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ORIGINAL PAPER

Gene expression profile of *Clonorchis sinensis* metacercariae

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Abstract *Clonorchis sinensis* develop through miracidium, sporocyst, redia, cercaria, and metacercaria stages before becoming egg-laying adult flukes. The authors undertook this analysis of gene expression profiles during developmental stages to increase our understanding of the biology of *C. sinensis* and of host–parasite relationships. From a *C. sinensis* metacercariae complementary deoxyribonucleic acid library, 419 expressed sequence tags (ESTs) of average length of 668 bp were collected and assembled into 322 genes containing 70 clusters and 252 singletons. The genes were annotated using BLAST searches and categorized into ten major functional categories. Genes expressed abundantly were those of proteases and metabolic, transcription, and translation housekeeping proteins. Genes expressed higher in *C. sinensis* metacercariae than in adults coded structural and cytoskeletal proteins, transcription and translation machinery proteins, and energy metabolism-related proteins. This EST information supports the notion that *C. sinensis* metacercariae in fish hosts have a physiology and metabolism that is quite different from that of its adult form in mammals.

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Introduction

Clonorchis sinensis is endemic in the Far East Asia, i.e., in China, Korea, Japan, Taiwan, northern Vietnam, and the Russian far east. It is estimated that about 35 million people are infected with this fluke in these endemic areas (Lun et al. 2005). People are infected by eating the flesh of cyprinoid fish (the second intermediate host) infected with *C. sinensis* metacercariae. Metacercariae excyst in the duodenum and then migrate up into the intrahepatic biliary duct and grow to ovigerous adult flukes. In vivo flukes provoke hyperplastic and metaplastic changes in the biliary epithelium and in some cases promote a neoplastic outcome namely, cholangiocarcinoma (Rim 2005).

The genome-sequencing project of *Schistosoma mansoni* resulted in the genome assembly and a gene structure annotation, which included 11,787 protein-coding gene structures. Gene structures were annotated by combining all evidence of ab initio gene prediction, expressed sequence tag (EST) and protein alignments, and *S. japonicum* conserved regions (Haas et al. 2007). The genome structures of parasites can be explored cost effectively by analyzing ESTs. Moreover, collections and analyses of large EST data sets of parasites have produced discoveries in the fields of pathogenesis, metabolism, host–parasite interactions, host adaptation, and gene expression and regulation. More than 1,462,000 ESTs have been registered from human and animal parasites in the publicly accessible dbEST datasets, which includes about 264,000 ESTs from human-infecting trematodes such as *S. amnsoni*, *S. japonicum*, *C. sinensis*, *Opisthorchis viverrini*, and *Paragonimus westermani* (http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html). High-throughput analyses of the ESTs of parasites have produced a large amount of genetic and molecular biological information, which has broadened and strengthened our

understanding of the pathobiological mechanisms of parasites (Hu et al. 2003; Verjovski-Almeida et al. 2003; Merrick et al. 2003; Kim et al. 2006).

Three secretory proteins, with known roles in tumorigenesis, were identified by large-scale EST analysis of a sister fluke, *O. viverrini* in Thailand, which is known to induce cholangiocarcinoma (Laha et al. 2007). To date, 2,551 ESTs have been collected from *C. sinensis* and deposited in public databases (Lee et al. 2003; Cho et al. 2006). To identify novel gene products and new drug targets and to identify vaccine candidates to control *C. sinensis* infections, a large amount of genetic information data should be available on diverse biological topics. The present study was performed to collect ESTs from *C. sinensis* metacercaria to provide a fundamental genetic basis for the juvenile fluke.

