Clonorchis sinensis can cause the important food borne zoonosis, clonorchiasis, leading to cholangitis, bile duct obstruction, and the serious complication of cholangiocarcinoma in humans. In the world, 20 million people are infected with C. sinensis, about 12 million in China, mostly in Guangdong, Guangxi provinces of south China and Jilin, Heilongjiang province of northeast China. Clonorchiasis is aggravated because this disease is often misdiagnosed as cholangitis or bile duct obstruction and can cause cancer. The public health and economic impact of clonorchiasis has evoked us to study on the following two projects. One is Genetic variation among C. sinensis isolates from different hosts and geographical locations revealed by sequence analysis of mitochondrial and ribosomal DNA regions (published in Mitochondrial DNA. 2013 Oct; 24), the other is molecular cloning and characterization of taurocyamine kinase from Clonorchis sinensis: a candidate chemotherapeutic target (published in PLoS Negl Trop Dis. 2013 Nov 21;7).

1. Genetic variation among Clonorchis sinensis isolates from different hosts and geographical locations revealed by sequence analysis of mitochondrial and ribosomal DNA regions

The present study examined genetic variability among Clonorchis sinensis isolates from four different geographical localities (Guangzhou, Nanning, Jiamusi and Daqing) and hosts species (cats, dogs, human and rabbits) in Mainland China by sequence analysis of two mitochondrial DNA (mtDNA) genes, namely NADH dehydrogenase subunits 2, 5 (nad2 and nad5) and ribosomal internal transcribed spacer 1 (ITS-1). A portion of the ITS1, nad2 (pnad2) and nad5 (pnad5) were amplified by polymerase chain reaction (PCR) separately from adult C. sinensis individuals and the amplicons were subjected to sequencing from both directions. The length of the sequences of ITS1, pnad2 and pnad5 were 643 bp, 666 bp and 771 bp, respectively. The intraspecific sequence variations within C. sinensis were 0-1.7% for ITS1, 0-1.4% for pnad2 and 0-0.9% for pnad5. The A+T contents of the sequences were 45.26-45.88% (ITS1), 62.91-63.51% (pnad2) and 58.24-58.63% (pnad5). Phylogenetic analyses using ribosomal and mitochondrial sequence dataset, with three different computational algorithms (Bayesian inference, maximum parsimony and maximum likelihood), all revealed distinct groups with high statistical support. These findings demonstrated the existence of low level intraspecific variation in ribosomal DNA (rDNA) and mitochondrial DNA (mtDNA) sequences among C. sinensis isolates from four provinces and hosts in China, and elucidated that mitochondrial DNA sequences and ribosome DNA sequences provided reliable genetic markers for phylogenetic studies of zoonotic trematodes.
2. Molecular Cloning and Characterization of Taurocyamine Kinase from Clonorchis sinensis: A Candidate Chemotherapeutic Target

Phosphagen kinases (PK) constitute a highly conserved family of enzymes, which play a role in ATP buffering in cells, and are potential targets for chemotherapeutic agents since variants of PK are found only in invertebrate animals, including helminthic parasites. This work is conducted to characterize a PK from C. sinensis and to address further investigation for future drug development.

A cDNA clone encoding a putative polypeptide of 717 amino acids was retrieved from a C. sinensis transcriptome. This polypeptide was homologous to taurocyamine kinase (TK) of the invertebrate animals and consisted of two contiguous domains. C. sinensis TK (CsTK) gene was reported and found consist of 13 exons intercalated with 12 introns. This suggested an evolutionary pathway originating from an arginine kinase gene group, and distinguished annelid TK from the general CK phylogenetic group. CsTK was found not to have a homologous counterpart in sequences analysis of its mammalian hosts from public databases. Individual domains of CsTK, as well as the whole two-domain enzyme, showed enzymatic activity and specificity toward taurocyamine substrate. Of the CsTK residues, R58, I60 and Y84 of domain 1, and H60, I63 and Y87 of domain 2 were found to participate in binding taurocyamine. CsTK expression was distributed in locomotive and reproductive organs of adult C. sinensis. Developmentally, CsTK was stably expressed in both the adult and metacercariae stages. Recombinant CsTK protein was found to have low sensitivity and specificity toward C. sinensis and platyhelminth-infected human sera on ELISA.

CsTK is a promising anti-C. sinensis drug target since the enzyme is found only in the C. sinensis and has a substrate specificity for taurocyamine, which is different from its mammalian counterpart, creatine.