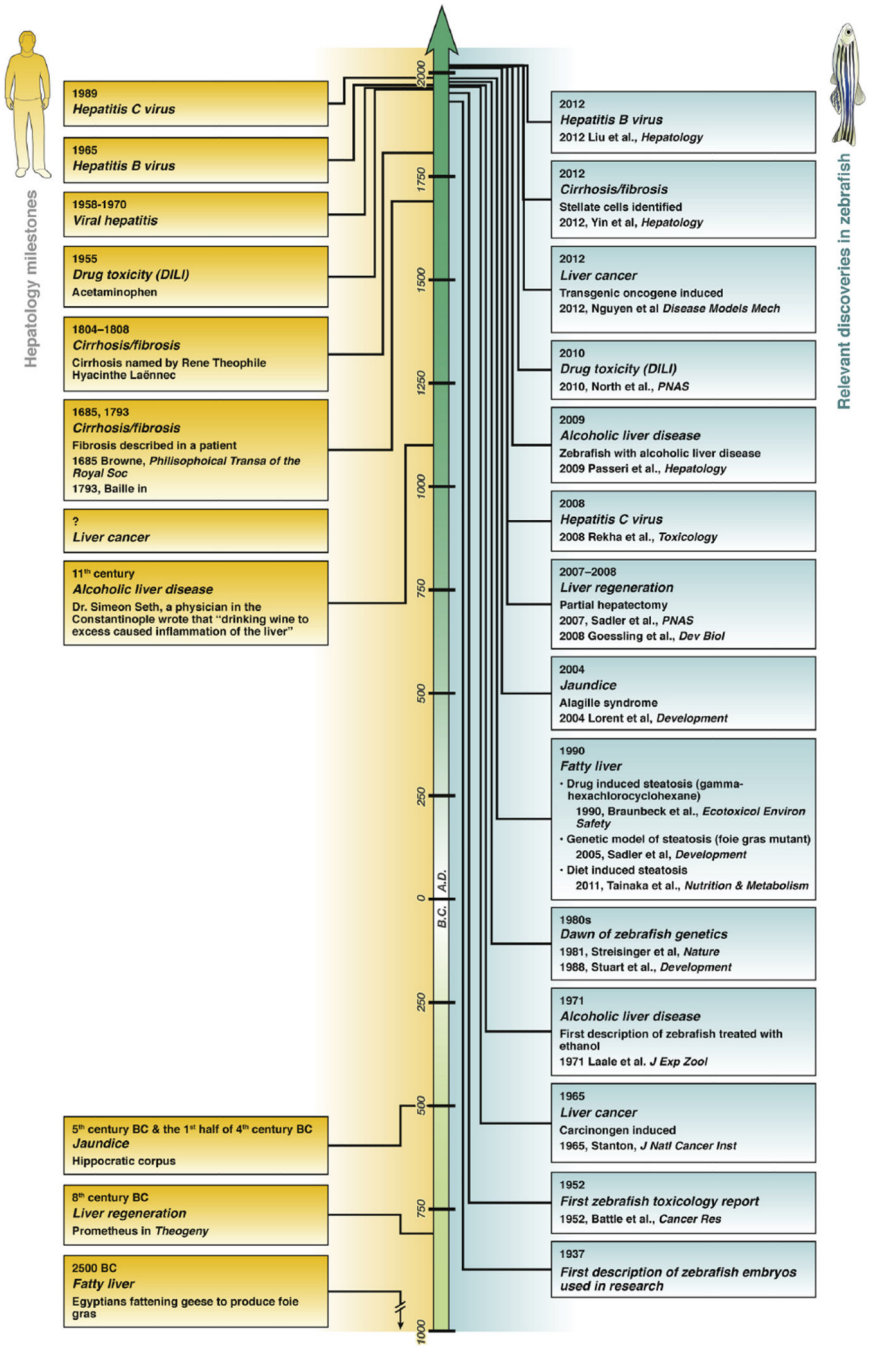



**Figure 1.**

Timeline of hepatology advances in zebrafish research. Major milestones or historic events in human liver pathology are indicated in *yellow*, and corresponding milestones in zebrafish research are indicated in *green*. The timeline indicates years B.C. and A.D.

