CHAPTER I

INTRODUCTION

1.1   Background and rationale of the study

Cholangiocarcinoma (CCA) is a primary liver cancer originating in the bile duct epithelium. Although CCA is a rare tumor worldwide, it has a high incidence in Southeast Asia, especially in northeastern Thailand. *O. viverrini* infection is transmitted by the consumption of raw or undercooked freshwater fish in regional dishes which contain the metacercarial (i.e, infective) stage of the fluke. Once consumed, the immature flukes migrate up the ampulla of Vater to the biliary tree and mature in the small intrahepatic bile ducts (Sripa et al., 2008). The life span of *O. viverini* in human is not known, however, it may be over 25 years as recorded in *C. sinesis* (Attwood et al., 1978). The geographical pattern of liver fluke infection is not uniform, however, with the greatest prevalence in the north (19.3%) and northeast (15.7%) compared with the central (3.8%) and southern regions (0%). Despite wide-spread treatment with praziquantel, an estimated 6 million people infected with the liver fluke (calculated from overall 9.4% prevalence within the population in the year 2001) (Jongsuksuntigul et al., 2003). As a result of *O. viverini* infection can present with a number of hepatobiliary diseases, including cholangitis, obstructive jaundice, hepatomegaly, cholecystitis and cholelithiasis (Harinasuta et al., 1984). Moreover, both experimental and epidemiological evidence strongly implicate the liver fluke infection in the etiology of CCA (Sripa et al., 2007; Sripa et al., 2008). Indeed, there may be no stronger link between an eukaryotic organism and a malignant neoplasm than that between *O. viverrini* and CCA, which led the World Health Organization's International Agency for Research on Cancer to classify *O. viverrini* as a Group 1 carcinogen (Bouvard et al., 2009).

Advanced CCA has an extremely poor prognosis. In the case of diffuse-type CCA, the median survival time is only 65 days (Sripa et al., 2008). Over the past 30 years, much effort has been devoted to improving the survival rate of CCA patients. Currently, the complete surgical resection of all detectable tumors from the liver and

bile duct has been shown to improve the five-year survival rate, but surgical resection must be done before advanced stages of CCA. Unfortunately, the majority of patients present with an advanced stage of CCA that is not amenable to the surgical intervention. Hence, the discovery of novel biomarkers to further refine prognosis and response to treatment is of great importance.

Compared with genomic technologies (such as serial analysis of gene expression or DNA microarrays), the major advantage of proteomics is the potential for detecting changes in the expression of the actual effectors of molecular functions (proteins instead of mRNAs) (Molloy et al., 2002). The use of several methodological aspects in MS based proteomics to determine the protein expression profiles of both *in vitro* models and clinical materials, is now an essential strategy for the understanding of the molecular basis of the disease development as well as the discovery of diagnostic and/or prognostic markers (Pitteri et al., 2010; Dunn et al., 2011). For instance proteomic approaches have been successfully utilized in studies for tumor marker discovery of cancers such as hepatocellular carcinoma and breast cancer (Bisca et al., 2004; Tan et al., 2005).

In the present study, we attempted to characterize protein profiles of both CCA cell lines as well as primary clinical specimens utilizing two-dimensional gel electrophoresis (2-DE), matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS), OFFGEL electrophoresis, laser capture microdissection (LCM) and liquid chromatography-tandem mass spectrometry (LC-MS/MS). The diagnostic and/or prognostic significance of protein candidates were subsequently investigated by immunohistochemistry (IHC).

1.2   Objectives of the study

The overall objective of this study was to find novel diagnostic and/or prognostic markers for CCA focusing on the protein level. More specifically we aimed at:

1.2.1 Investigating protein profiles of human CCA cell lines and tissues using proteomic approaches.

1.2.2 Assessing the utility of candidate protein(s) in relation to diagnosis and/or prognosis for CCA.

1.3 Scope and limitation of the study

This research dissertation was performed in both human CCA cell lines and CCA tissues. High throughput protein expression profiles using cutting edge proteomic strategies including 2-DE, MALDI-TOF MS, OFFGEL and LC-MS/MS were employed for molecular study. With regard to the budget constraints, only differential expressed spots in 2-DE gels were selected for further protein identification using MALDI-TOF MS. In addition, validation of interesting proteins was obtained by Western blotting and immunohistochemistry.

1.4 Anticipated outcomes

The anticipated outcome of this study is the differential protein alteration patterns of CCA subgroups not only in both type of human CCA cell lines but also in clinical CCA tissues. Furthermore, validation of candidate protein(s) may reveal the novel potential markers for differential diagnosis and/or prognosis of CCA.