Materials and methods

cDNA library construction

C. sinensis metacercariae were collected by artificially digesting (Hong et al. 2000) *Pseudorasbora parva* collected in Shenyang, Lyoning, China. Metacercariae (1.5 g) were homogenated in guanidium thiocyanate buffer, and total ribonucleic acid (RNA) was extracted using a CsCl-ultracentrifugation method, as described previously (Hong et al. 2000). The extracted total RNA (0.49 mg) had an A260/280 ratio of 1.77 and was qualified by RNA–gel electrophoresis. A complementary deoxyribonucleic acid (cDNA) library was constructed using 0.17 mg of total RNA in HybriZAP-2.1XR Vector, according to the manufacturer's instructions (Stratagene, CA). The resultant cDNA library had a titer of 2.0×10^5 plaque-forming units per 1 μg of bacteriophage DNA. The cDNA library was checked for quality by plaque polymerase chain reaction employing forward and reverse primers of the library vector. The cDNA insertion rate of the library was 86.2%, and the length of the cDNAs ranged from 0.75 to 2.5 kb. The cDNA library in the bacteriophage was then transcribed into phagemid DNA by massive in vivo excision and converted into a plasmid cDNA library.

Sequencing and homology searches

To qualify insertion rate and lengths of cDNA inserts in the library before massive sequencing, 96 colonies were randomly selected and sequenced from the *C. sinensis* cDNA library. Plasmid DNA was extracted and sequenced once with a forward primer of pAD-GAL4 vector (5′-ATGATGAAGATACCCCACCAAA-3′) and a Big-Dye reaction mix using an automatic sequencer (ABI Prism 3700, Perkin-Elmer). This preliminary sequencing revealed a cDNA insertion rate of 86.5%, a redundancy of 19.3%, and an average cDNA length of 600 bp. On this basis, another 504 plasmid colonies were randomly selected from the same cDNA library and sequenced, as described previously Cho et al. 2006).

cDNA reads were trimmed off vector and adaptor sequences and assembled into clusters using TIGR assembler version 2.0 (www.tigr.org). Clusters were subjected to homology searches in GenBank using BLASTX and annotated as corresponding cDNAs of *C. sinensis* when their *e* values were smaller than 10^{-5}. Homologues of the remainder clusters were searched using BLAST N and annotated as described above. Nonannotated cluster were viewed as unknown or ESTs.

Comparison with the adult *C. sinensis* EST pool

To obtain an insight in the developmental gene expression and regulation of *C. sinensis*, the metacercariae ESTs collected here were compared with an EST pool comprised of 2,387 ESTs, which were previously collected from adult *C. sinensis* by our colleagues (Cho et al. 2006). The two EST pools were compared in terms of annotation rates, common ESTs, and functional groups.

Results

Annotation and classification

From the cDNA library of *C. sinensis* metacercariae, 600 sequences were read and processed to 419 ESTs after trimming off vector and adaptor sequences and excluding reads smaller than 100 bp. ESTs were registered in GenBank under accession numbers EV523942–EV524360. The ESTs were 660 bp long on average and assembled into 322 clusters. Clusters shorter than 200 bp were 11.2%, clusters longer than 1,000 bp were 32.9%, and the longest cluster was of 1,495 bp. Of the clusters, 252 (78.3%) were singletons, 48 (14.9%) consisting of two ESTs, 13 (4.0%) consisted of three ESTs, 4 (1.2%) consisted of four ESTs, and 2 (0.6%) consisted of five ESTs (Fig. 1). There were three clusters assembled each with 6, 9, and 12 ESTs, respectively. The biggest cluster containing 12 ESTs was a hypothetical protein of *Neurospora crasa*.

Among the whole clusters, 186 (57.8%) clusters were found to have homologues in databases and were annotated accordingly. Some were classified into ten functional groups as reported elsewhere (Cho et al. 2006; Table 1). The clusters constituting each functional group were as follows. The largest group of structural and cytoskeletal

