CHAPTER I
INTRODUCTION

1.1 Background and rationale

The microenvironment of carcinomas, the epithelial-derived cancers, is composed of cancer-associated fibroblasts, endothelial cells, inflammatory cells and abundant extracellular matrix which can be called tumor stroma. It is hypothesized to be functionally organized to promote survival of cancer cells and generate a favorable environment for cancer cells in both primary and metastatic sites. Myofibroblasts, a specialized type of fibroblast with high expression of α-smooth muscle actin (α-SMA), are the predominant cell types in cancer stroma. In addition to their role in creating the structural matrix around cancer cells, these fibroblasts have been revealed their strong contribution in cancer development, invasion, metastasis, and treatment resistance (Liotta and Kohn, 2001). Cholangiocarcinoma (CCA) or bile duct cancer originated from biliary epithelial cells is quite unique in northeastern Thailand. Several lines of evidences suggest opisthorchiasis as an important factor in the etiology of CCA in the endemic areas of Thailand (IARC, 1994), while in Korea and Japan, the risk factor for CCA is *Clornorchis sinesis* infection (Choi et al., 2006). In Western countries, the genesis of CCA is associated with chronic inflammation of the bile duct from a variety of etiologies such as sclerosing cholangitis, choledochal cysts and congenital hepatic fibrosis (Scott et al., 1980; Lipsett et al., 1994; Broome et al., 1996). Despite the different etiologies of CCA, it is well recognized that CCA contains abundant fibrous stroma. Fibrous stromal cells are α-SMA positive fibroblasts and their numbers in CCA show a significant positive correlation with the degree of tumor fibrosis (Terada et al., 1996). The high α-SMA fibroblasts have been recently shown the significantly correlated with poor patient survival and large tumor size (Chuaysri C et al., 2009). In addition, *in vitro* study indicated concordant results that primary culture fibroblasts derived from CCA tissues could induce human biliary epithelial cell proliferation more than that of non-tumorigenic fibroblasts (Chuaysri C et al., 2009).

All the above data lead to the hypothesis that CCA-associated fibroblast may play some critical roles in particular cancer progression. To a better understanding of the molecular mechanism of CCA-associated fibroblast-driven cancer progression, the genome wide expression analysis of fibroblasts in CCA stroma is of great interest to explore. In this study, the expression profile of cancer-related genes in **primary culture fibroblast (Cf)** isolated from CCA tissues compared to that of **primary culture non-tumorigenic liver fibroblast (Lf)** isolated from the grossly normal liver tissue was demonstrated. The altered genes encoding secreted proteins with established tumorigenic functions were selected. Among these, periostin (PN) which has been shown several functions involved in cancer progression was explored its roles and activated signal transduction pathway in CCA.

PN exhibits normal functions involving regulation of osteoblast adhesion, development of periostium, periodontal ligament (Horiuchi et al., 1999) and heart valves tissue (Kuhn et al., 2007). PN, a multifunctional extracellular matrix protein, has been detected in several cancers in which is secreted from either cancer cells or stromal cells. It has been proposed as a marker-associated cancer aggressiveness and plays critical roles in genesis and progression of several cancers (Ruan, Bao, and Ouyang, 2009). However, PN has never been mentioned in CCA, it is of great interest to explore roles of fibroblast-derived PN in the progression of this cancer. In hepatocellular carcinoma, chemotherapy has been demonstrated to be more effective, if therapies against the underlying fibrosis are also employed (Friedman et al., 2000). The results herein may highlight roles of fibroblasts in CCA and the fibroblast-derived PN activated signal transduction pathway may serve as therapeutic targets to obtain a better treatment in CCA patients. It will support the fact that targeting the tumor as an organ would be more effective than targeting the tumor alone.

1.2 Research questions

1.2.1 What is the differential gene expression profile of Cf compared to Lf?

1.2.2 What are cancer-related genes in Cf involving in CCA progression?

1.2.3 What is (are) the specific signal transduction pathway(s) in biliary epithelial cell induced by Cf-derived secreted protein and the way to inhibit?

1.3 Hypothesis

CCA-associated fibroblast has differential gene expression profile in comparison to that of normal fibroblast. These differential genes encoded, in particular, secreted proteins which may have functions related to cancer progression including proliferation, invasion and metastasis. The fibroblast-derived substances can activate intrabiliary cell signal transduction pathway by which specific inhibitors can be applied to inhibit this activation. The taken knowledge would highlight the application of targeting fibroblasts in CCA to attenuate cancer progression.

