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Ancestral genetic complexity of arachidonic acid metabolism in Metazoa

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ABSTRACT

Eicosanoids play an important role in inducing complex and crucial physiological processes in animals. Eicosanoid biosynthesis in animals is widely reported; however, eicosanoid production in invertebrate tissue is remarkably different to vertebrates and in certain respects remains elusive. We, for the first time, compared the orthologs involved in arachidonic acid (AA) metabolism in 14 species of invertebrates and 3 species of vertebrates. Based on parsimony, a complex AA-metabolic system may have existed in the common ancestor of the Metazoa, and then expanded and diversified through invertebrate lineages. A primary vertebrate-like AA-metabolic system via cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P450 (CYP) pathways was further identified in the basal chordate, amphioxus. The expression profiling of AA-metabolic enzymes and lipidomic analysis of eicosanoid production in the tissues of amphioxus supported our supposition. Thus, we proposed that the ancestral complexity of AA-metabolic network diversified with the different lineages of invertebrates, adapting with the diversity of body plans and ecological opportunity, and arriving at the vertebrate-like pattern in the basal chordate, amphioxus.

1. Introduction

Eicosanoids, oxygenated metabolites of certain polyunsaturated fatty acids (PUFAs), have been identified in all major phyla and participate in diverse crucial physiological actions [1–3]. Also, eicosanoids are present in all tissues and body fluids of mammals, and play important roles in physiological processes and combating diseases [4–6]. In mammals, eicosanoids are synthesized through the major enzymatic oxidative pathway and non-enzymatic oxidative pathway [5]. An outline of eicosanoid biosynthesis in mammals, including COX, LOX, and CYP pathways, is summarized in Fig. 1. Vertebrate COX catalyzes the rate-limiting step in the production of prostaglandins (PGs) from arachidonic acid (AA). In two reaction steps, AA is firstly converted to prostaglandin G2 by the cyclooxygenase activity, subsequently converted to the prostaglandin H2 via the peroxidase activity. LOX is non-heme iron-containing dioxygenase that catalyzes the stereo-specific peroxidation of AA to a variety of eicosanoids such as lipoxins (LXs), leukotrienes (LTs), and hydroxyeicosatetraenoic acid (HETEs) [7]. The oxygenation reaction of LOX generally consists of four elementary steps, including hydrogen abstraction, radical rearrangement, oxygen insertion, and peroxy radical reduction [8]. The CYP activity consists of two main branches of ω-hydroxylation and epoxygenase reaction. ω-Terminal hydroxylation forms C_{16}–C_{20} alcohols of AA, such as 16-, 17-, 18-, 19-, and 20-HETEs; and epoxygenase reaction results in the production of *cis*-epoxyeicosatrienoic acids (EETs), such as 14,15-, 11,12-, 8,9-, and 5,6-EETs [7,9,10]. Furthermore, some CYP epoxygenases have also the strong ω-hydroxylase activity [7], which directly determines the complexity of eicosanoid production from CYP pathway.

In invertebrates, eicosanoids play important roles at the cellular, organismal, and ecological levels in fundamental biological processes, including oocyte maturation, salt and water transport, cellular immune defenses, and mediating certain host–parasite and predator–prey interactions [11–15]. In some invertebrates, patterns of eicosanoid production are phylum- or class-specific processes. In several species of coral, high concentrations of endogenous PG esters have been identified [16,17], and the functions of coral COX in catalyzing the transformation of AA into PGs are also well-characterized [18–21]. Coral 8R-LOX participates in prostanoids (clavulones) production likely through allene oxide intermediate pathway for chemical defense [22]. In sea squirt, the wide tissue distribution of 12-hydroxyeicosapentaenoic acid (12-HEPE) and 8-HEPE imply the activities of 8- and 12-LOX enzymes [23]. In *Hydra vulgaris*, 11(R)-HETE is produced by strong LOX activity to enhance the average number of tentacles [24]. In starfish, 8*R*-HETE isomer from AA is produced by oocyte to trigger the oocyte maturation [25]. In starfish, no classic PGs, including PGE_2 or PGD_2, exist in ionophore-challenged coelomocytes, but 8-HETE and the lower level of 8-HEPE are found [26]. *Drosophila melanogaster* lacks the homologous enzymes

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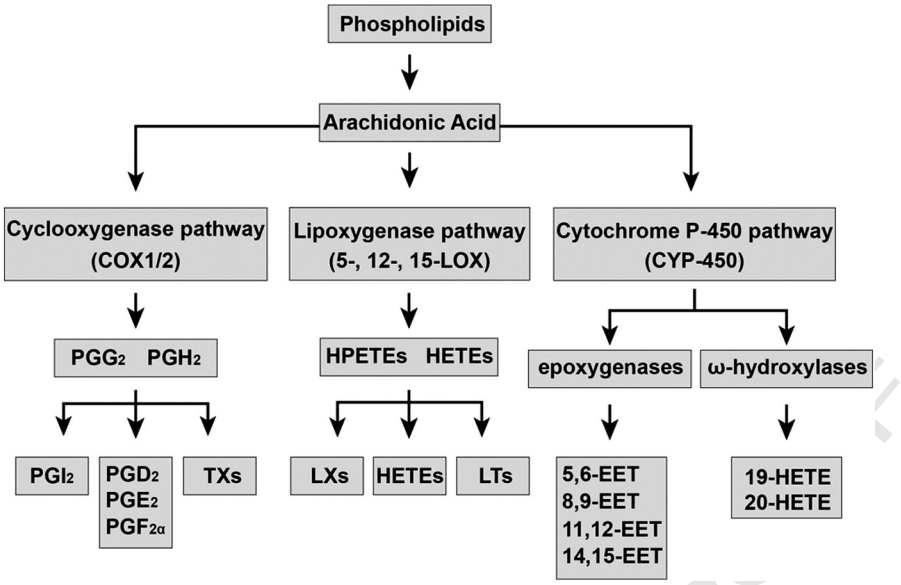

