CHAPTER I: INTRODUCTION

1.1 Introduction

Opisthorchis viverrini is a human liver fluke that causes serious public health concern in some parts of Southeast Asia, including Thailand, Laos PDR, Vietnam and Cambodia. The prevalent O. *viverrini* infection in regions where consumption of raw freshwater fish is commonly found (Mulvenna et al., 2010). The infection of *O. viverrini* mainly causes opisthorchiasis associated with several hepatobiliary diseases including cholangitis, obstructive jaundice, hepatomegaly, cholecystitis, and cholelithiasis. In Northeast Thailand, 6 million people were estimated to be infected with *O. viverrini* (Sripa et al., 2012). Moreover, *O. viverrini* infection is strongly associated with cholangiocarcinoma (CCA), bile duct cancer; that is unprecedentedly high in these regions.

Cholangiocarcinoma in developing countries including Thailand showed at rates 50-100 times incidence compared in developed countries, for instance in Korea with CCA caused by *C. sinensis*, however, the rates at more than 5 times lower than in Northeast Thailand. There are three main proposed mechanisms of *O. viverrini* infection that are hypothesized to contribute to CCA: (1) mechanical damage; (2) immunopathology; (3) toxic effects from the fluke proteins (Fig. 1.1) (Smout et al., 2011). The mechanical damage from the parasite suckers wound the epithelial lining of the bile duct and lead to the constant activation of wound repair signals, thus, alter the cell states and lead to DNA damage (Smout et al., 2011). *O. viverrini* found to induce the Th2 response, one of the major subsets of $CD4^+$ T cells. Th2 cells produce IL-5, IL-6, and IL-10 which are responsible for immediate type hypersensitivity involving IgE, eosinophils and mast cells (Wongratanacheewin et al., 2003). Thus, production nitric acid synthase increased due to the promotion of immediate type hypersensitivity, and may contribute to an increase in *N*-nitroso compounds that increase the risk of CCA in inflamed tissue through alkylation and deamination of DNA (Mulvenna et al, 2010). However, the information of the significance of immune response generated during the liver fluke infections is limited. Moreover, the long term inflammatory reactions due to the irritation by the parasites proteins also contribute to carcinogenesis. There are two main sources of proteins that have a critical role in *O. viverrini* survival, reproduction, pathogenesis and carcinogenesis: (1) the excretory/secretory (ES) products (2) the tegument. The ES product is a complex mixture of proteins, carbohydrates and lipids secreted by the parasites from the surface, oral openings or gut. The ES products have the ability to down regulate apoptosis, prevent DNA repair, and induce proliferation. Meanwhile, the tegument is the syncytial outermost surface of the parasite that plays a crucial role in parasitic survival, modulation of host immune responses, reproduction, and host tissue invasion (Fig. 2.1.1) (Mulvenna et al., 2010).

