

Thesis Title	Identification of Tumor Specific Antigens from Human Cholangiocarcinoma Cell Line and Evaluation of its Potential in the Diagnosis of Cholangiocarcinoma
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ABSTRACT

Cholangiocarcinoma (CCA), a malignant tumor of the epithelial lining of the biliary system, is more common in the northeastern part of Thailand where the liver fluke infection caused by *Opisthorchis viverrini* is endemic. Patients with CCA are often diagnosed too late to be managed successfully because there is no specific laboratory tests for diagnosis of this tumor at its early stage of development. The objective of this study was to identify and characterize a possible CCA-associated soluble antigen with potential for use in early detection by using CCA cell line.

In this study, a CCA cell line (HuCCA-1), established from malignant tissue and subsequently propagated into nude mouse, was used. The transplanted tumor was removed and was used for the establishment of tumor cell line, HuCCA-1Nu. Homogenate and secreted products from HuCCA-1Nu were used for the production of monoclonal antibodies (mAbs). A specific clone, mAb 3B1, was screened and selected, using indirect ELISA against the cell line homogenate. By Western blotting, the antibody reacted with 71, 75-82 and 87-kDa components which also stained prominently with wheat germ agglutinin and concanavalin A, indicating their

glycoprotein nature. The immunoblotting reaction of these components resisted periodate oxidation but was digested by trypsin treatment. These data showed that the mAb obtained reacted with the epitope associated with the protein core and not the carbohydrate moiety of the glycoprotein molecule. Immunohistological staining showed the antigen to be present in both the cytoplasm and membrane of the tumor cell. The mAb 3B1 was used to set up a sandwich ELISA for the quantification of these antigens in the serum and bile specimens. This assay system could detect the cell line homogenate antigen at a concentration as low as 3 $\mu\text{g/ml}$. The system used could detect antigens present only in the homogenate of HuCCA-1Nu and in the secreted products of HuCCA-1 and HuCCA-1Nu. However, it failed to give positive reaction with the serum or bile specimens from the patients.

The data from this study suggested that the CCA-associated antigen as defined by mAb 3B1 was a specific marker for HuCCA-1Nu cell line. Although the antibody to epitope present on the 71, 75-82 and 87-kDa components could not be directly used to detect CCA tumor specific antigens in specimens, it nevertheless provides relevant and valuable information regarding the antigens of this tumor and stimulates further development of diagnosis for its detection.