1.4 Objectives

1.4.1 To study genome expression profile of CCA-associated fibroblast and compare to that of non-tumorigenic liver fibroblast

1.4.2 To identify the cancer-related genes in fibroblasts playing roles in CCA progression

1.4.3 To identify and inhibit the specific intrabiliary cell signal transduction pathway activated by CCA-associated fibroblast-derived substances

1.4.4 To determine the possible therapeutic target to attenuate CCA-associated fibroblast-driven cancer progression

1.5 Conceptual framework

In CCA, alterations of biliary epithelial cells directly develop to cancer. In addition, the alterations of CCA stromal cells especially fibroblasts have been demonstrated. Our group has recently reported the significance of CCA-associated fibroblasts for their abundance related to patient survival, and the proliferation induction capacity (Chuaysri C et al., 2009). With stimulation by cancer fibroblasts, the disease would be more aggressive (Fig 1-1). The mechanisms of how CCA-associated fibroblasts affect biliary epithelial are the results of altered gene expression in cancer fibroblasts from normal one. The genes encoding secreted proteins of which having tumorigenic functions including cell proliferation, anti-apoptosis, invasion, metastasis and angiogenesis may help in concert with mutations in cancer cells to promote cancer progression. Fibroblast-derived PN, for example, may affect cancer cell through integrin (ITG)-related intrabiliary epithelial cell signal transduction

pathway leading to induction of cell proliferation and invasion. The specific inhibitor to this activated pathway when applied may help inhibit PN-induced CCA progression. In addition, this will highlight the impact of fibroblasts, fibroblast-derived substance, and intra-CCA signal pathways activated by fibroblast-derived substance as future theraptic target to inhibit tumor progression.