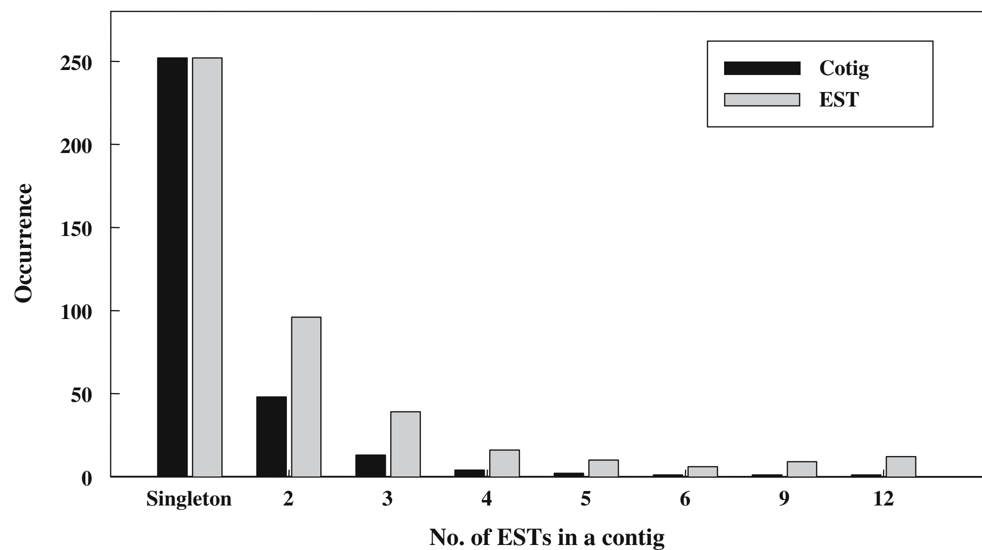

Fig. 1 Frequency distribution of ESTs in each contig

proteins comprised 21 proteins, e.g., myosin, myosin heavy and light chains, microtubule-associated protein tau, gap junction protein (pannexin), and T complex protein 1 α-subunit. The second group of transcription and translation proteins contained ten clusters, e.g., transcriptional regulation, DnaJ (HSP40 homologue), reverse transcriptase, 60S ribosomal protein L34, and 40S ribosomal protein 527. The third group of kinases and phosphatases consisted of eight clusters, e.g., protein kinase C type beta, protein kinase G, protein phosphatase A-2, and pyruvate kinase. The fourth group associated with energy metabolism had eight clusters, e.g., glycogen phosphorylase, fumarate hydratase, glycezatdehyde-3-phasphatedehydrogenase type 2, adenosine diphosphate/adenosine triphosphate (ATP) carrier, ATP synthase lipid-binding protein-like protein, and malate dehydrogenase. The fifth group of metabolic proteins and enzymes contained eight clusters, e.g., disulfide isomeras-related protein, β-*N*-acethylhexosamidase, bile acid β-glucosidase, amidase, and histone deacetylase 3. The sixth group of DNA scaffold and DNA-binding proteins contained seven clusters, e.g., heterochromatin protein 1 beta, poly(A)-binding protein, single-stranded DNA-binding protein, and TAR DNA-binding protein. The seventh group of regulatory and single proteins comprised seven clusters, e.g., proliferation associated protein 1, erbB3-binding protein EBP1, receptor-mediated endocytosis RME-1, and retinoblastoma-binding protein. The eighth group containing proteases and inhibitors had six clusters, e.g., cathepsin B, carboxypeptidase H precursor, and serpin-like protease inhibitor. The ninth group contained transporters and channels, e.g., clusters of high voltage-activated calcium channel α subunit $Ca_v\alpha$, Na/Ca exchanger, transient receptor potential cation channel, and FMRFamide-gated and pH-modulated sodium channel. Furthermore, the tenth and final group contained a cluster of T cell-recognized antigens.

Genes expressed in metacercariae and adult stages

The annotated 186 EST clusters were compared with the annotated 848 clusters of adult *C. sinensis* analyzed previously (Cho et al. 2006). The messenger RNAs (mRNAs) expressed in both metacercariae and adults fell into ten clusters of DnaJ (Hsp40 homologue), endoplasmin, peptide chain release factor 3, GAPDH, leukotriene A-4 hydrolase, HMG1-like protein, and four unidentified clusters (Table 2).

