CYCLOOXYGENASE ISOFORMS IN ZEBRAFISH (*DANIO RERIO*)

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Cyclooxygenase is a rate-limiting enzyme for biosyntheses of prostaglandins that are a well known mediator of physiologic function in many mammalian tissues. Two isoforms of cyclooxygenase have been identified by molecular approaches, a constitutive form (COX-1) and an inducible form (COX-2). The two forms share similar enzymatic properties, but differ markedly with respect to cellular expression pattern and regulation (DeWitt, D., et al., Cell 83:345-348, 1995). In general, COX-1 is expressed constitutively in a wide variety of tissues and is considered to have "house keeping" functions. COX-2 is much more restricted in its expression and can be dramatically induced by cytokines as well as mitogenic factors. Recent studies suggest that COX-2 is also involved in regulation of physiological processes in different organ systems including kidney. Within the kidney, both COX isoforms are predominately expressed in renal medulla where COX-2 expression is stimulated by high salt diets or dehydration but COX-1 is not, suggesting distinct function in regulation of salt and water excretion (Yang, T., Am. J. Physiol. 274:F481-F489, 1998; Yang, T. at al., Am. J. Physiol. 277:F1-F9, 1999). Little is known about this pathway in fish. The aims of this study is to identify COX isoforms and to further study their expression in zebrafish.

Analysis of 2 EST clones obtained from available zebrafish ESTs permitted determination of the full length zebrafish COX-2 cDNA including 80 bp 5'UTR, 1803 bp reading frame, and 150 bp 3'UTR. The deduced amino acid sequence (601 AA) is 68-72% identical to mammalian COX-2 and 58-60% identical to mammalian COX-1. The active site containing S530, Y385, H386, and H388 is conserved between zebrafish and mammalian COX-2. RT-PCR using zebrafish COX-2-specific primers was performed on total RNA isolated from various organs from adult zebrafish, and mRNA was detected in kidney and fin, but not in brain, heart, muscle, and gill. A single EST clone (2 kb insert) was identified in the zebrafish EST database as highly homologous to COX-1. Preliminary sequencing analysis indicates that this clone is comprised of 70 bp 5'UTR, 1800 bp coding region, and 120 bp 3'UTR. Analysis of the coding region is currently underway. Comparison of available nucleotide sequence with mammalian COX isoforms reveals an identity of 71% for mammalian COX-1 and 57 % for mammalian COX-2. In adult zebrafish, RT-PCR amplified COX-1 mRNA only in kidney, but not in fin.

To study nephron localization of COX-2 in zebrafish, RT-PCR was performed on total RNA isolated from microdissected nephron segments including glomerulus (Glom), proximal tubules (PT), and colleting ducts (CD). Zebrafish ß-actin was used for normalization. COX-2 was abundantly expressed in CD, and was detected at lower levels in PT, but not in Glom. COX-1 mRNA was exclusively present in CD, but not in Glom and PT.

In summary, we identified two COX isoforms, COX-1 and COX-2, from the zebrafish EST database. Both isoforms are constitutively expressed in the kidney and further localized to collecting ducts. Further studies are underway to characterize the regulatory profile of the two COX isoforms in response to inflammatory stimuli and to study the possible role of COX-2 in nephrogenesis in zebrafish.

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