

Ratana Thartarporn. 2000. *Establishment and characterization of human cholangiocarcinoma cell line.*

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Abstract

Cholangiocarcinoma (CHCA) is a rare tumor but it is highly prevalent in Northeast Thailand. However, biology of this cancer has been less studied. Human solid tumor cell lines are an important source for studies of tumor biology including tumor cell growth, differentiation, cytogenetics, molecular pathogenesis and drug responses. To date, only 13 reports about CHCA cell lines have been described in the literature including one from a Thai case. Therefore, our goal to establish more CHCA cell lines from patients living in endemic areas of *Opisthorchiasis* in Northeast Thailand which are more represent those of CHCA in this region, both in cell biology and molecular pathogenetics, than those obtained from other countries.

Fresh tumor tissue from hepatectomy specimens were obtained from 2 patients with CHCA who admitted to the Faculty of Medicine, Srinagarind Hospital. After trypsinization, the cells were cultured in Ham's F12 with the presence of 10% fetal bovine serum and antibiotics at 37 °C in CO₂ incubator. Contaminating fibroblasts were periodically scraped out. Established cell lines were obtained after subculturing of the pure colonies at least 4-5 times. Characterization was then performed including cell morphology, growth kinetics, ultrastructure, immunocytochemistry and cytogenetics. The primary tumors of the two cases were histologically diagnosed as adenosquamous and moderately differentiated adenocarcinoma. Two cell lines were established at approximately 4 months after primary culture and were designated as KKU-M139 and KKU-M156, respectively. Doubling times of both cell line were 7 days. Immunocytochemically, these cell lines exhibited cytokeratin, CEA, EMA and CD44 but not alphafetoprotein, and desmin. In addition, KKU-M156 were positive for CA19-9 and CA125 whereas KKU-M139 were negative. The expression of these proteins was similar to those of the original primary tumors. Ultrastructurally, KKU-M139 showed dense tonofilament of squamous origin and microvilli of adenocarcinoma cells while KKU-M156 had secretory granules and prominent microvilli and junctional complex. Cytogenetics analysis revealed aneuploidy karyotypes with a modal chromosome numbers of 74 and 124 for KKU-M139 and KKU-M156, respectively. Marked chromosomal structural changes were noted in both cell lines. These cell lines have been maintained in our laboratory for more than 6 months.

KKU-M139 and KKU-M156 are two additional cell lines established in our laboratory. Moreover, KKU-M139 is the second adenosquamous subtype of cholangiocarcinoma cell line in the world. These

cell lines will be further characterized in details. Future study in all aspects using these cell lines have been planned including molecular biology, growth factors, metastasis and drug responses