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Phylogenetic characterization of *Clonorchis sinensis* proteins homologous to the sigma-class glutathione transferase and their differential expression profiles

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ABSTRACT

Glutathione transferase (GST) is one of the major antioxidant proteins with diverse supplemental activities including peroxidase, isomerase, and thiol transferase. GSTs are classified into multiple classes on the basis of their primary structures and substrate/inhibitor specificity. However, the evolutionary routes and physiological environments specific to each of the closely related bioactive enzymes remain elusive. The sigma-like GSTs exhibit amino acid conservation patterns similar to the prostaglandin D synthases (PGDSs). In this study, we analyzed the phylogenetic position of the GSTs of the biocarcinogenic liver fluke, *Clonorchis sinensis*. We also observed induction profile of the GSTs in association with the parasite's maturation and in response to exogenous oxidative stresses, with special attention to sigma-class GSTs and PGDSs. The *C. sinensis* genome encoded 12 GST protein species, which were separately assigned to cytosolic (two omega-, one zeta-, two mu-, and five sigma-class), mitochondrial (one kappa-class), and microsomal (one membrane-associated proteins in eicosanoid and glutathione metabolism-like protein) GST families. Multiple sigma GST (or PGDS) orthologs were also detected in *Opisthorchis viverrini*. Other trematode species possessed only a single sigma-like GST gene. A phylogenetic analysis demonstrated that one of the sigma GST lineages duplicated in the common ancestor of trematodes were specifically expanded in the opisthorchiids, but deleted in other trematodes. The induction profiles of these sigma GST genes along with the development and aging of *C. sinensis*, and against various exogenous chemical stimuli strongly suggest that the paralogous sigma GST genes might be undergone specialized evolution to cope with the diverse hostile biochemical environments within the mammalian hepatobiliary ductal system.

1. Introduction

Glutathione transferase (GST; EC 2.5.1.18) plays a major role in detoxification of diverse xenobiotics and endogenously derived hydrophobic substances by conjugating reduced glutathione (GSH) with an electrophilic center of the harmful molecules (phase II detoxification process) [1]. The soluble and non-toxic peptide derivatives are also excreted or compartmentalized by phase III detoxification proteins [2,3]. Certain GST proteins exhibit biochemical properties similar to those of peroxidase [4], maleylacetoacetate isomerase [5], thiol transferases [6], and heme-/bile acid-binding proteins [7,8]. The non-catalytic functions of GSTs are associated with signaling processes [9]. The detoxifying proteins also appear to mediate synthesis of prostaglandin and leukotrienes [10], and catabolism of aromatic amino acids [11]. Mammalian GSTs are grouped into diverse classes including alpha, mu, pi, omega, sigma, theta, and zeta, mainly on the basis of their amino acid conservation patterns in the catalytic sites and substrate/inhibitor specificity, although some GSTs show mosaic patterns of enzymatic properties [12]. Members of multiple GST classes have been identified in the trematode parasites including *Clonorchis* and *Fasciola* spp. [13–15].

Of the multiple GST classes, sigma-class GSTs have attracted special attention, since the proteins share structural properties with

Abbreviations: C-H_2O_2, cumene hydroperoxide; CDS, coding DNA sequence; ESP, excretory-secretory products; GSH, reduced glutathione; GST, glutathione transferase; I-3-C, indole-3-carbinol; MAPEG, membrane-associated proteins in eicosanoid and glutathione metabolism; PB, piperonyl butoxide; PG, prostaglandin; PGDS, PGD synthase; PGS, PG synthase.

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the GSH-dependent, hematopoietic prostaglandin D synthases (PGDSs) [10,16]. Prostaglandins (PGs) are a group of oxygenated eicosanoids associated with the control of various defense and homeostatic responses in animals. Several species of PGs including PGI2, PGF2α, PGD2, and PGE2 are synthesized from polyunsaturated fatty acids, most notably arachidonic acid, via the actions of cyclooxygenases and a series of PG synthases (PGS), and demonstrate specialized biological functions [17]. PGD2 produced from PGH2 by PGDS in mast cells largely exerts its role in inflammatory responses by recruiting Th2 cells, eosinophils, and basophils. The molecule also participates in the pathogenesis of allergic diseases, vasodilatation, and male baldness in mammals. In nematodes, excretory PGDS or sigma GST might modulate host immune responses through PGD2 production [18]. *Schistosoma mansoni* cercariae also utilize PGs during the penetration into the definitive host [19]. The actual activity of sigma GST for the generation of PGD2 has been empirically demonstrated with the *Schistosoma haematobium* and *Fasciola hepatica* proteins [20,21]. However, the general roles and evolutionary episodes of helminth parasites largely remain elusive.

Clonorchis sinensis is a hermaphroditic trematode that resides in the bile ducts of mammalian hosts including humans. Human infections occur by eating raw or undercooked freshwater fish harboring infective metacercariae. Clonorchiasis is prevalent in several areas of Asian countries, where it causes great socio-economic and public health burdens [22]. Approximately 35 million people suffer from clonorchiasis [23]. More importantly, recent epidemiological studies have indicated that chronic infections with *C. sinensis* are closely related with the development of a biliary ductal carcinoma (cholangiocarcinoma) in humans [24,25]. The liver is a special organ where exogenous and endogenous xenobiotics/drugs accumulated and are converted into diverse metabolic derivatives. It seems reasonable to consider that hepatobiliary parasites, such as *C. sinensis*, *Opisthorchis viverrini*, and *Fasciola* spp., have been equipped with specialized mechanisms to cope with the harmful oxidative/xenobiotic environments within the hosts.