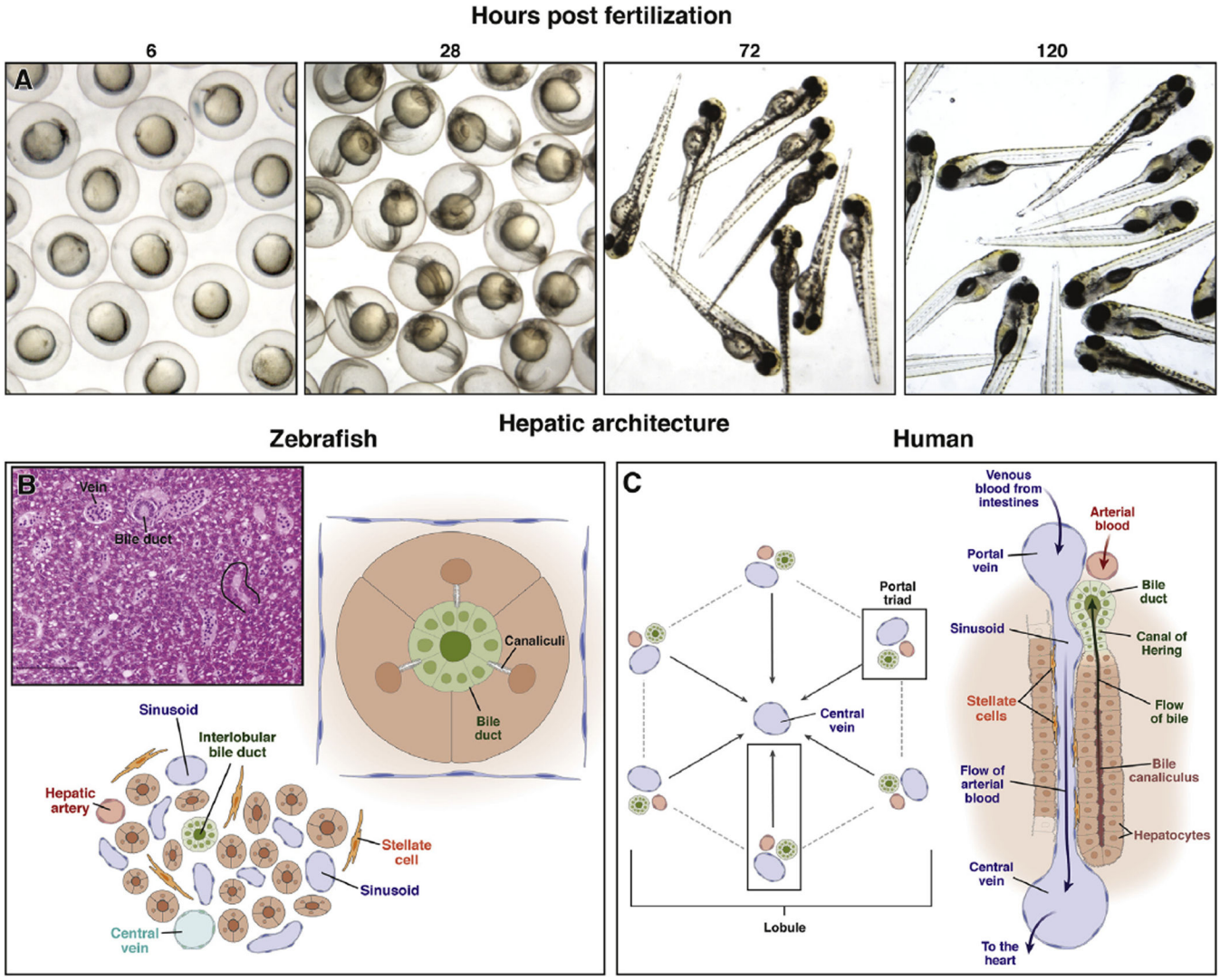



**Figure 2.**

Comparative anatomy of human and zebrafish livers. (*A*) Live zebrafish at 6, 28, 72, and 120 hours postfertilization show that a large number of synchronously developing larvae can be easily cultured. Cellular anatomy and architecture of the liver in (*B*) zebrafish and (*C*) humans. (*B*, *inset*) A histological section of adult zebrafish liver stained with H&E.