The most abundantly expressed genes in metacercariae were a group of structural and cytoskeletal proteins, followed by transcription and translation machinery proteins, and a group of energy metabolism proteins. In contrast, abundant mRNA clusters of adult *C. sinensis* contained regulatory and signal proteins, other metabolic proteins and enzymes, and structural and cytoskeletal proteins in descending order, which differed from that

Table 1 Functional classification of the annotated clusters of *C. sinensis* metacercaria ESTs

Category	Number of clusters
Structural and cytoskeletal proteins	21
Transcription and translation machinery	10
Kinases and phosphatases	8
Energy metabolism	8
Other metabolism and enzymes	8
DNA scaffold and DNA binding proteins	7
Regulatory and signal proteins	7
Proteases and inhibitors	6
Transporters and channels	4
Antigen	1
Subtotal	[80]
Not enough information to classify	106
Similarity not enough to be annotated	136
Total	322

Table 2 List of clusters shared by *C. sinensis* metacercariae and adults

Cluster ID	Accession number	Putative annotation	Organism	*e* value
CsMc-01-E10	AAF66929	Endoplasmin	*Schistosoma mansoni*	1.0E−27
CsMc-Contig55	AAH36077	Peptide chain release factor 3	*Homo sapiens*	2.0E−10
CsMc-04-G01	NP 076454	Glyceraldehyde-3-phosphate dehydrogenase type 2	*Rattus norvegicus*	2.0E−72
CsMc-03-G04	P30349	Leukotriene A-4 hydrolase	*Rattus norvegicus*	2.0E−19
CsMc-Contig97	AAP06024	DnaJ (Hsp40 homologue)	*Schistosoma japonicum*	9.0E−51
CsMc-Contig68	AAP06425	HMG1-like protein	*Schistosoma japonicum*	3.0E−09
CsMc-Contig10	AAP06411	AF099012 QM protein	*Bombyx mori*	4.0E−87
CsMc-05-F07	AAP06007	AF151804 CGI-46 protein	*Homo sapiens*	9.0E−35
CsMc-Contig6	XP310308	ENSANGP00000015236	*Anopheles gambiae*	8.0E−22
CsMc-Contig5	AAK35217	unknown	*Paragonimus westermani*	7.0E−22

observed in metacercariae (Fig. 2). The genes of structural and cytoskeletal proteins, DNA scaffolds, energy metabolism enzymes, and kinases and phosphatases were expressed more frequently in metacercariae than in adults. The genes expressed more abundantly in adults than in metacercariae coded regulatory and signal proteins, other metabolic proteins and enzymes, proteases and inhibitors, and antigenic proteins (Fig. 2).

Discussion

In this study, 419 ESTs were collected from a *C. sinensis* metacercariae cDNA library and assembled into 322 clusters, which consisted of 78.3% singletons and 21.7% contigs. By homology searches, more than half (57.8%) of the clusters were annotated to putative proteins with high probabilities, while the others remained unidentifiable because homologies (*e* values) were unacceptably high or no match was found. Moreover, clusters assembled with more than four ESTs appeared homologous with hypothetical proteins or unnamed proteins or did not match any record in the public data bases searched. Genes expressed highly in metacercariae were supposed to be unique. Metacercariae parasitize cold-blooded intermediate hosts, which provide a complete different environment from that encountered during the adult stage. Metacercariae in the muscles of freshwater fishes are in a resting stage wherein they simply maintain a basal metabolic status. In contrast, adult *C. sinensis* have a high metabolic rate and produce large numbers of eggs in mammalian hosts (Rim 2005). Thus, it might be expected that the physiological features of metacercariae are likely to be unique. Research on helminthic parasites has been focused largely on the adult stage, which provokes clinically significant disease. Many proteins, cDNAs, and ESTs have been identified in *C. sinensis* adults and reported to public domains, but scarcely any have been identified in metacercariae. The physiological uniqueness and limited biological information about metacercariae may support the notion that ESTs encoding hypothetical proteins are unique. EST pools provide a solid dataset to further the understanding of the stage-specific physiology, metabolism, and gene expression of metacercaria and adult *C. sinensis*.

In the present study, ESTs were found in *C. sinensis* metacercariae that encode proteins homologous with EBP1,