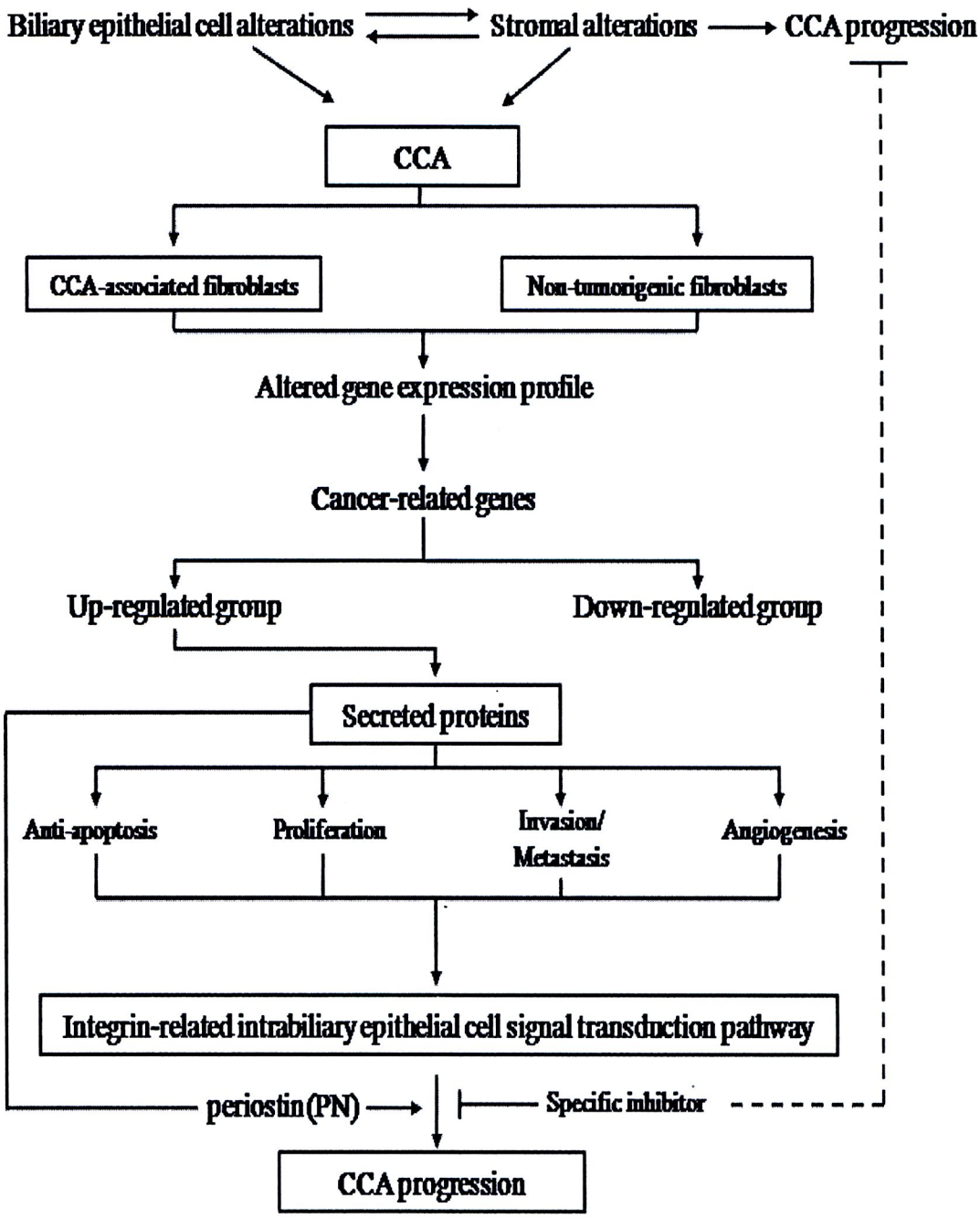

Figure 1-1 Conceptual framework of this study

1.6 Research design

The research design of this study is divided into 4 phases (Fig 1-2).

Phase I: *Transcriptomic study of Cf*

Whole gene expression profile of Cf was studied by cDNA microarray in comparison to that of Lfs. Lists of genes with either up-regulated or down-regulated expressions were analysed and indicated their tumorigenic effects by literature review. The cancer-related genes encoding secreted proteins in particular those controlling cancer cell proliferation, invasion, metastasis and angiogenesis were mainly considered and selected to validate their expression levels.

Phase II: *Selection and validation of cancer-related genes in fibroblasts*

To validate the expression of selected genes, real time PCR was performed to semi-quantitate the mRNA level. From this step, *PN* was selected because of its several carcinogeneic properties and importantly has never been reported its roles in CCA.

Phase III: *Verification of PN expression and investigation of its tumorigenic effects on biliary epithelial cells*

PN was verified at the protein level in Cfs using western blot analysis. Immunohistochemistry was used to confirm its expression and localization in CCA tissues in comparison to the tissues of benign liver disease and hepatocellular carcinoma. The tumorigenic effects of PN on non-tumorigenic biliary epithelial cells and tumorigenic CCA cells were explored including the induction of cell proliferation, cell growth and invasion.

Phase IV: *Identification of PN-induced intrabiliary cell signal pathway*

ITGα5β1-associated signal pathway was explored in cells exposed to PN. RNAi technique using si*ITGα5* and inhibitors of PN-activated pathway were applied to determine the mechanism of PN-driven CCA progression.