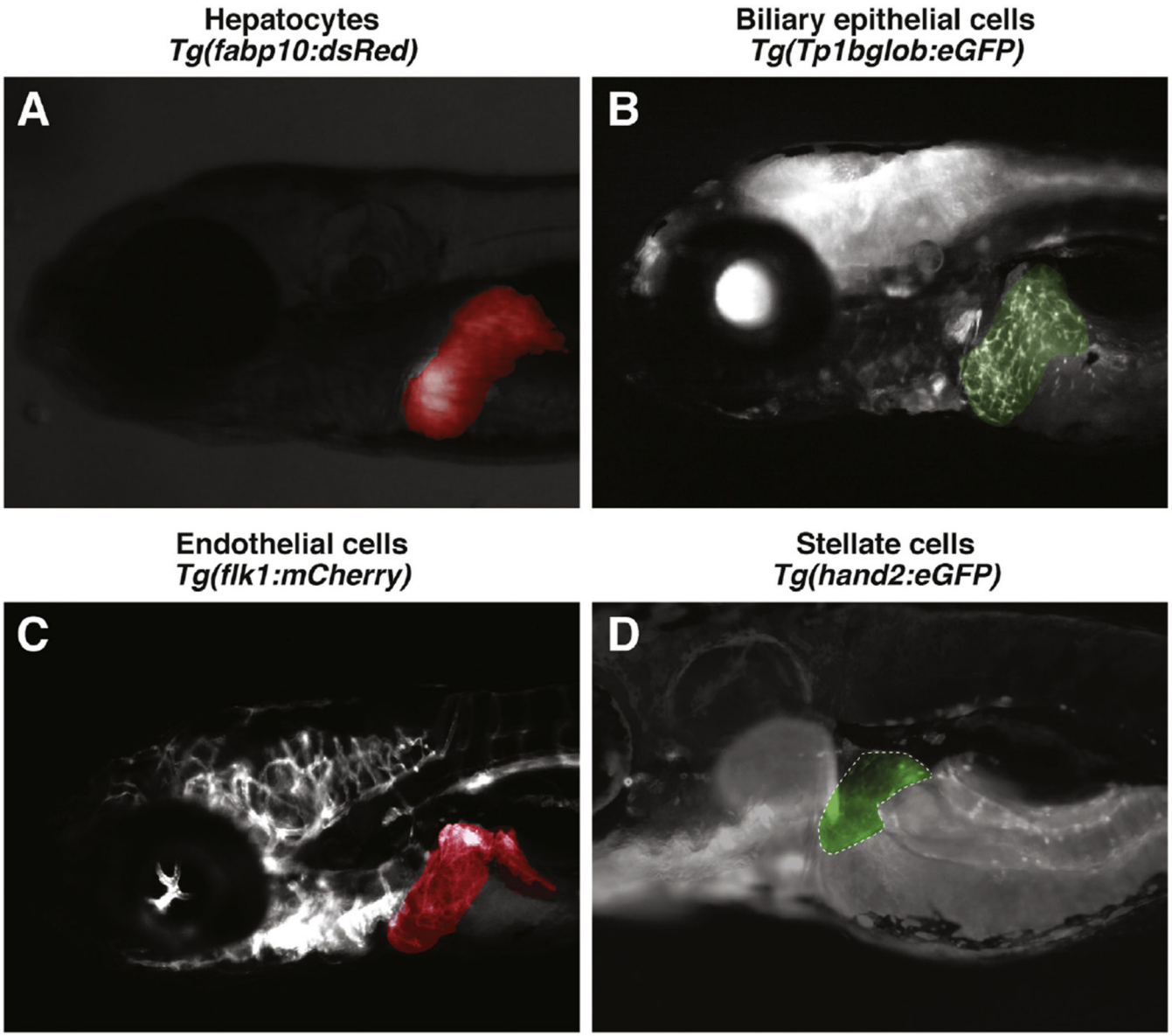



**Figure 3.**

Fluorescent zebrafish with live markers of liver cells. Images of live transgenic zebrafish on 5 dpf with fluorescent proteins expressed in (*A*) hepatocytes (*fabp10*), (*B*) biliary cells (Tp1), (*C*) endothelial cells (*flk1*), and (*D*) stellate cells (*hand2*). The image of *Tg(hand2:GFP)* larvae was provided by C. Yin.

