2010 Master Thesis Abstract

Biological Functions and Characterization of Soluble Epoxide Hydrolase (sEH) in *Xenopus laevis* Embryos

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The soluble Epoxide Hydrolase (sEH) has two catalytic domains, *N*-terminal phosphatase domain and *C*-terminal EH domain. The endogenous substrate for EH activity is identified as epoxyeicosatrienoic acid (EET) which is produced by cytochrome P450 from arachidonic acid but the substrate for phosphatase activity is unknown although some candidates are reported. EET has multi-functions and thought to be an important clinical target. In previous study of our laboratory, EET was proved to induce the growth of cancer cells and sEH contributes to the function. However, the functions of EET and roles of sEH in cell growth remain to be elucidated. As sEH contributes to cell growth, it is easy to speculate the contribution of sEH to embryonic development. Final goal of this study is to clear the mechanism of sEH function in the embryonic development. In the developmental study, *Xenopus laevis* was using as an animal model but *Xenopus* sEH was not characterized. First in this study, sEH was isolated from *Xenopus laevis* embryos to characterize its enzyme activity and to investigate biological functions. Homology percent of deduced amino acid sequence from *Xenopus sEH* (*xsEH*) cDNA with human sEHis 56%. Using embryos at various stages (st. 0, 10.5, 18, 29 and 38), expression of *xsEH* mRNA level was increased by development. In the whole mount *in situ* hybridization (WISH) at the 29/38 stage, strong expression of *xsEH* was observed in lens, optic vesicle, cement gland, hatching gland, brachial arches/gill and somite. Secondly, catalytic activity of xsEH was investigated. Originally isolated *xsEH* cDNA in this study had amino acid substitutions at 29th Thr to Asn and 146th Arg to His, compared with *xsEH* cDNA reported in GenBank (designated wild type *xsEH*). Originally isolated cDNA designated mutant 1 and the wild *xsEH* cDNA was prepared by site-directed mutation from mutant 1. Their proteins were expressed in *E. coli* and purified. Mutant 1 and wild xsEH had significant EH activity but lacked phosphatase activity. Contribution of the epoxide hydrolase and phosphatase domain play an important role in *VEGF* expression from previous study of our laboratory. Thirdly, the effects of xsEH over-expression on *VEGF* mRNA expression was observed with a human hepatoma cell line, Hep3B. As expected, over-expression of human sEH reduced the *VEGF* mRNA expression but over-expression of xsEH, which lacks phosphatase activity, did not affect *VEGF* mRNA expression. Start from this investigation will give contribution to clarification of the physiological role of this enzyme in development.