GENES AND CHOLANGIOCARCINOMA

Kanlayanee Sawanyawisuth

Department of Biochemistry, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand

Abstract. Cholangiocarcinoma (CCA) is a major public health problem in the northeastern part of Thailand where the prevalence of this cancer is highest in the world. It is a slow growing tumor but highly metastatic with a poor prognosis. The traditional habit of eating raw fish, repeated exposure to liver fluke and consumption of nitrosamine-contaminated food are major risk factors. Understanding of carcinogenesis and metastasis of cholangiocarcinoma at the molecular level will provide tools for better prevention, diagnosis and treatment. This review focuses on CCA-associated genes and their possible applications.

INTRODUCTION

The term “cholangiocarcinoma” is used for primary tumor of the bile duct, including intrahepatic, perihilar, and distal extrahepatic tumors (Nakeeb et al., 1996). In Thailand, cholangiocarcinoma (CCA) refers to malignant tumor of the biliary tree including the intrahepatic and extrahepatic portions, but not carcinoma of the gall bladder and the ampulla of Vater (Uttaravichien et al., 1999).

Intrahepatic CCA, originating from intrahepatic biliary tree including right and left hepatic ducts, is classified according to the main location along the biliary tree as peripheral and central types. Peripheral intrahepatic CCA is defined as carcinoma of the intrahepatic bile duct peripheral to the bifurcation of the right and left hepatic ducts and the tumor mass is usually located within the liver (Uttaravichien et al., 1999). Central type refers to CCA that originates from intrahepatic portion of the right or left hepatic duct (lobar duct) and the tumor is located mainly at the hepatic hilus, usually derived from the right or left hepatic duct or its direct branches or in the vicinity of hepatic duct confluence.

EPIDEMIOLOGY

In humans, CCA is a relatively rare cancer that almost always presents with an extremely poor prognosis (Carriaga and Henson, 1995; Farley et al., 1995). Even in those that have undergone complete surgical resection, the recurrence rate remains quite high and consequently the 5-year survival rate ranges only from 0-40% (Gores, 2003; Anderson et al., 2004). Apart from certain regions of Asia, in particular Northeast Thailand where CCA is endemic, worldwide frequencies of this malignant neoplasm have generally been reported to range from 5%
to 30% of all liver cancers (Parkin et al., 1993). Although CCA is relatively infrequent compared to hepatoma, attention to CCA is now growing because both incidence and mortality rate of CCA are increasing in the United States, the United Kingdom, Australia and overall worldwide (Patel, 2001; Khan et al., 2002). The highest incidence rate, 188 per 100,000 of CCA, is in Khon Kaen, Thailand (Sriamporn et al., 2004).

### RISK FACTORS

Primary sclerosing cholangitis (PSC) is a well-known risk factor for CCA in Western countries (Rosen et al., 1991) and associates with ulcerative colitis. The lifetime risk of CCA among ulcerative colitis patients is 10-15% (Lee and Kaplan, 1995). In addition, thorotrast used as a radiologic contrast agent in x-ray diagnosis was reported to cause CCA several decades after exposure (Liu et al., 2002).

In Asian countries, liver fluke infection is the major risk for CCA. Clonorchis sinensis, a liver fluke endemically infested in China, Korea, Japan and Taiwan, has been proposed to be a probable cause of CCA (Hou, 1956). In Northeast Thailand, the traditional habit of eating raw freshwater and salt-fermented fish on a daily basis results in a local population repeatedly exposed to liver fluke (Opisthorchis viverrini, OV) infection and nitrosamine-contaminated food from early in life. Epidemiological studies have revealed a correlation between high prevalence of CCA and liver fluke infection in this region (Khan et al., 2008). In addition, experimental animal models have demonstrated that dietary contamination of nitrosamines and opisthorchiasis are strong predisposing factors for the genesis of CCA (Thamavit et al., 1993; Mitacek et al., 1999). Apart from exogenous nitrosamines, endogenous nitrosamines were also shown as risk factors (Ohshima et al., 1994; Satarug et al., 1996).