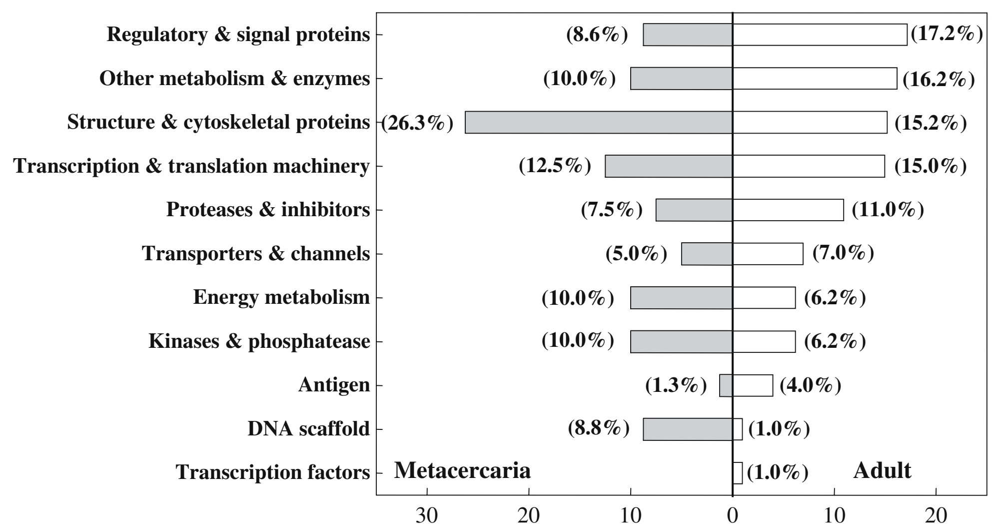

Fig. 2 Comparison of *C. sinensis* metacercariae and adults to identify developmental gene expression using annotated and classified clusters. The scale represents the percentage frequencies of ESTs

histone deacetylase, and retinoblastoma-binding protein. EBP1 is a transcription factor or transcriptional coregulator belonging to the proliferation-regulated protein family (Zhang et al. 2003) and can bind retinoblastoma protein and histone deacetylase-2 and inhibit transcription from cell cycle-regulating promoters to reduce cell proliferation and induce cell differentiation (Squatrio et al. 2004; Zhang and Hamberger 2004; Zhang et al. 2005). In *C. sinensis*, EBP1 is to regulate cell differentiation during development from metacercariae to the adult stage.

To survive in bile juice, *C. sinensis* probably needs to neutralize and eliminate potentially toxic bile acids from its body, and in man, bile acids are conjugated by glycosylation, sulfation, and amidation and excreted in urine or bile (Momose et al. 1997). *C. sinensis* metacercariae were found to have a bile acid β-glucosidase (Matern et al. 2001), which could catalyze the hydrolysis of bile acid 3-*O*-glucosides (Matern et al. 1997) and facilitate the elimination of the glycosylated conjugated form of bile acids.

The FMRFamide-gated sodium channel, a ligand-gated channel, is activated by Phe-Met-Arg-Phe-NH2 (a neuropeptide), and can be blocked by amiloride or FMRFamide analogues in a pH-dependent manner (Green and Cottrell 1999; Jeziorski et al. 2000). This channel has been found in neurons and in the nervous system (Marks et al. 1995; Davey et al. 2001) and has been reported to be responsible for muscle contraction and parasitic movement (Nelson et al. 1998; Perry et al. 2001).

In the present study, a high voltage-gated Ca^{2+} channel (VGCC) protein was found to be expressed in *C. sinensis* metacercariae. VGCCs couple changes in membrane potential to the influx of Ca^{2+} that is necessary to elicit intracellular signals, such as excitation–contraction coupling, excitation–secretion coupling, and other Ca^{2+}-dependent processes. They are found in muscle, nerves, and other excitable cells. VGCCs are multisubunit protein complexes and are composed of a pore-forming subunit α_1 and modulatory subunits β, $\alpha_2\gamma$, and δ. Phylogenetic analysis divides the α_1 subunits into three clusters known as Ca_v1, Ca_v2, and Ca_v3 (Jeziorski and Greenberg 2006). The α_1 subunit contains four homologous domains including six transmembrane segments, which form the membrane pore (Doyle et al. 1998), whereas the β subunit ($Ca_v\beta$) increases current density and ligand binding to the α_1 subunit and modulates various kinetic properties of the channel. Moreover, β subunits contain a conserved site, the 30-residue β interaction domain (BID), which spans the SH3 and guanylate-kinase (GK)-like domains. The modified GK domain binds to the α interaction domain of the α_1 subunit (Chen et al. 2004). Two variant subtypes of $Ca_v\beta$s ($Ca_v\beta$var) were cloned from both *S. mansoni* and *S. japonicum*, which lacked two highly conserved serine residues in BID (Kohn et al. 2001). The two serines each constitute consensus protein kinase C phosphorylation sites, and schistosome $Ca_v\beta$var subunits have been shown to confer praziquantel sensitivity to α_1 subunits (Kohn et al. 2001), which results in rapid Ca^{2+} influx and sustained Ca^{2+}-dependent muscle contraction (Andrews 1985). *C. sinensis* juveniles and adults are highly susceptible to praziquantel (Rim et al. 1980), which suggests that $Ca_v\beta$var could be expressed and confer praziquantel sensitivity to the α_1 subunit in *C. sinensis*.