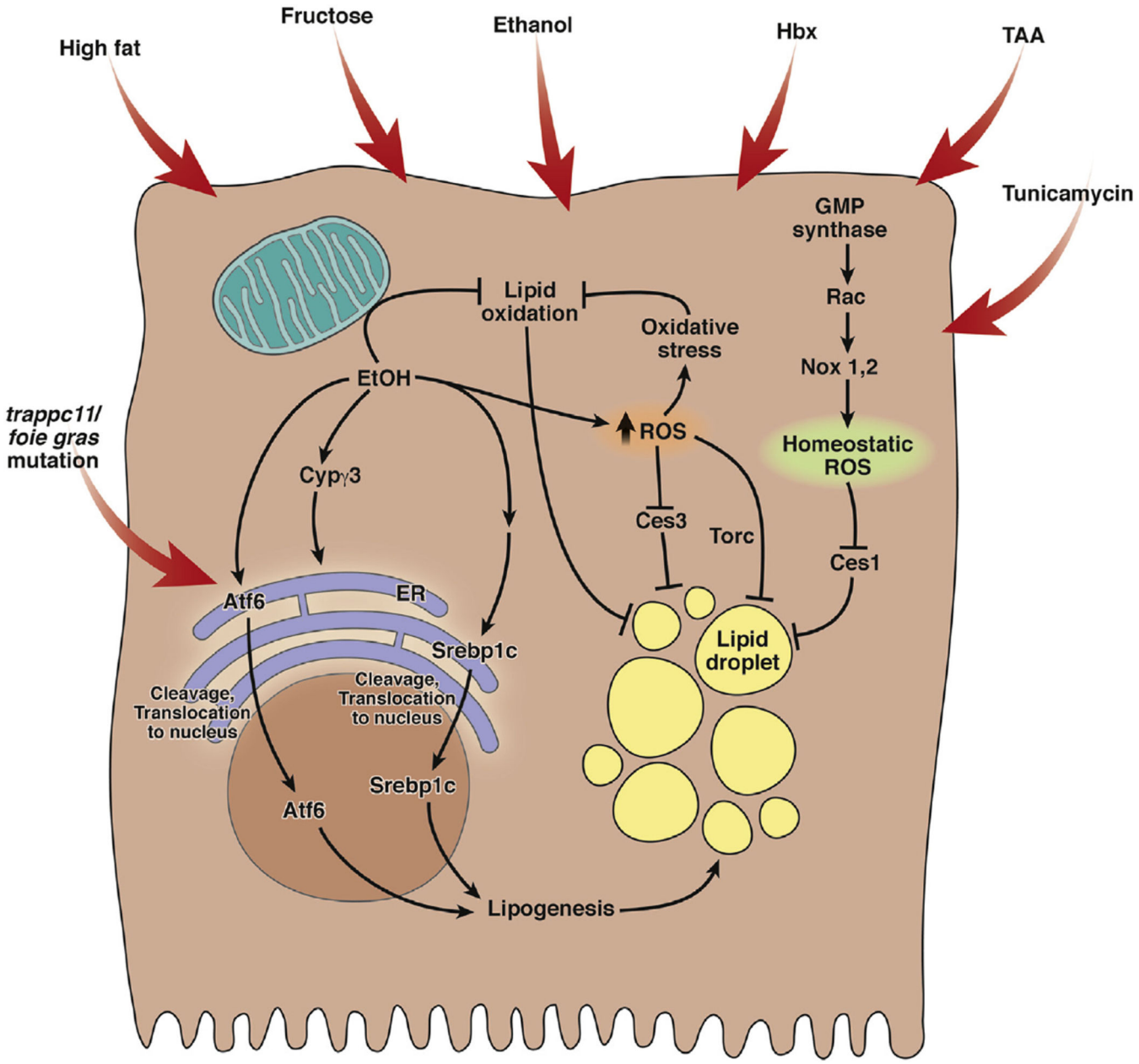



**Figure 4.**

Cell signaling and lipid metabolism insights into FLD from studies in zebrafish. Research using zebrafish has identified several factors that cause fatty liver, as indicated by the *red arrows*. The