CHAPTER IV
CONCLUSIONS AND PERSPECTIVES

4.1 Conclusion

Cholangiocarcinoma (CCA), the malignant tumor arising from bile duct epithelia, is the common cancer and a major public health problem in northeast Thailand. CCA is a highly invasive/metastasis malignancy that is difficult to be diagnosed until the advanced or disseminated stage, resulting in poor prognosis. Therefore, CCA treatment is still a challenging task. Although, the details of its molecular carcinogenesis have been elusive, making it difficult to identify possible therapeutic targets.

Our group has demonstrated that the overexpression of PRKAR1A in the liver fluke *(Opirthorchis viverrini*, Ov) and *N*-nitrosodimethylamine (NDMA) induced hamster CCA tumors (Loilome et al., 2006). We also observed PKA isozyme switching, which indicated that the PRKAR1A/PKAI pathway might have contributed to the induction of biliary cell transformation and proliferation in Ov and NDMA-induced progressive cholangiocarcinogenesis in hamster model (Loilome et al., under revision) as well as human cholangiocarcinoma tissues (Loilome et al., under revision). However, the mechanism by which PRKAR1A/PKAI contributes in CCA development is not yet clear. Therefore, the present study was aimed at investigating possible molecular mechanism by which PRKAR1A/PKAI regulates human CCA cell growth. This information could help to determine if PRKAR1A/PKAI is a suitable target for inhibiting CCA cell growth either as single-drug or in combination with other antitumor drugs. In summary, there are 2 main points of conclusion as following

4.1.1 PRKAR1A is over-expressed and represents a possible therapeutic target in human cholangiocarcinoma cell lines

PRKAR1A is overexpressed in both mRNA and protein level in human CCA cell lines. Functional analysis of PRKAR1A by shRNA transfection to CCA cell lines, M156 and OCA17, reveals that down regulation of PRKAR1A expression has led to a decrease of PKA activity and subsequently reduces CCA cells growth of both

M156 and OCA17 via decreased expression of cell cycle regulation proteins. Furthermore, suppression of PRKAR1A expression also dramatically increases apoptosis of both cell lines by increase caspase 3/7 activity as well as increase pro-apoptotic proteins and decrease anti-apoptotic proteins expression. These results suggest that PRKAR1A regulates CCA cell survival by inducing cell proliferation and preventing cell apoptosis. Therefore, targeting PRKAR1A may be benefit for CCA treatment.

Moreover, depletion of PRKAR1A expression results in a decrease in phosphorylation of proteins involved in MAPKs and PI3K/AKT signaling pathway in both cell lines. Additionally, decrease phosphorylated proteins involved in JAK/STAT pathway are observed in OCA17 whereas proteins involved in Wnt/β-catenin pathway are observed in M156. It implies that these proteins are downstream of PRKAR1A/PKAI pathway in CCA. Our results suggest that PRKAR1A/PKAI may regulate CCA cell growth mainly via MAPKs as well as in crosstalk with the PI3K/AKT, JAK/STAT and Wnt/β-catenin signaling pathways.

We investigated also if the small molecule inhibitors of PKA would inhibit CCA cell growth. Proliferation of CCA cell lines was significantly suppressed in the presence of the small molecule inhibitor of PKA, the isoquinoline H89 in a dose-dependent manner. Moreover, the site-selective cAMP analogues, 8-Cl-cAMP and 8-Br-cAMP also showed a growth inhibitory effect, however, high concentrations of drugs were needed. The high concentration of drug used for inhibiting CCA cell growth suggests combining 8-Cl-cAMP with other antitumor drugs, in order to get a better therapeutic response. Moreover, the more effectiveness PKA inhibitors need to be developed.

4.1.2 Suppression of PRKAR1A expression in CCA cell lines enhances antiproliferative and apoptosis effects of protein kinase inhibitors and chemotherapeutic drugs

The PRKAR1A silencing in combination with protein kinase inhibitors as well as chemotherapeutic drugs caused additive growth inhibitory and apoptosis effect in CCA cell lines. These results suggest that suppression of PRKAR1A expression increases the effectiveness of the conventional chemotherapy and protein kinase inhibitors. Thus, combination of down-regulation of PRKAR1A and these

anticancer drugs could be the therapy of choice in CCA treatment. However, the particular drug combinations need to be considered.

4.2 Suggestions for further study

In this study, shRNA stable transfection strategy was used to find out the candidate functions of PRKAR1A in CCA cell lines. PRKAR1A functions implicated in CCA development with cell proliferation and anti-apoptosis advantages. PRKAR1A associated proteins were elucidated by kinase array and the possible molecular mechanism of PRKAR1A/PKAI in CCA were revealed. This study has provided the basic information directly involved with suppression of PRKAR1A in CCA cell lines and has opened further investigations. We demonstrated the crosstalk between PKAI and MAPKs, PI3K/AKT, JAK/STAT and Wnt/β-catenin signaling pathways. Therefore, targeting other signal transduction pathways which associated with PKAI needs also be further investigated.