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References

Andrews P (1985) Praziquantel: mechanisms of anti-schistosomal acitivity. Pharmacol Ther 29:129–156

Chen YH, Li MH, Zhang Y, He LL, Yamada Y, Fitzmaurice A, Shen Y, Zhang H, Tong L, Yang J (2004) Structural basis of the a_1-b subunit interaction of voltage-gated Ca^{2+} channels. Nature 429:675–680

Cho PY, Lee MJ, Kim TI, Kang SY, Hong SJ (2006) Expressed sequence tag analysis of adult *Clonorchis sinensis*, the Chinese liver fluke. Parasitol Res 99:602–608

Davey F, Harris SJ, Cottrell GA (2001) Histochemical localization of FMRFamide-gated Na^+ channels in *Helisoma trivolvis* and *Helix aspersa* neurons. J Neurocytel 30:877–884

Doyle DA, Cabral JM, Pfuetzner RA, Kuo A, Gulbis JM, Dohen SL, Chair BT, MacKinnon R (1998) The structure of the potassium channel: molecular basis of K^+ conduction and selectivity. Science 280:69–77

Green KA, Cottrell GA (1999) Block of the helix FMRFamide-gated Na^+ channel by FMRFamide and its analogues. J Physiol 519:47–56

Haas BJ, Berriman M, Hirai H, Cerqueira GG, LoVerde PT, El-Sayed NM (2007) *Schistosoma mansoni* genome: closing in on a final gene set. Exp Parasitol (in press), DOI 10.1016/j.expara.2007.06.005

Hong SJ, Seong KY, Sohn WM, Song KY (2000) Molecular cloning and immunological characterization of phosphoglycerate kinase from *Clonorchis sinensis*. Mol Biochem Parasitol 108:207–216

Hu W, Yan Q, Shen DK, Liu F, Zhu ZD, Song HD, Xu XR, Wang ZJ, Rong YP, Zeng LC, Wu J, Zhang X, Wang JJ, Xu XN, Wang SY, Fu G, Zhang XL, Wang ZQ, Brindley PJ, McManus DP, Xue CL, Feng Z, Chen Z, Han ZG (2003) Evolutionary and biomedical implications of a *Schistosoma japonicum* complementary DNA resource. Nat Genet 35:139–147

Jeziorski MC, Greenberg RM (2006) Voltage-gated calcium channel subunits from platyhelminths: potential role in praziquantel action. Int J Parasitol 36:625–632

Jeziorski MC, Green KA, Sommerville J, Cottrell GA (2000) Cloning and expression of a FMRFamide-gated Na^+ channel from *Helisoma trivolvis* and comparison with the native neuronal channel. J Physiol 526:13–25

Kim TS, de Guzman JV, Kong HH, Chung DI (2006) Comparison of gene representation between diploid and triploid *Paragonimus westermani* by expressed sequence tag analyses. J Parasitol 92:803–816

Kohn AB, Anderson PAV, Roberts-Misterly JM, Greenberg RM (2001) Schistosome calcium channel b . subunits. : unusual modulatory effects and potential role in the action of the antischistosomal drug praziquantel. J Biol Chem 276:36873–36876

Laha T, Pinlaor P, Mulvenna J, Sripa B, Sripa M, Smout MJ, Gasser RB, Brindley PJ, Loukas A (2007) Gene discovery for the carcinogenic human liver fluke, *Opisthorchis viverrini*. BMC Genomics 8:189