pathways illustrated in this figure are described in the text. Hbv, hepatitis B X antigen; TAA, thioacetamide; *trappc11*, trafficking protein particle complex 11.

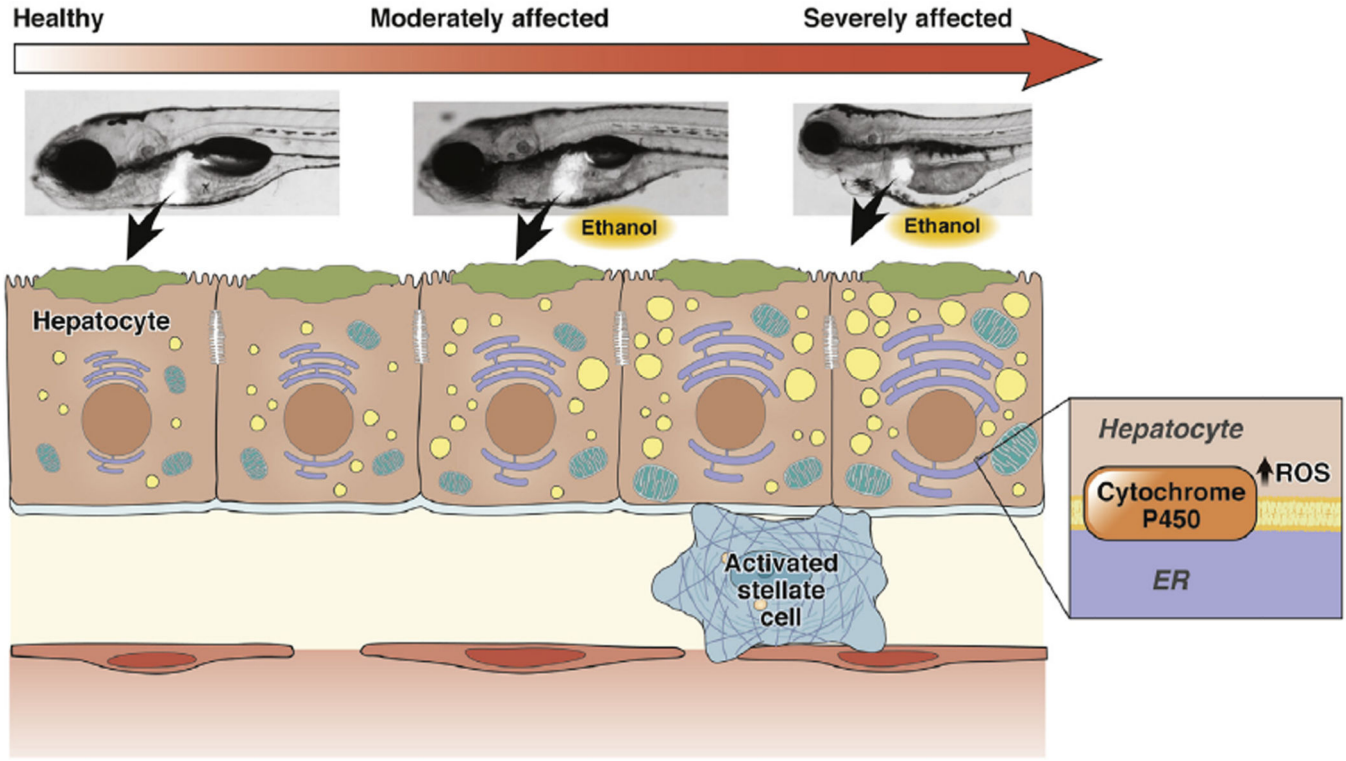
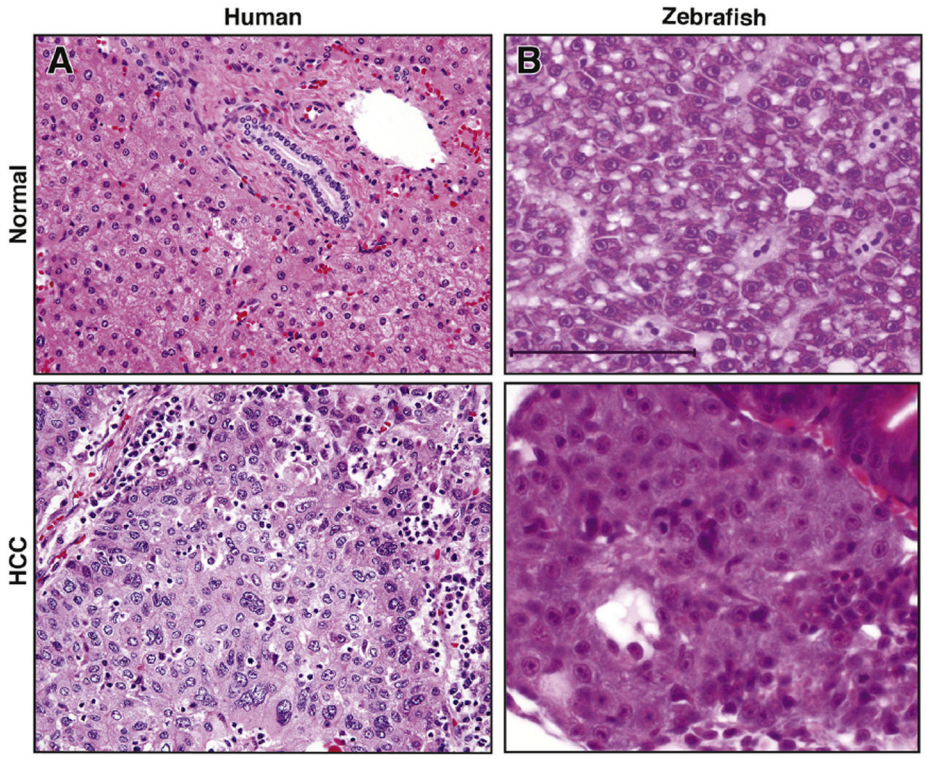



