Zebrafish models of dyslipidemia: relevance to atherosclerosis and angiogenesis

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Lipid and lipoprotein metabolism in zebrafish and in humans are remarkably similar. Zebrafish express all major nuclear receptors, lipid transporters, apolipoproteins and enzymes involved in lipoprotein metabolism. Unlike mice, zebrafish express *cetp* and the Cetp activity is detected in zebrafish plasma. Feeding zebrafish a high cholesterol diet, without any genetic intervention, results in significant hypercholesterolemia and robust lipoprotein oxidation, making zebrafish an attractive animal model to study mechanisms relevant to early development of human atherosclerosis. These studies are facilitated by the optical transparency of zebrafish larvae and the availability of transgenic zebrafish expressing fluorescent proteins in endothelial cells and macrophages. Thus, vascular processes can be monitored in live animals. In this review article, we discuss recent advances in using dyslipidemic zebrafish in atherosclerosis-related studies. We also summarize recent work connecting lipid metabolism with regulation of angiogenesis, the work that considerably benefited from using the zebrafish model. These studies uncovered the role of *aibp*, *abca1*, *abcg1*, *mtp*, *apoB*, and *apoC2* in regulation of angiogenesis in zebrafish and paved the way for future studies in mammals, which may suggest new therapeutic approaches to modulation of excessive or diminished angiogenesis contributing to the pathogenesis of human disease. (Translational Research 2014;163:99–108)

Abbreviations: AIBP = apoA-I binding protein; CE = cholesterol ester; CETP = cholesterol ester transfer protein; DsRed = red fluorescent protein from Discosoma sp.; EC = endothelial cells; EGFP = enhanced green fluorescent protein; HCD = high cholesterol diet; HDL = high-density lipoprotein; LDL = low-density lipoprotein; LPL = lipoprotein lipase; MDA = malondialdehyde; OxCE = oxidized CE; OxPC = oxidized phosphatidylcholine; PLA2 = phospholipase A_2; SeA = segmental artery; SIV = subintestinal vein; VLDL = very low-density lipoprotein

Consumption of a high cholesterol, high fat diet and genetic mutations contribute to dyslipidemia, which, in turn, is a major risk factor for atherosclerosis and cardiovascular disease. Disorders of lipid metabolism also result in dysregulation of important physiological processes and play a role in the pathogenesis of adipose tissue inflammation and insulin resistance, diabetes, steatohepatitis, renal disorders, and neurodegenerative diseases. Rodent animal models were instrumental in uncovering causes of dyslipidemia and elucidating mechanisms of diseases linked to altered lipid metabolism. Work from our laboratory contributed to the development of a zebrafish model of hypercholesterolemia and vascular lesion formation,

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Conflict of interest: None.

Work in the authors' laboratory is supported by the NIH grants HL093767 (Y.I.M.), HL055798 (Y.I.M.) and HL114734 (L.F.). All authors have read the journal's policy on conflicts of interest and wish to disclose that Y.M. has received an investigator-initiated grant from Merck and is a co-inventor of a patent owned by the University of California for the use of the hypercholesterolemic zebrafish model. Y.M. and L.F. are co-inventors of a patent owned by the University of California related to therapeutic applications of apoA-I binding protein.

Submitted for publication July 8, 2013; revision submitted September 7, 2013; accepted for publication September 10, 2013.

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1931-5244/$ - see front matter

http://dx.doi.org/10.1016/j.trsl.2013.09.004

Table I. Transgenic zebrafish and fluorescent tracers

Tools	Targets	Applications	References
Transgenic zebrafish			
fli1:EGFP	Endothelial cells	Angiogenesis, vascular lipid accumulation and inflammation	3,53
lyz:EGFP, *lyz:DsRed2*	Myeloid cells	Vascular inflammation	3,25
mpeg1:EGFP	Macrophages	Vascular inflammation, foam cell formation	24,26
Lipoprotein/lipid tracers and enzyme substrates			
DiI-LDL	LDL in circulation	LDL distribution in flow	28
cholesteryl BODIPY-C11	CE trafficking, LDL, lipid deposits	Vascular lipid accumulation	3,18
BODIPY-cholesterol	Cholesterol trafficking,	Vascular lipid accumulation,*	17,18,20
NBD-cholesterol	Lipid deposits	Intestinal cholesterol absorption and trafficking	
BODIPY-C12	Fatty acid trafficking, Lipid droplets	Intestinal fatty acid absorption and trafficking	17
PED6	PLA_2 activity	Digestive tract and vascular PLA_2 activity	3,29

