CHAPTER I
INTRODUCTION

1.1 Rational and background

Cholangiocarcinoma is the highest incident primary liver cancer in Northeast Thailan (Vatanasapt *et al.*, 1990) and still main health problem of people in this area. It has recently been reported that the incidence of CCA in developed countries is now increased (Blendis and Halpern, 2004). This malignant tumor is highly fatal and poor prognosis because there is no method for early detection and lack of effective treatments. At present, only surgical resection of all detectable tumors is correlated with the improvement in five-year survival (Ota *et al.*, 1995; Uttaravichien *et al.*, 1999). Evidence from clinical studies demonstrated that the response rate of this cancer to chemotherapy is relatively poor in which partial responses have been observed in only approximately 10-20% of cases (Lee *et al.*, 2004; Martin and Jarnagin, 2003; Patt *et al.*, 2001). A single chemotherapy for unresectable CCA give a poor response rate of about 10% for mitomycin C and 5-FU, 8% for cisplatin. There are many reports shown that clinical trials in CCA of combination regimen give a response rate of about 10-34% (Choi *et al.*, 2000; Ducreux *et al.*, 1998; Patt *et al.*, 1996; Sanz-Altamira *et al.*, 1998). The poor response rate of this cancer to chemotherapy suggest that this cancer may resist to a number of chemotherapeutic agents. In order to improve efficiency in chemotherapeutic treatment of CCA patients, a new strategy is required.

Recently, prediction of the response of chemotherapy has become of great interest, with numerous attempts being made to accomplish personalized treatment of cancer patients (Tanaka *et al.*, 2004; Yoshida *et al.*, 2003). Of the chemotherapeutic agents, 5-FU is one of the most widely used drugs in a variety of tumors, including CCA. Following uptake into the cells, 5-FU is metabolized into two different active forms: 5-fluorouridine triphosphate (FUTP) and 5-fluorodeoxyuridine monophosphate (FdUMP). These active forms produce the anticancer effects by significantly inhibiting RNA function and DNA synthesis, respectively. The inhibition of DNA synthesis occurs when 5-FU within the tumor is converted by thymidine phosphorylase (TP) through the intermediary of 5-fluorodeoxyuridine (FUdR) or 5-fluorouridine monophosphate (FUMP) to 5-fluorouridine diphosphate (FUDP) and then to

5-fluorodeoxyuridine diphosphate (FdUDP) to form FdUMP. Together with the coenzyme 5,10-methylene tetrahydrofolate (M-THF), FdUMP forms a covalent ternary complex with the DNA de novo synthesizing enzyme thymidine synthetase (TS), bloking the conversion of deoxyuridine monophosphate to thymidine monophosphate (dTMP) and thus inhibiting DNA synthesis. RNA function is inhibited when 5-FU is phosphorylated by orotate phosphoribosyl transferase (OPRT) to form FUMP, or converted by 5-fluorouridine phosphorylase (UP) to 5-fluorouridine (FUR) and then phosphorylated by 5-fluorouridine kinase (UK) to form FUMP, followed by FUDP conversion to FUTP and subsequent uptake by RNA. In the RNA, this substance competes with the essential substrate uridine triphosphate (UTP) to block RNA polymerase. Recent research has suggested that of the three known phosphorylation pathways, the primary pathway involves OPRT (Fukushima *et al.*, 1996). There are also reports that this enzyme plays a major role with regard to 5-FU sensitivity (Inaba *et al.*, 1996). In addition, the degradation pathway for 5-FU involves conversion by dihydropyrimidine dehydrogenase (DPD), which is present in large quantities in the liver, to 5-fluoro-dihydrouracil (DHFU) and then to 2-F-3-ureidopropionate (FUPA), leading to decomposition into α-fluoro-β-alanine (FBAL) and excretion through the urine.

In addition to the establishment of standards of care, researchers now seek for methods to adequately identify patients who may benefit from a given treatment. Approaches for individualizing treatment include prediction of response against chemotherapy through *in vitro* drug sensitivity test, in which viability of cancer cells in relation to the controls is quantified after exposure to anticancer drugs for a predetermined period of time (Samson *et al.*, 2004). The histoculture durg response assay (HDRA) is an *in vitro* chemosensitivity test which uses a three-dimensional tumor tissue in culture instead of a culture of free cells. This assay maintains intercellular contact and interaction with stromal cells in a condition of native architecture which is closely related to the *in vivo* condition (Hasegawa *et al.*, 2007). Drug sensitivity evaluated by HDRA (Furukawa *et al.*, 1995), was observed to predict response against adjuvant chemotherapy in stage III/IV gastric cancer (Kubota *et al.*, 2003) and seems to be predictive of chemosensitivity also for other cancer types (Iwahashi *et al.*, 2005; Kakimoto *et al.*, 2005; Kanasugi *et al.*, 2006; Suda *et al.*, 2002).

In addition to the drug sensitivity test, enzymes that are related to metabolism of 5-FU, including TP, OPRT, TS and DPD, have been implicated to predict chemosensitivity. Expression of these enzymes alone and in combination, have shown potential as predictive parameters for sensitivity against 5-FU-based chemotherapy in gastric cancer (Ishikawa *et al.*, 2000; Ma *et al.*, 2004; Toriumi *et al.*, 2004) as well as metastatic colorectal cancer (Adlard *et al.*, 2002; Ichikawa *et al.*, 2003a; Ichikawa *et al.*, 2003b; Ochiai *et al.*, 2006).

However, study on TS, TP, DPD and OPRT gene expression in relation to 5-FU sensitivity in CCA has not yet been determined. Therefore in the present study, we measured mRNA expression of the four enzymes using real-time RT-PCR and compared the results with our findings from an *in vitro* 5-FU chemosensitivity using the HDRA, in order to search for useful molecular markers for predicting 5-FU sensitivity.

1.2 Objectives of the study

2.1 Establish histoculture drug response assay (HDRA) for testing the response of chlangiocarcinoma to 5-FU *in vitro.*

2.2 To examine intratumoral TS, TP, DPD and OPRT gene expression in CCA by using real-time PCR.

2.3 To determine the relationship between the 5-FU sensitivity of CCA tissues and intratumoral expression levels of TS, TP, DPD and OPRT genes.

1.3 Scope and limitation of the study

Tumor tissues from CCA patients were included in this study. An *in vitro* drug sensitivity to 5-FU was performed using HDRA and the intratumoral TS, TP, DPD and OPRT gene expressions were performed using real time PCR. Then the correlations of these drug metabolizing enzyme gene expressions with the *in vitro* drug sensitivities were analyzed.

This study was an *in vitro* study. However, the results cannot directly extrapolate to the patients. Further clinical trial study are warranted.

1.4 Anticipated outcomes

4.1 Application of the HDRA to determine drug sensitivity test with CCA tissues.

4.2 Understanding molecular mechanism of drug sensitivity of CCA tissues by determining the relationship between the sensitivity of CCA tissues and intratumoral expression levels of TS, TP, DPD and OPRT genes.

4.3 Find a predictive assay *in vitro* that can be routinely used to predict the response to chemotherapy.