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BMP and RA signaling cooperate to regulate Apolipoprotein C1 expression during embryonic development

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ABSTRACT

Apolipoproteins, the major components of lipoproteins, play physiological roles in lipoprotein metabolism. Contrary to the well-documented effects on plasma lipid, little is known about the function and regulation of Apolipoproteins during embryonic development. Here we have shown that *apolipoprotein C1* (*apoc1*) gene is highly expressed in the yolk syncytial layer, a structure implicated in embryonic and larval nutrition. The *apoc1* transcripts are also observed in the deep cell layer at the ventral and lateral region during gastrulation, and in the tail paraxial mesoderm during somitogenesis. By whole-mount in situ hybridization and quantified RT-RCR, we further demonstrate that *apoc1* expression is induced by bone morphogenetic proteins (BMPs) signaling, while retinoic acid (RA) signaling suppresses the expression of BMP ligands and inhibits the BMP effect in this process.

1. Introduction

Lipids play many fundamental roles during animal lifetime. These include providing substrate fuel for metabolism, use as architectural components in membrane, protection for organ insulation, and acting as chemical messengers for signaling transduction (Ryan and van, 2000; Eaton, 2008; Fahy et al., 2009; Nohturfft and Zhang, 2009; Subramaniam et al., 2011). In lower vertebrates, such as fish and frog, lipids are also prominent components of egg yolk, which consists of mass lipoproteins, phosphoproteins and lipid inclusions (Mommsen and Walsh, 1988; Gui and Zhu, 2012). The embryonic development completely depends on endogenous nutrition and energy supplied by the yolk repository. Very-low-density lipoprotein (VLDL), the major lipoprotein in teleost yolk, is rich in neutral lipids (Endo et al., 2011). As one of the protein components in VLDL, Apoc1 is transferred between VLDL and high density lipoprotein (HDL), and serves as an important mediator of VLDL metabolism (Grundy, 1990; Jong et al., 1999; Westerterp et al., 2007). Recently, we observed that teleost embryos express considerable Apoc1 (Wang et al., 2008, 2013). However, little is known about its regulative mechanism and function in yolk development and embryo nutrition.

Retinoic acid (RA), a vitamin A derivative, plays critical roles in a number of physiological processes (Duester, 2008; Niederreither and Dolle, 2008; Vilhais-Neto and Pourquié, 2008). Evidence in mammalian has established that RA acts as a ligand of nuclear receptors (Giguere et al., 1987; Petkovich et al., 1987; Mangelsdorf and Evans, 1995; Shaw et al., 2003), which regulate transcription of a wide range of target lipoprotein genes. For example, RA status regulates expression of hepatic Apolipoprotein A-I and Apolipoprotein A-II genes, both of which are associated with HDL (Grenier et al., 2007). HDL and oxidized-LDL uptake is increased by RA through upregulating expression of CD36, a type of scavenger receptor (Wuttge et al., 2001). RA induces expression of Apolipoprotein CIII that delays the catabolism of triglyceride-rich lipoprotein (VLDL and chylomicrons) (Vu-Dac et al., 1998). Furthermore, RA inhibits lipoprotein lipase activity, which hydrolyzes triglyceride from VLDL (Davies et al., 2001). Therefore, RA administration results in hypertriglyceridemia by increasing VLDL production and decreasing VLDL utilization (Vajreswari and Jeyakumar, 2008).

Bone morphogenetic proteins (BMPs) are important class of morphogens that regulate development and maintain normal tissue functions (Plouhinec et al., 2011; Guo and Wu, 2012). Recently, their roles in lipoprotein metabolism have begun to be revealed (Schulz and Tseng, 2009). Bmp2 up-regulates Apolipoprotien E, which associates with several lipoproteins and mediates their uptakes, to inhibit smooth muscle cell growth (Bachner et al., 1999; Yao et al., 2008). Apolipoprotein B100 secretion increases in HepG2 cells treated with Bmp2, and Bmp signaling regulates LDL cholesterol metabolism (Derwall et al., 2012). Additionally, Bmp1 promotes HDL formation and following