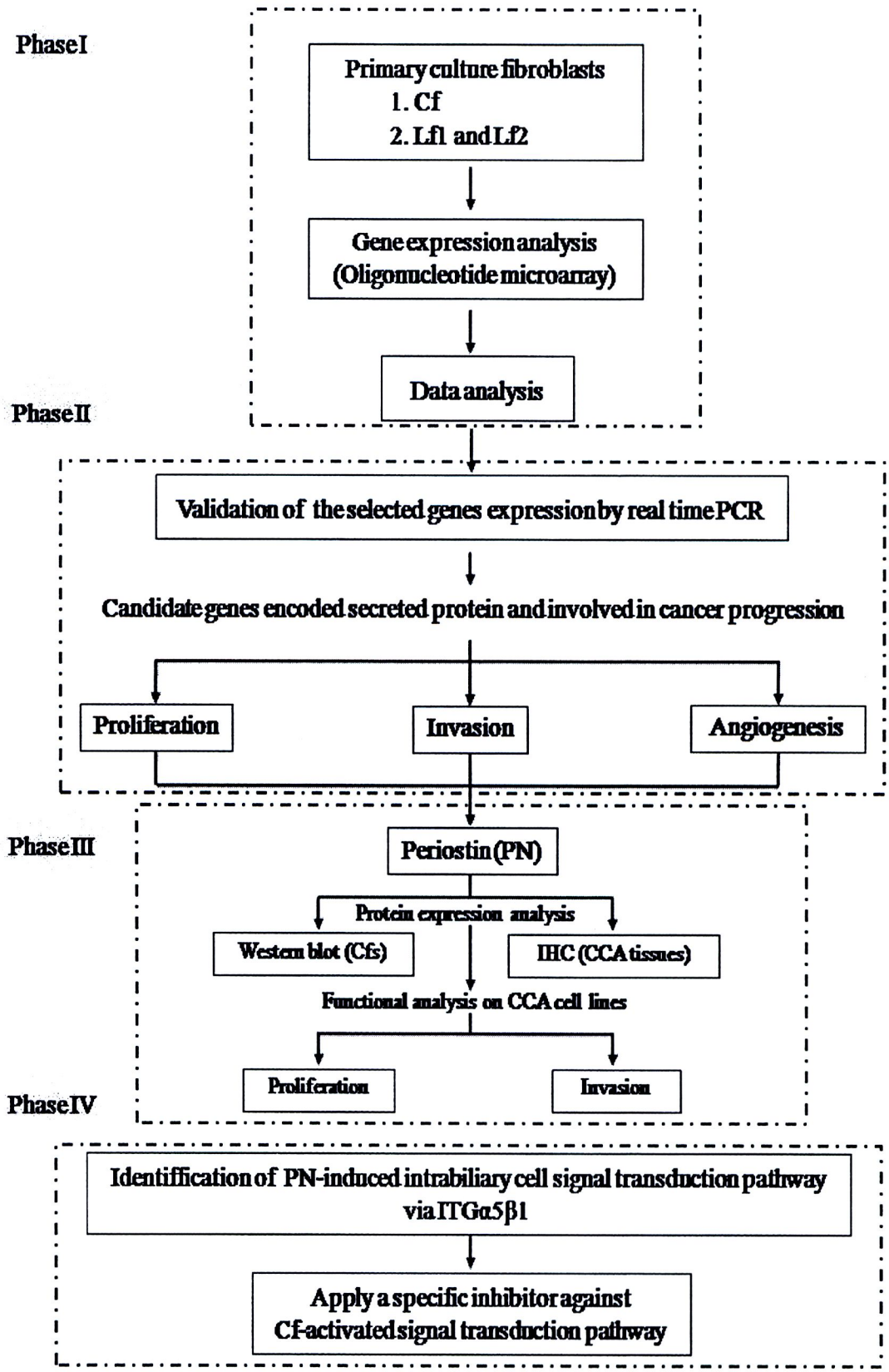

Figure 1-2 The 4-phase of research design

1.7 Anticipated outcomes

1.7.1 The whole gene expression profile of CCA-associated fibroblast was obtained. The up- and down-regulated genes involved in CCA were listed and discussed their possible tumorigenic roles especially in cancer progression. This is the important source of cancer-related gene list in fibroblasts which needed further experiments to explore and confirm their impacts in CCA.

1.7.2 Roles of fibroblast-derived PN in promotion of biliary epithelial cell proliferation and invasion were confirmed. The ITG signaling pathway induced by PN was proposed and may be used as the future therapeutic targets in CCA patients.

1.7.3 The data obtained from this study was published in the international peer-reviewed journals as following;

(1) **Utispan K**, et al. Gene expression profiling of CCA-derived fibroblast reveals alterations related to tumor progression and indicates periostin as a poor prognostic marker, ***Mol Cancer*** 2010; 9; 13 doi: 10. 1186/1476-4598-9-13 (Impact factor = 5.36)

(2) **Utispan K**, et al. Periostin modulates CCA cell invasion through integrin and PI3K/AKT signaling pathway (under preparation)

1.8 Applications

1.8.1 Transcriptomic profile of CCA-associated fibroblasts

Lists of cancer-related genes in CCA-associated fibroblasts will be helpful for researchers to make further studies on these genes to confirm the impact of fibroblasts in CCA.

1.8.2 Molecular mechanism of cancer-associated fibroblast-derived PN in CCA

This may open new aspect regarding role of fibroblasts in progression of CCA and proposed PN-associated pathway in cancer cell as a new therapeutic target to inhibit fibroblast-driven CCA progression.