CHAPTER I
INTRODUCTION

1. Rationale and background

Although cholangiocarcinoma (CCA) is a rare malignancy of the biliary tract, interest in this tumor is rising as incidence and mortality rates for intrahepatic tumor increase markedly worldwide [1-3]. In the Northeast of Thailand, CCA accounts for the highest incidence of this cancer worldwide with truncated age-standardized incidence of 317.6 per 100,000 population for ages >35 years [4]. CCA remains an important public health problem in Thailand because the incidence and fatality rates are still very high. The disease is notoriously difficult to diagnose and is usually fatal because of the late clinical stage and the lack of effective non-surgical therapy [5]. Surgical resection can be potentially curative, but few patients are candidates for surgery [6]. Thailand has a high prevalence of infection of the liver fluke, *Opisthorchis viverrini* (OV), and an approximated six million people are infected [7]. The experimental and epidemiological evidences implicate opisthorchiasis in the etiology of CCA in this endemic area [8-10]. It is possible that chronic inflammation induced by the infection enhances the susceptibility of biliary tract to carcinogenesis leading to genetic and epigenetic aberrations in the epithelial cells. However, the molecular events involved in the initiation, progression and drug resistance of CCA are not well understood. Like other tumors, the development of CCA is hypothesized to be a multistep processes with the accumulation of genetic and epigenetic aberrations in regulatory genes. These alterations lead to the activation of oncogenes and the inactivation or loss of tumor suppressor genes.

Genetic defects such as mutation and deletion can lead to the dysfunction of genes. Although epigenetic alteration does not change the DNA sequence, it can affect gene expression by chemical modifications including DNA methylation and histone modification (acetylation and methylation). Hypermethylation of regulatory regions near many gene promoters, known as CpG islands, is associated with transcriptional inactivation [11-13]. Genes which can become epigentically silenced

by this mechanism include tumor suppressor genes which are involved in important molecular pathways, e.g., cell cycle control, apoptosis, DNA repair and cell adhesion.

DNA methylation, the addition of a methyl group to the carbon-5 position of cytosine residues, occurs almost exclusively at cytosines that are followed by a guanine (so-called CpG dinucleotides). The bulk of the genome displays a clear depletion of CpG dinucleotides, and those that are present are nearly always methylated. By contrast, small stretches of DNA, called CpG islands, are comparatively rich in CpG nucleotides and are normally unmethylated. These CpG islands are frequently located within the promoter regions of human genes, and methylation within the islands has been shown to be associated with transcriptional repression of the corresponding gene [11-14]. Alterations in DNA methylation is important in the development of most cancers [14]. As mentioned previously, CpG-island methylation or hypermethylation is an important molecular mechanism to development of cancers Therefore, DNA methylation has become the focus in many studies of the development of cancers including CCA.

Moreover, acquired drug resistance is a major limitation for successful treatment of cancer. One mechanism by which chemotherapy kills cancer cells is via apoptotic signaling pathways. Epigenetic silencing by DNA methylation of CpG-island in cancer cells is associated with transcriptional suppression of many genes involved in apoptotic signaling pathways and cell cycle arrest [190]. This status leads to acquired chemotherapeutic drug resistance and cancer cell growth. Re-expression of tumor suppressor genes epigenetically inactivated can result in the suppression of cancer cell growth or sensitization to epigenetic and chemotherapeutic agents. This study aims to investigate DNA methylation in CCA using firstly a candidate CpG island approach of genes implicated in other tumor types and secondly a genome-wide approach. The aim is to identify DNA methylation changes which may provide insight into this disease and provide biomarkers which may help improve diagnosis, prognosis and treatment of the tumor.

2. Research questions

Part I: CpG-island methylation profiling in liver fluke related-cholangiocarcinoma

2.1 Does DNA methylation change of examined CpG-islands at candidate genes play the important role in CCA?

2.2 Is the methylation status at the studied CpG-islandschanged in CCA compared to adjacent normal samples?

2.3 Is methylation at CpG-island(s) significantly related to cinicopathological data of the patients in diagnostic or prognostic aspect?

2.4 Are candidate CpG-island(s) epigenetically silenced through DNA methylation?

Part II: Genome-wide DNA methylation signature in cholangiocarcinoma

2.5 What is DNA methylation signature of CCA as defined by Illumina's Infinium® HumanMethylation27 BeadChip?

2.6 Are there the difference of DNA methylation changes between CCA from Thailand and the UK?

2.7 Are there difference of DNA methylation between short and long survival groups of CCA patients?

2.8 Are there any gene ontology terms enriched in the differential methylation in CCA?

3. Hypothesis of the study

DNA methylation of CpG-island in cancer cells is associated with transcriptional suppression of many tumor suppressor genes leading to the development of cancer cells. In CCA, hypermethylation of CpG-islands related to molecular mechanisms might be a common event and play a role in cholangiocarcinogenesis. Then, it could be an interesting study to evaluate the changes of epigenetic signature both hypermethylation and hypomethylation in genome-wide scale where specific and be significant functional terms to cholangicarcinoma.

4. Objectives of the study

4.1 To study CpG-island methylation profiling of 26 CpG-islands* involved in apoptosis, cell cycle and DNA repair using methylation-specific polymerase chain reaction (MSP) in CCA and adjacent normal biliary tissues with appropriate clinicopathological data.

Note * 26 CpG-islands; *APAF-1, BLU, BRCA1, CASP8, DAPK, DcR1, DcR2, DR4, DR5, FancF, Fas, GSTp1, HIC1, MGMT, MINT25, OPCML, p16, p21, p73, PTEN, RASSF1A, SFRP-1, SOCS-3, Survivin, TMS1* and *14-3-3σ*.

4.2 To confirm positive results of CpG-island methylation by pyrosequencing.

4.3 To examine for clinicopathological associations and methylation profiling of 25 CpG-islands.

4.4 To compare candidate genes indentified to gene expression analysis by immunohistochemistry.

4.5 To perform genome-wide DNA methylation profiling of cholangiocarcinoma using the high throughput technology of Illumina's Infinium® HumanMethylation27 BeadChip and identify methylation changes specific to CCA.

4.6 To identify specific functional analysis of gene ontology enriched in the differential methylation in CCA?

4.7 To evaluate the difference of DNA methylation between short and long survival group of CCA patients.

5. Scope and limitation of the study

In this research, CpG-island methylation profiling of 26 CpG-islands involved in cell cycle arrest, apoptosis, cell adhesion and DNA repairing system were screened in 102 CCA and 29 adjacent-normal tissues from Thailand using MSP and confirmed by pyrosequencing and/or combined bisulfate restriction analysis (COBRA). The correlation between methylation status of each gene and clinicopathological data was analysed. The candidate gene expression was performed using immunohistochemistry.

For genome-wide scale, 32 primary CCA from Thailand and the West, 6 matched adjacent normal samples, 5 CCA cell lines and a normal biliary cell lines were investigated DNA methylation level using HumanMethylation27 BeadChip

based on Infinium® technology. Then, differentially hypermethylated and hypomethylated changes were analysed by using bioinformatics.

6. Anticipated outcomes

6.1 The pattern of DNA methylation profiling of certain CpG-islands in CCA patients is obtained.

6.2 The association between methylation of candidate regulatory genes and clinicopathological data is clarified which might serve as the biomarkers in CCA patients.

6.3 DNA methylation signature with hypermethylation and hypomethylation in the genome-wide scale of CCA is obtained providing a useful data resource of methylation biomarkers of this fatal malignancy.