Abbreviations: Apo, apolipoprotein; BMP, bone morphogenetic protein; RA, retinoic acid; VLDL, very-low-density lipoprotein; HDL, high density lipoprotein; ATRA, all-trans retinoic acid; DMSO, dimethyl sulfoxide; DEAB, 4-diethylaminobenzaldehyde; WISH, whole mount in situ hybridization; YSL, yolk syncytial layer; qPCR, and real-time quantitative PCR; Alk8, activin receptor-like kinase 8; SMAD, drosophila mothers against decapentaplegic protein.

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reverse cholesterol transport through stimulating the maturation of newly secreted proapo A1 (Chau et al., 2007; Zhu et al., 2009). However, the function and underlined mechanism of BMPs involved in lipid metabolism is still largely unknown, and its value in the field of developmental biology is underappreciated. In this study, zebrafish was used to study the regulation of Apoc1, since this fish provides an opportunity to combine embryological, genetic and molecular analyses in vivo (Hong et al., 2014). We revealed a crosstalk between BMP and RA signaling to regulate the expression of *apoC1* during zebrafish embryogenesis.

2. Materials and methods

2.1. Fish maintenance

Zebrafish (*AB strain) stocks and embryos were raised at 28.5 °C and stage-matched based on morphological criteria (Mei et al., 2009; Wang et al., 2011). The animal treatments were approved by the Institute of Hydrobiology Institutional Animal Care and Use Committee (Approval ID: keshuizhuan 0829).

2.2. Whole-mount in situ hybridization and immunofluorescence staining

In situ hybridization and immunofluorescence staining were carried out as previously described (Li et al., 2013; Xu et al., 2014). Riboprobes for *bmp2b* (GenBank: BC114256) (Nikaido et al., 1997), *bmp4* (GenBank: BC078423) (Chin et al., 1997) and *apoc1* (GenBank: CN326047) (Wang et al., 2013) were made by using DIG RNA labeling kit (Roche). Anti-phosphorylated Smad1/5/8 antibody (CST) was used at 1:200.

2.3. Morpholino oligonucleotides, constructs and mRNA injections

Morpholino oligonucleotides of *bmp2b* (Lele et al., 2001), *bmp4* (Leung et al., 2005), *bmp7* (GenBank: AF201379) (Lele et al., 2001), *alk8* (GenBank: AF038425) (Bauer et al., 2001) were obtained from Gene Tools. *smad1* and *smad5* morpholinos were kindly provided by Yong-hua Sun (IHB). The mRNA was synthesized by using Message Machine-Kit (Ambion). The microinjections were performed as described previously (Liu et al., 2009; Zhong et al., 2014).

2.4. RA treatments

Embryos at 4 hpf (hours post fertilization) or 12 hpf were incubated in 0.5 μM ATRA (Sigma), 16 μM DEAB (sigma) or DMSO (control) in E3 embryo media. Early embryos were collected and fixed for in situ hybridization at 85% epiboly, while late embryos at 26 hpf.

3. Results

3.1. Expression pattern of apoc1 during embryogenesis in zebrafish

Zebrafish *apoc1* is a maternal gene and the zygotic message is initially transcribed at the blastula stage as the previous report (Wang et al., 2013), in which we examined its expression in early embryogenesis. In this study, we first analyzed the expression pattern later than late gastrulation by using whole mount in situ hybridization (WISH). During gastrulation, *apoc1* is expressed gradually in the blastoderm at the epiboly stage, showing the highest levels on the ventral side and progressively lowering levels toward the dorsal axis (Fig. 1A and B). The ventral *apoc1* is mainly resided in the margin (Fig. 1B), where is presumptive ventral mesoderm. As embryo develops, it becomes more posterior and is restricted in the paraxial mesoderm at the somite stages (Fig. 1C–E). The caudal expression of *apoc1* decreases gradually and disappears at the high-pec stage (Fig. 1F and G). At the pec-fin stage, *apoc1* is expressed in the liver primordium (Fig. 1H), which will develop to be liver, an adult organ with the highest expression level of *apoc1* (Lauer et al., 1988).