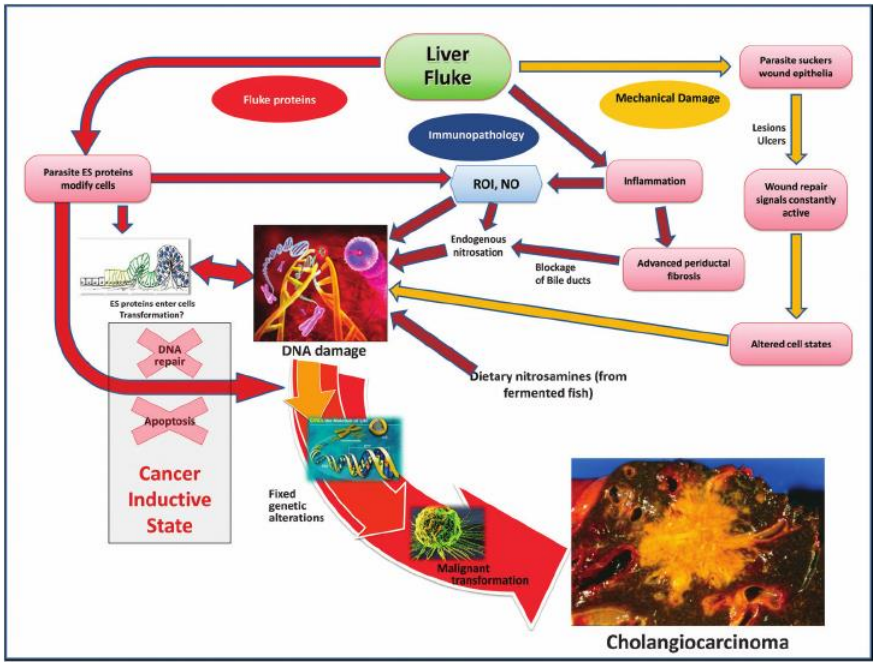

Figure 1.1 Proposed mechanism of opisthorchiasis associated cholangiocarcinoma. The infection of *O. viverrini* damage the bile duct tissues through three distinct pathways: (1) mechanical damage from the parasite suckers that wound the epithelial lining of the bile duct; (2) immunopathology due to the inflammation, and production of reactive oxygen intermediates (ROI) and nitric oxide (NO); (3) toxic effects from fluke proteins that secreted on the biliary epithelia. These three pathways will alter the cell states and cause DNA damage, and results in cholangiocarcinoma. Adapted from " Infection with human liver fluke, *Opisthorchis viverrini*," by M.J. Smout et al, 2011, Molecular Biosystems, 7, p. 1370. Copyright 2011 by The Royal Society of Chemistry.

Due to the serious public health concern caused, surveillance programs were conducted throughout the years in order to decrease the prevalence and incidence of both opisthorchiasis and CCA in the affected regions. The surveillance programs include the parasite control in the definitive hosts and intermediate hosts, health campaign and education, as well as treatment and prevention through the mass consumption of praziquantel. Currently, it was reported that the prevalence of opisthorchiasis, especially in northeast Thailand has already below 1% (Sripa et al., 2015).

Although the prevalence has decreased, however, there are many researches about *O. viverrini* due to the disease that has caused poor prognosis, and awareness of possible resistance towards the current used treatment will be developed. Proteomic approach is one of the scope of research that is commonly used to study the drug target of *O. viverrini*, pathogenesis, immunodiagnostic and vaccine candidate. Several proteins of *O. viverrini* have been discovered as potential drug targets. This proposed study will identify the key role protein that is important in carcinogenesis, pathogenesis, and survival of the parasite from the tegument as a potential vaccine

candidate, since the tegument has been reported to be involved in immunopathological processes that results in CCA. In addition, some studies considered the tegument as a vaccine candidate in schistosomiasis (Fig. 2.2.1) (Piratae et al., 2012; Chaiyadet et al., 2017).

1.2 Problem statement

O. viverrini infection has been a serious public health concern in some parts of Southeast Asia due to the high prevalence of opisthorchiasis associated with CCA in these regions. *O. viverrini* infection associated with CCA has been proposed through three pathways such as mechanical damage, immunopathology, and proteins from the parasite. There are two main sources that are important for the parasites survival, but also have been suggested to be involved in regulating immune response, such as ES products and tegument. Tegument is the outermost surface that is critical for the parasites and has been studied as a vaccine candidate in other parasitic infections such as schistosomiasis. However, information regarding the significant immune response generated during *O. viverrini* infection is seemingly limited though this information may facilitate the efforts in developing a vaccine in order to reduce the use of antiparasitic drugs that might potentially cause the parasite to resist and reduce the effectiveness of the treatment.

1.3 Objectives

This proposed study aimed to identify the tegumental proteins of the *O. viverrini* as potential vaccine candidates, which are capable of induce protection. The research was employed several proteomic approaches in identification of the tegumental protein and analyzed the response of the antibody that produced by the susceptible host and non-susceptible host against the target protein; which may help to elucidate the mechanism of host immune response towards the *O. viverrini* infection, since the information regarding the significance of immune response generated during the liver fluke infections is limited.