

Thesis Title Identification and Characterization of Tumor Markers with
Potential for Diagnosis of Cholangiocarcinoma in a Hamster Model

Name Nalinee Prempracha

Degree Doctor of Philosophy (Microbiology)

Thesis Supervisory Committee

Stitaya Sirisinha, D.M.D., Ph.D.

Kavi Ratanabanangkoon, Ph.D.

Somnate Boonpacknavig, M.D.

Petcharin Srivatanakul, Ph.D.

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ABSTRACT

Liver cancer is a major health problem in many Asian-Pacific countries. Of these, cholangiocarcinoma (CCA) is one of the two main types of primary cancers of the liver found in these countries. It is particularly more common in areas where the liver fluke infection caused by *Opisthorchis viverrini* and *Clonorchis sinensis* are endemic. In Thailand, the prevalence of CCA closely parallels that of opisthorchiasis which is most common in Northeastern part of the country. At present, presumptive diagnosis of CCA can be made with a certain degree of accuracy in advanced stages but most often at a stage too late to be managed successfully. The objective of this study was to identify and characterize a possible CCA-associated soluble antigen with potential for use in early detection using experimental hamster model. With this animal model, more tumor specimens and more detailed analysis could be carried out to obtain relevant information that will give valuable pointers for research study on CCA in humans.

In this study, a liver fluke-associated CCA, comparable to that occurring in humans, was induced by exposing *O. viverrini*-infected hamsters to dimethylnitrosamine (DMN). Tumor masses were removed, histopathologically identified and one portion was transplanted

to adult hamsters and maintained by subsequent intramuscular transplantation. The transplanted tumor tissues were extracted for antigen used for the production of monoclonal antibodies (mAbs). The CCA-associated antigen identified by specific mAbs obtained appeared to be a mucin-associated glycoprotein with a relative molecular weight of 200 kDa. This newly described antigen appeared to be immunologically distinct from other tumor markers that has been described previously. When the tumor sections were examined by immunoperoxidase method using a specific mAb 6E5, the strongest reaction was noted at the apical surface of the tumor cells and in secretions formed inside the lumen of proliferating biliary tubules. The mAb 6E5 was used to set up a sandwich ELISA for the quantification of this antigen in the serum and bile of tumor-bearing animals. The assay system was sensitive enough to detect the antigen at concentrations below 10 ng/ml. The serum and biliary levels of this antigen were markedly elevated in animals with progressive tumors when compared with untreated controls. The serum taken serially from each animal that subsequently developed CCA showed a gradual elevation of antigen as carcinogenesis progressed. A few isolated animals also exhibited a significant elevation of antigen at a time as early as the end of DMN treatment, when the CCA should not have yet developed, judging from microscopic examination.

The data from this animal model suggested that the CCA-associated soluble antigen defined by mAb 6E5 was a useful marker for the detection of tumors at an early stage of development and it may be possible to obtain a similar result with human CCA. The data presented provides a relevant and valuable information that will help in simplifying the ongoing research on human CCA.