Lee JS, Lee JW, Park SJ, Yong TS (2003) Analysis of the genes expressed in *Clonorchis sinensis* adults using the expressed sequence tag approach. Parasitol Res 91:283–289

Lun ZR, Gasser RB, Lai DH, Li AX, Zhu XQ, Yu XB, Fang Y (2005) Clonorchiasis: a key foodborne zoonosis in China. Lancet Infect Dis 5:31–41

Marks NJ, Halton DW, Maule AG, Brennan GP, Shaw C, Southgate VR, Johnston CF (1995) Comparative analyses of the neuropeptide F (NPF)- and FMRFamide-related peptide (FaRP)-immunoreactivities in *Fasciola hepatica* and *Schistosoma* spp. Parasitology 11 (pt4):371–381

Matern H, Heinemann H, Legler G, Matern S (1997) Purification and characterization of a microsomal bile acid beta-glucosidase from human liver. J Biol Chem 272:11261–11267

Matern H, Boermans H, Lottspeich F, Matern S (2001) Molecular cloning and expression of human bile acid beta-glucosidase. J Biol Chem 276:37929–37933

Merrick JM, Osman A, Tsai J, Loverde PT, Lee NH (2003) The *Schistosoma mansoni* gene index: Gene discovery and biology by reconstruction and analysis of expressed gene sequences. J Parasitol 89:261–269

Momose T, Maruyama J, Iida T, Goto J, Nambara T (1997) Comparative abilities and optimal conditions for b-. glucosidase enzymes to hydrolyse the glucuronide, glucoside, and . *N*-acetylglucosaminide conjugates of bile acids. Biol Pharm Bull 20:828–833

Nelson LS, Rosoff ML, Li C (1998) Disruption of a neuropeptide gene, *flp-1*, causes multiple behavioral defects in *Caenorhabditis elegans*. Science 281:1686–1690

Perry SJ, Straub VA, Schofield MG, Schofield MG, Burke JF, Benjamin PR (2001) Neutonal expression of an FMRFamide-gated Na^+ channel and its modulation by acid pH. J Neurosci 21:5559–5567

Rim HJ (2005) Clonorchiasis: an update. J Helminthol 79:269–281

Rim HJ, Ha JH, Kim SJ (1980) Experimental study on the therapeutic effect of praziquantel (Embay 8440) in rats infected with *Clonorchis sinensis*. Korean J Parasitol 18:65–80

Squatrio M, Mancino M, Donzelli M, Areces LB, Draetta GF (2004) EBP1 is a nucleolar growth-regulating protein that is part of pre-ribosomal ribonucleoprotein complexes. Oncogene 23:4454–4465

Verjovski-Almeida S, DeMarco R, Martins EAL, Guimaraes PEM, Ojopi EPB, Paquola ACM, Piazza JP, Nishiyama MY Jr, Kitajima J, Adamson RE, Ashton PD, Bonaldo MF, Coulson PS, Dillon GP, Farias LP, Gregorio SP, Ho PL, Leite RA, Malaquias LCC, Marques RCP, Miyasato PA, Nascimento ALTO, Ohlweiler FP, Reis EM, Ribeiro MA, Sa RG, Stukart GC, Soares MB, Gargioni C, Kawano T, Rodrigues V, Madeira AMBN, Wilson RA, Menck CFM, Setubal JC, Leite LCC, Dias-Neto E (2003) Transcriptome analysis of the acoelomate human parasite *Schistosoma mansoni*. Nat Genet 35:148–157

Zhang Y, Hamberger AW (2004) Heregulin regulates the ability of the ErbB3-binding protein Ebp1 to bind E2F promoter elements and repress E2F-mediated transcription. J Biol Chem 279:26126–26133

Zhang Y, Woodford N, Xia X, Hamberger AW (2003) Repression of E2F1-mediated transcription by the ErbB3 binding protein Ebp1 involves histone deacetylases. Nucleic Acids Res 31:2168–2177

Zhang Y, Akinmade D, Hamberger AW (2005) The ErbB3 binding protein Ebp1 interacts with Sin3A to repress E2F1 and AR-mediated transcription. Nucleic Acids Res 33:6024–6033