Fig. 1. The pathways of vertebrate AA metabolism. PGX — prostaglandin G_2, H_2, I_2, D_2, E_2, $F_2\alpha$; TXs — thromboxanes; LXs — lipoxins; LTs — leukotrienes; EETs — epoxyeicosatrienoic acids; HETE — hydroxyeicosatetraenoic acid.

for the synthesis of eicosanoids, and also lacks the substrates C_{20} and C_{22} PUFAs [27,28]. In *Caenorhabditis elegans*, COX-independent F series of PGs are produced to promote sperm guidance [12,29]. Thus, eicosanoids evolved their roles concurrently with the diversification and elaboration of metazoan body plans.

However, the dynamic patterns of AA-metabolic system in the evolution of multicellular organisms remain elusive. Elucidating the evolution of AA-metabolic system in the metazoan will aid in understanding eicosanoid recruitment in the diversification and elaboration of the metazoan body plan. Comparison of genomic and EST sequences is a powerful approach to exploring this topic. We conducted analyses of AA-metabolic enzymes via genomic and EST comparisons of four protostome phyla: Mollusca, Annelida, Arthropoda, and Nematoda; three major deuterostome phyla: Echinodermata, Hemichordata, and Chordata. We also included the basic metazoan Cnidaria (corals and sea anemones) and the basal eumetazoan Placozoa, *Trichoplax adhaerens* for the analyses. Consequently, a series of analyses on sequences and domain comparisons, sequence-based phylogenesis, and functional sites were conducted to elucidate the ancient precursor of AA-metabolic system in the evolution of Metazoa and the origin of this system in vertebrates.

2. Materials and methods

2.1. Identification and annotation of AA-metabolic gene homologs in amphioxus

Three approaches were used to identify the AA-metabolic genes in the amphioxus, *Branchiostoma floridae*. The assembly release v.1 and v.2 of its genome were obtained from the DOE Joint Genome Institute (http://genome.jgi-psf.org/Brafl1/Brafl1.home.html). The whole-genome shotgun (WGS) assembly from an individual male Chinese amphioxus *Branchiostoma belcheri* was obtained from http://222.200.186.228/cgi-bin/gbrowse?source=Branchiostoma belcheri V2. Domain analyses of AA-metabolic genes, with other functional genes, were conducted by IprScan (InterPro Domain Scan). The genes were further identified via tBLASTn. Subsequently, the KEGG lipid metabolism and GO term annotations in JGI were used to analyze AA-metabolic genes. The gene models involved in AA metabolism were identified and manually curated based on the haplotype of *B. floridae* v.2.0, and validated by National Center for Biotechnology Information (NCBI) BLAST and our *B. belcheri* genome sequences. Homologous searches were conducted to verify the reliability of non-conserved domains and novel protein architectural sequences with the BLASTALL program in conjunction with the NCBI *B. floridae* EST data set and our *B. belcheri* genome and EST data set.

2.2. Identification and annotation of the homologs in other species

Protein sequences of *Ciona intestinalis* (sea squirt), *D. melanogaster* (fruitfly), *C. elegans*, and human were downloaded from the Ensemble database, and then the same sequences were removed. The *Strongylocentrotus purpuratus* (sea urchin) and *Saccoglossus kowalevskii* (acorn worm) genomes were searched in the genome project of Baylor College of Medicine (http://www.hgsc.bcm.tmc.edu). The genomes of *Lottia gigantean* (gastropod), *Capitella teleta* (polychaete), *Helobdella robusta* (leech), *Daphnia pulex* (water flea), *Nematostella vectensis* (sea anemone), and *Trichoplax* were searched in the DOE Joint Genome Institute (http://genome.jgi-psf.org). The orthologs of *Acropora digitifera* (coral) were obtained from the OIST Marine genomics unit (http://marinegenomics.oist.jp/genomes/viewer?project_id=3). Domain analyses were conducted by IprScan (InterPro Domain Scan) database. The proteins were further identified by the NCBI GenBank and UCSC database (http://genome.ucsc.edu/cgi-bin/hgGateway). Finally, we used tBLASTn and profile SMART (http://smart.embl-heidelberg.de/) analyses to identify the proteins in these organisms. Although the tBLASTn method can identify an ortholog with the most homologous hits in a specific organism through the organism restricted search options, tBLASTn analysis may not detect AA-metabolic genes with limited similarity to known genes, such as lineage-specific genes. Thus, SMART searching was used to curate the domain of AA-metabolic genes. The partial genomes of *Mus musculus* (house mouse), *Xenopus laevis* (African clawed frog), *Oncorhynchus mykiss* (rainbow trout), *Danio rerio* (zebrafish), *Gersemia fruticosa* (coral), and *Bos taurus* (bovine) were downloaded from NCBI GenBank.

2.3. Comparative and phylogenetic study

Phylogenetic analyses were based on protein sequences and the alignments were obtained with the CLUSTALX V2 program. Sequences of poor quality were reciprocally deleted, and neighbor-joining (NJ) methods for the phylogenetic analyses were performed based on the

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