**Figure 5.**

Progressive hepatocyte response to ethanol in zebrafish. Zebrafish exposed to ethanol show signs of liver toxicity as well as gross morphological and behavioral changes that increase over time. The progression of steatosis is illustrated by the *yellow droplets* in the cytoplasm, and the activation of stellate cells at advanced stages of toxicity is illustrated in *blue*.




**Diagnostic criteria for HCC in zebrafish**

**C**

| Histological Criteria | Normal | Atypical cells | Dysplastic foci | HCC |
|---|---|---|---|---|
| **Cytological features** | | | | |
| Vacuolization | Yes | Yes | Yes/no | No |
| Mitotic figures | No | No | Rare | Yes |
| Cellular degeneration | Rare | Some | Yes | Yes |
| Pleomorphic nuclei | No | Yes | Yes | Yes |
| Prominent nucleolus | No | Yes | Yes | Yes |
| Hyperchromatic nuclei | No | Some | Some | Yes |
| Coarse chromatin | No | Yes | Yes | Yes |
| Increased N:C ratio | No | Some | Yes | Yes |
| Eosinophilic cytoplasm | Yes | Some | No | No |
| **Architecture** | | | | |
| Focal lesion | No | n/a | <10 cells | >10 cells |
| Hepatocyte crowding | No | n/a | Yes | Yes |
| Thickened trabecluae | No | n/a | Some | Yes |

**Figure 6.**

Comparative histology of HCC and the diagnostic criteria for liver cancer in zebrafish. H&E-stained sections from (*A*) normal human liver and (*B*) 20 dpf normal zebrafish liver are compared with sections from HCC in both species. (*C*) Criteria for diagnosing HCC in zebrafish.