### CHOLANGIOCARCINOGENESIS

Several mechanisms by which OV infection may enhance the genesis of cholangiocarcinoma have been proposed (Sripa and Pairojkul, 2008). The primary pathological change, ie epithelial desquamation, may be due to mechanical irritation caused by the liver fluke and/or its excretory/secretory (ES) products. However, immunopathological processes may also contribute to the long-standing hepatobiliary damage. Following liver fluke infection, inflammation, periductal fibrosis and proliferative body responses, including epithelial hyperplasia, goblet cell metaplasia, adenomatous hyperplasia, may result in predisposing lesions that enhance a greater susceptibility of DNA to carcinogens.

In Northeast Thailand, several N-nitroso compounds and their precursors exist in low levels in fermented food, such as fermented fish ("pla-ra"), which is a daily northeastern diet (Sripa et al., 2007). These compounds are possible primary carcinogens leading to CCA in this region. Apart from exogenous carcinogens, endogenous nitrosation caused by the liver fluke infection has been extensively studied both in animals and humans (Pairojkul et al., 1991; Srivatanakul et al., 1991; Bartsch et al., 1992; Thamavit et al., 1994). Several human studies indicated that infected individuals have a higher endogenous nitrosation level than uninfected individual (Srivatanakul et al., 1991). OV infection in hamsters can induce nitric oxide synthase production in macrophages, mast cells and eosinophils in the inflamed areas surrounding the bile ducts. Recently, biomarkers of DNA damage, 8-oxo-7,8-dihydro-2′-deoxyguanine (8-oxodG) and 8-nitroguanine were demonstrated in inflammatory cells and bile duct epithelium of OV infected hamsters (Pinlaor et al., 1999).
et al, 2003). Moreover, repeated infection with OV mediates oxidative and nitrosative DNA damages sooner than a single infection (Pinlaor et al, 2004).

Both exogenous and endogenous nitrosamine formation may lead to DNA alkylation and DNA base deamination in predisposed and inflamed tissues. Excess nitric oxide produced by the infection could also exert direct cytotoxic and mutagenic effects and increase cell proliferation. DNA damaged biliary epithelium may then be transformed to malignant CCA.

A recent study has demonstrated the effect of OV ES product(s) in inducing NIH-3T3 mouse fibroblast cell proliferation in vitro by stimulating the expression of phosphorylated retinoblastoma (pRB) and cyclin D1, key proteins driving cells through the G1/S transition point of the cell cycle (Thuwajit et al, 2004). Furthermore, gene expression profile obtained from cDNA array analysis showed significant changes of gene expression in several functional categories including genes related to cell proliferation (Thuwajit et al, 2006). TGF-beta and EGF signal transduction pathways were indicated as possible pathways of OV-driven cell proliferation (Thuwajit et al, 2006).

TUMOR SPREAD AND METASTASIS IN CCA

Various routes of tumor spread are seen in CCA. The recognized patterns include direct invasion, infiltration along the biliary tree, vascular and lymphatic permeation, and perineural and intraneural invasion (Sripa and Pairojkul, 2000). Direct invasion to the adjacent liver parenchyma through the sinusoid is common in CCA. This may occur with or without inflammatory cell infiltration. On the other hand, infiltration along the biliary tree usually shows periductal infiltration and variable desmoplastic reaction. Lymphatic permeation in CCA is more common than vascular invasion. Spreading along nerves is encountered particularly in tumor at the large portal tracts. Intrahepatic metastasis develops in nearly all cases at relatively advanced stage. The incidence of regional lymph node metastasis is high in CCA. Blood-borne spread occurs later, particularly to the lungs, but other sites include bone, adrenals, kidney, spleen and brain (Sripa and Pairojkul, 2000).

