

Identification and characterization of novel antigens from *Opisthorchis viverrini*

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Abstract

Opisthorchis viverrini is the causative agent of human opisthorchiasis in Thailand. Long lasting infection with the parasite has been correlated to the development of cholangiocarcinoma. This kind of cancer is a major public health problem in North- and Northeast-Thailand. At present, the knowledge which parasite antigens are involved in the establishment of infection and tumorigenesis is quite limited. We have, therefore, screened the published parasite transcriptome sequences to identify new antigens which might be involved in the host/parasite interaction. Two related transcripts were selected due to a suspected function of the encoded proteins (OvDM9A, OvDM9B) in the transport of hydrophobic molecules. The corresponding cDNAs were isolated from an adult stage *O. viverrini* cDNA library and subcloned into bacterial expression vectors. Recombinant proteins were produced in *E. coli* but were insoluble. Only OvDM9A was selected for further analyses and the purified recombinant protein has been used for production of polyclonal antisera in mice. The antisera will be used to localize native OvDM9A in parasite antigen extracts and tissues. To study its biochemical function OvDM9A will be expressed in yeast to obtain soluble protein which will be tested in binding assays with hydrophobic molecules. The biological function will be studied in vivo using RNAi to analyze if the protein is essential for parasite survival in the host. If a role in binding and transport of hydrophobic molecules can be established these novel proteins could be useful for drug targeting.

Keywords: *Opisthorchis viverrini*, cholangiocarcinoma, transcriptome, transport, drug targeting

Introduction

Opisthorchiasis caused by *Opisthorchis viverrini* is mainly prevalent in Thailand, Lao People's Democratic Republic, and Cambodia. It is highly endemic in Northeast Thailand, where the occurrence of cholangiocarcinoma (CCA) is the highest in the world (IARC 1994, 1997). Infection occurs when raw or inadequately cooked infected freshwater fish are ingested. The fluke is residing in the bile ducts and gall bladder. Infection with many parasites can produce morbidity including abdominal pain, dyspepsia, and fatigue and in very heavily infected cases, pyogenic cholangitis, biliary calculi, obstructive jaundice, and even cholangiocarcinoma in long lasting infections (Harinasuta et al., 1984; Pungpak et al., 1994; Schwartz, 1980). Since the early 1980s, when the good safety and therapeutic profile of praziquantel against opisthorchiasis had been established, treatment and control of opisthorchiasis relies on this drug (Keiser and Utzinger, 2007). Although treatment failures have not yet been reported for praziquantel in *O. viverrini* infected patients, the search for alternative trematocidal drugs is warranted. The aim of this study is discover novel antigens which are involved in host/parasite interaction and could be applied for drug targeting.

Methods

Database analysis

The published parasite transcriptome sequences were searched to identify novel proteins and to design primer pairs for cloning of their cDNAs

Cloning and sequence analysis of OvDM9 genes

We isolated cDNAs for OvDM9A, OvDM9B from an adult stage *O. viverrini* cDNA library and subcloned them into bacterial expression vectors

Expression and purification of recombinant OvDM9

Recombinant proteins were produced in *E. coli* and purified by Ni-NTA affinity chromatography

Results

Database analysis

Two sequence related transcripts were selected due to a suspected function of the encoded proteins (OvDM9A, OvDM9B) in the transport of hydrophobic molecules.

Cloning and sequence analysis of OvDM9 genes

The size of both OvDM9 cDNAs is 470 bp.

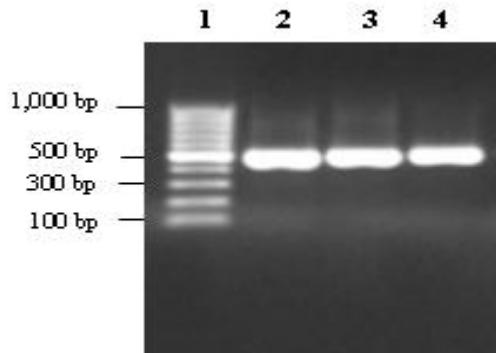


Figure 1. PCR products of the OvDM9 cDNAs subcloned into the expression vector, lane 1: 100 bp DNA ladder, lane 2-3: OvDM9B cDNA, lane 4: OvDM9A cDNA.

Expression and purification of recombinant OvDM9A protein

The molecular mass of rOvDM9 is approximately 17 kDa

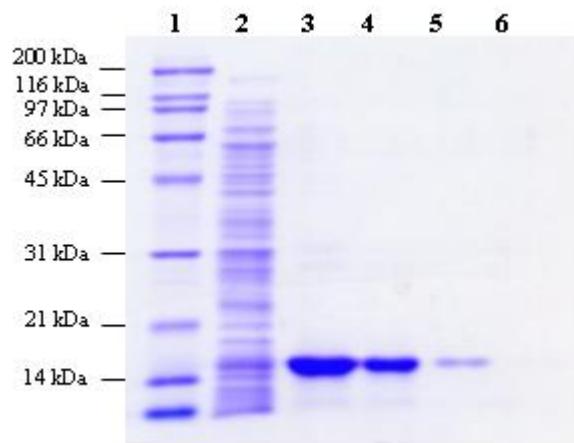


Figure 2. Purification of rOvDM9A under denaturing conditions, lane 1: broad range marker, lane 2: flow through, lanes 3-6: elution fractions.

Discussion and conclusion

The cDNAs of two related genes from *O. viverrini* were cloned. They encode proteins which may act as transporters of hydrophobic molecules analogous to cytosolic fatty acid binding proteins. The recombinant OvDM9 proteins were not correctly folded in *E. coli* and insoluble. Only rOvDM9A was purified and used for production of polyclonal antisera in mice. These sera will be used for immunohistochemical analyses and in immunoblots. To analyze the biochemical function of the protein it will be expressed in yeast to obtain it in soluble form which can be used in binding assays with hydrophobic molecules. The biological function will be studied in cultured parasites using RNAi to analyze if the protein is essential for parasite survival. If a role in binding and transport of hydrophobic molecules can be established these novel proteins could be useful for drug targeting.

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References

1. Harinasuta T, Riganti M, Bunnag D. *Opisthorchis viverrini* infection: pathogenesis and clinical features. *Arzneimittelforschung* 1984; 34:1167–9.
2. Keiser J, Utzinger J. Food-borne trematodiasis: current chemotherapy and advances with artemisinins and synthetic trioxolanes. *Trends Parasitol* 2007; 23: 555–62
3. IARC. Infection with liver flukes (*Opisthorchis viverrini*, *Opisthorchis felineus* and *Clonorchis sinensis*). IARC Monogr Eval Carcinog Risks Hum 1994; 61: 121–75.
4. IARC. Working Group on the Evaluation of Carcinogenic Risks to Humans: polychlorinated dibenzo-para-dioxins and polychlorinated dibenzofurans. Lyon, France, 4–11 February 1997. IARC Monogr Eval Carcinog Risks Hum 1997; 69: 1–631.
5. Pungpak S, Chalermrut K, Harinasuta T, Viravan C, Schelp PF, Hempfling A, Schlattmann P, Bunnag D. *Opisthorchis viverrini* infection in Thailand: symptoms and signs of infection—a population-based study. *Trans R Soc Trop Med Hyg* 1994; 88: 561-4.
6. Schwartz, DA. Helminths in the induction of cancer: *Opisthorchis viverrini*, *Clonorchis sinensis* and cholangiocarcinoma. *Trop Geogr Med* 1980; 32: 95–100.