At the 85% epiboly stage, *apoc1* message begins to be detectable in the entire yolk syncytial layer (YSL) (Fig. 1B, arrow), similar to other apolipoproteins that have been reported in fish (Babin et al., 1997; Poupard et al., 2000; Xia et al., 2008). YSL is a layer consisting of a membrane-enclosed group of nuclei on top of the yolk cells and beneath the blastoderm cells. The expression in YSL becomes stronger at the following developmental stages (Fig. 1C–H). In addition, *apoc1* message is also detected in prechordal plate at the gastrulation (Fig. 1A and B, blank arrowheads). Therefore, *apoc1* is expressed in several territories during zebrafish development. In this paper, we selected the late epiboly stage and the 26-somite stage to investigate the regulation of *apoc1* gene expression, since the patterns at these stages are representative.

3.2. apoc1 is regulated by BMP signaling

The expression pattern of *apoc1* at the gastrula stage is similar as that of BMP ligands, which appear higher expression levels on the ventral side and lower levels on the dorsal side (Kishimoto et al., 1997; Nikaido et al., 1997; Dick et al., 2000). This suggests that *apoc1* expression would be involved with BMP signaling. In this study, two independent approaches, WISH and real-time quantitative PCR (qPCR), were used to evaluate gene expression level in response to the treatments. Activin receptor-like kinase 8 (Alk8) was identified in zebrafish as a

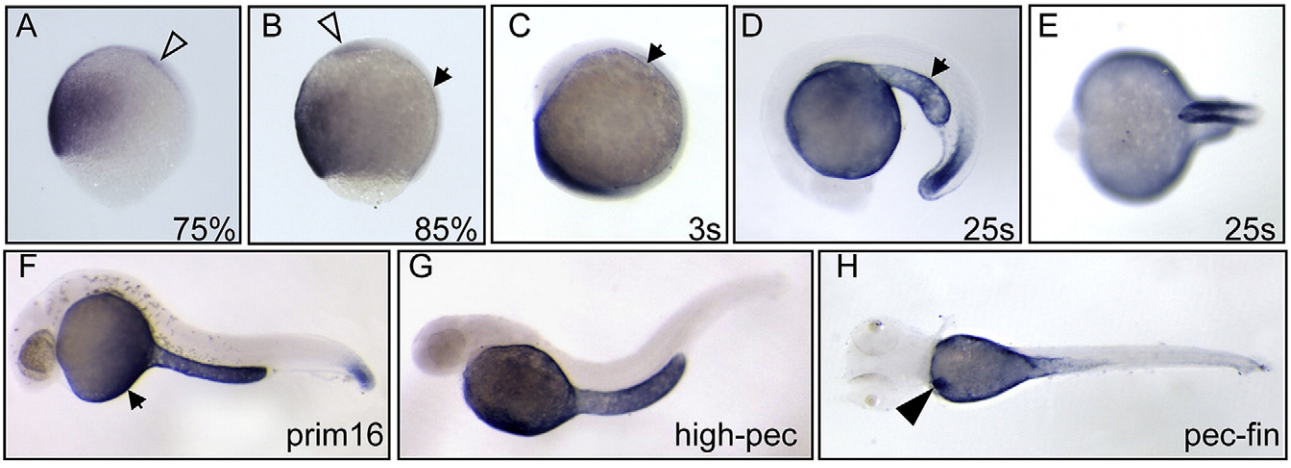

Fig. 1. Spatiotemporal expression pattern of *apoc1* in zebrafish embryos. Dorsal to the right at early stages (A–C) and head to the left at late stages (D–H). Lateral view unless indicated; H: dorsal view; E: ventral view. prechordal plate is indicated by blank arrowheads. YSL is indicated by arrows. Liver region is indicated by black arrowhead.