Excretory and/or tegumental GST proteins might constitute the front-line effector system against toxic or oxidative molecules derived from the hosts. Detoxifying enzymes are substantially detected in the excretory-secretory products (ESP) of *F. hepatica* [26], *O. viverrini* [27] and *C. sinensis* [15]. GST proteins are the

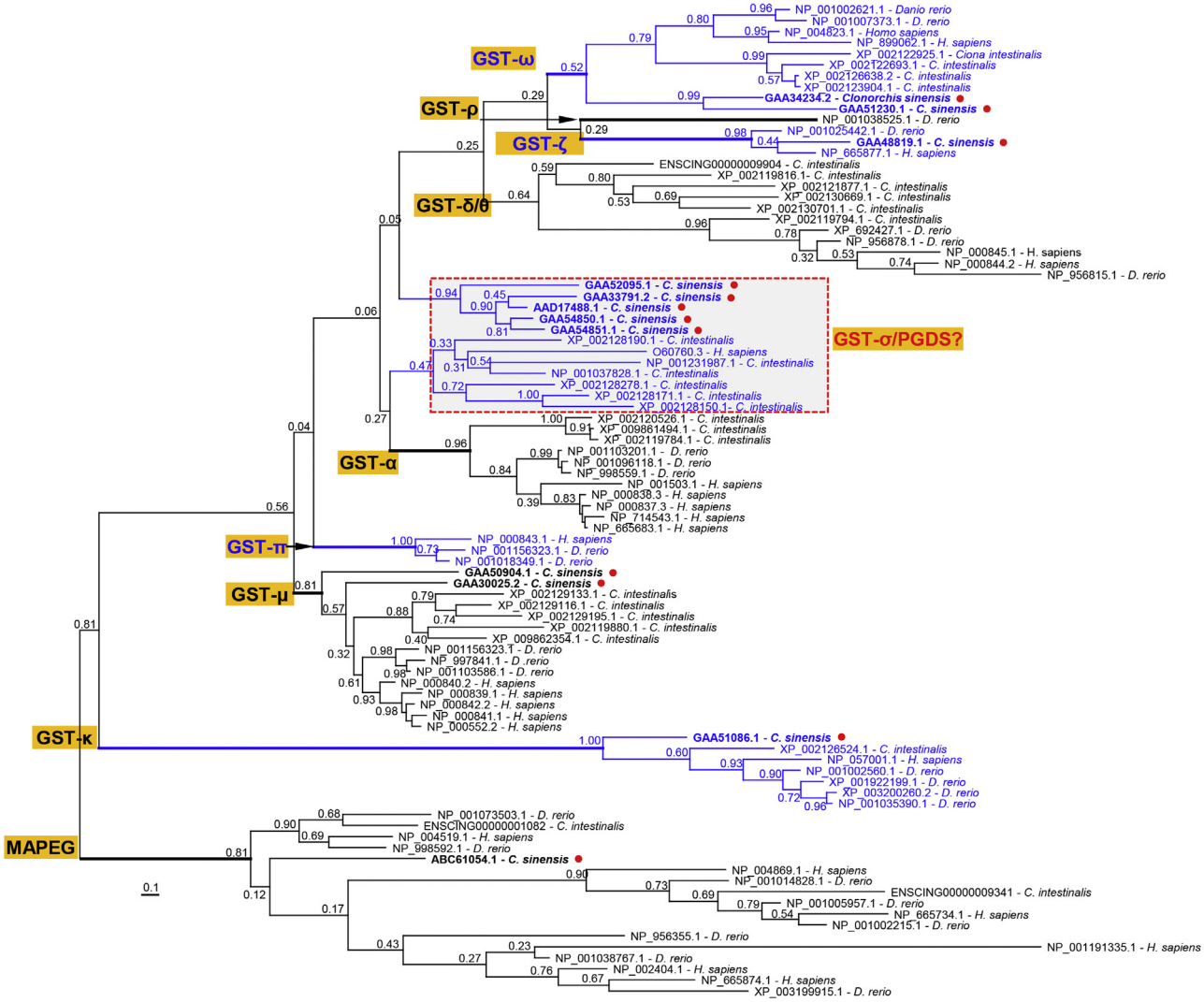

Fig. 1. Phylogenetic analysis of GST proteins in *Clonorchis sinensis* and the representative deuterostomian species. The maximum likelihood tree was constructed on the basis of the alignment of amino acid sequences with the PhyML program. Numerals at branching nodes indicate their percentages of appearance in 500 bootstrap replicates. The identity of each sequence was provided by the GenBank accession number followed by the species of donor organisms. Proteins isolated from *C. sinensis* were marked with bold letters and following red circles. GST sequences of *Danio rerio*, *Ciona intestinalis*, and human were taken from a previous study [30]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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