Abbreviations: CE, cholesterol ester; LDL, low-density lipoprotein; PLA_2, phospholipase A_2.
*Our unpublished observations.

relevant to early stages of human atherogenesis. Zebrafish were also used to model other aspects of lipid metabolism and to elucidate mechanisms of disorders associated with dyslipidemia. In this article, we will focus on recent work in which zebrafish were used to study the connection between lipid metabolism and vascular pathology, specifically, disorganized angiogenesis and vascular lipid accumulation and lesion formation.

The advantages of a zebrafish model include the optical transparency of zebrafish embryos and larvae and the availability of effective tools of genetic manipulation. Together, they enable *in vivo* monitoring of vascular processes in live transgenic zebrafish expressing fluorescent proteins in specific vascular cell types and/or fed a diet supplemented with fluorescent lipid tracers. For example, Table I lists several studies in which transgenic zebrafish with enhanced green fluorescent protein (EGFP) or red fluorescent protein from Discosoma sp. (DsRed) expressed in endothelial cells (EC), myeloid cells, or macrophages were used, often in conjunction with fluorescent lipids and lipoproteins. Other advantages include economic colony maintenance, large numbers of progeny obtained from a single mating, and the ability of zebrafish larvae to readily absorb small molecules from water.

More importantly, notable similarities in human and zebrafish lipid metabolism, propensity to hyperlipidemia and lipoprotein oxidation make zebrafish a particularly attractive animal model for studying mechanisms of diseases triggered by dyslipidemia. The studies related to dyslipidemia and vascular pathology, highlighted in this article, contribute to the growing trend of using zebrafish models for defining disease pathways and for discovering new therapies in a broad range of human disorders.[1]

LIPOPROTEIN AND LIPID METABOLISM IN FISH

Zebrafish express all the major classes of apolipoproteins, apoA, apoB, apoC, and apoE, which share high homology with human apolipoproteins.[2-4] Antibodies against human apoB-100 and apoA-I recognize proteins of a similar molecular mass in zebrafish plasma and were used in a plate-based immunoassay to capture zebrafish lipoproteins.[3,5] During zebrafish embryonic development, yolk syncytial layer actively synthesizes apoE as well as other apolipoproteins, and produces very low-density lipoprotein (VLDL) from yolk lipids, which subsequently enters the circulation and transfers nutrient lipids to the whole body.[2] As in mammals, zebrafish microsomal triglyceride transfer protein (Mtp) functions in the VLDL assembly in the yolk during early development.[6] The presence of VLDL, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) fractions was documented in rainbow trout and zebrafish plasma, using density gradient ultracentrifugation and agarose gel electrophoresis.[3,7] HDL dominates the lipoprotein profile in zebrafish fed a normal diet. However, feeding a high cholesterol diet (HCD) increases the proportion of VLDL and LDL fractions in zebrafish plasma and results in a lipoprotein profile closely resembling that of human plasma.[3]

Fish favor lipids rather than carbohydrates as the source of energy and are hyperlipidemic and hypercholesterolemic compared with mammals.[7] Apolipoproteins comprise as much as 36% of total proteins in fish plasma compared with only 10% in humans. Compared with humans, there is more triglycerides but less cholesterol esters (CE)s in fish LDL.[7] Zebrafish express *cetp*, homologous to the human cholesterol ester transfer protein gene *CETP*, and the Cetp activity in zebrafish plasma increases with