PROGNOSIS

Prognosis of intrahepatic CCA is generally unfavorable because loco-regional extension is usually advanced at the time of diagnosis. Even after a resection, the outcome for patients with advanced stage is extremely poor (Khuntikeo, et al, 2008). Several prognostic factors for patients with resection have been reported. The presence of vascular invasion, lymphatic invasion, and lymph node metastasis is significant poor prognostic predictor for intrahepatic CCA (Hanazaki et al, 2002). In addition, periductal infiltration, perineural invasion, portal vein invasion, intrahepatic metastasis, and two or more lymph node metastases predict shorter survival after surgery (Uenishi et al, 2001; Hirohashi et al, 2002; Suzuki et al, 2002).

Several studies also showed a significant relationship of survival rate to vascular permeation, extrahepatic metastases, and lymphatic, neural, and nodal involvement. Patients with lymphatic, neural, or nodal involvement die early after surgery (Namieno et al, 2001). Among the different routes of metastasis, the presence of lymph node metastasis has been reported in most studies to be the worst prognostic factor after a resection (Shirabe et al, 2002). The survival rate for intrahepatic CCA patients positive for lymph node metastasis is lower than those negative.

GENE EXPRESSION PROFILE AND CCA

Cancer phenotype reflects changes in the expression patterns of hundreds or even thousands of genes that occur as a consequence of the primary mutation of an oncogene or a tumor suppressor gene. Much of cancer research over the past 50 years has been devoted to analysis of genes that are expressed differently in tumor cells as compared with their normal counterpart parts.

To date, only a few studies of a comprehensive analysis of gene expression in CCA have been reported. Global gene expression analysis of primary resected tumors, biliary cell lines and non-neoplastic biliary epithelial scrapings using Affymetrix oligonucleotide microarrays has been conducted (Hansel et al, 2003). Two hundred and eighty-two known genes are up-regulated three-fold or more in cancer compared to normal epithelium, including proliferation and cell cycle antigens (cyclins D2 and E2, cdc2/p34, and geminin), transcription factors (homeobox B7 and islet-1), growth factors and growth factor receptors (hepatocyte growth factor, amphiregulin, and insulin-like growth factor 1 receptor), and enzymes modulating sensitivity to chemotherapeutic agents (cystathionine synthase, dCMP deaminase, and CTP synthase). Gene expression profiles of intrahepatic CCA using tumor cell populations purified by laser microbeam microdissection were analyzed (Obama et al, 2005). Fifty-two genes are commonly up-regulated and 421 down-regulated in intrahepatic CCA compared with noncancerous biliary epithelial cells. Among the 52 up-regulated genes, P-cadherin and survivin were investigated for enhanced expression in cancer tissues by immunohistochemical staining (Obama et al, 2005). The frequency of expressed sequence tags of intrahepatic cholangiocarcinoma cell lines was compared to normal liver tissues (Wang et al, 2006). One hundred and thirty-seven genes are differentially expressed, and among them, ANXA1, ANXA2, AMBP and SERPINC1 were verified in human ICC cell lines and tissues by semi-quantitative RT-PCR and immunohistochemical analysis (Wang et al, 2006).

METASTASIS-ASSOCIATED GENES IN CCA

To understand the molecular basis for metastasis, a comparison of gene expression profile of primary and metastatic tissues are necessary. In several tumors, these metastasis-associated genes have been defined (Reyes et al, 2007; Basak et al, 2008; Stein et al, 2009), but there are limited data of such genes in CCA.

Increased c-erbB-2 expression contributes to the development of CCA into an advanced stage associated with tumor metastasis (Aishima et al, 2002). Expression of thymidine phosphorylase, an important regulator of angiogenesis, shows a significant correlation with vascular invasion, lymphatic permeation, perineural invasion and lymph node metastasis of intrahepatic CCA (Aishima et al, 2002). In addition, sialyl Lewis (a) is related to vascular invasion and poor prognosis of CCA (Juntavee et al, 2005). On the other hand, expression of bcl-2 is inversely related to lymph node metastasis, vascular invasion and perineural invasion of CCA (Ito et al, 2000).

The incidence rate of lymph node metastasis has been shown to increase in proportion to an increase in the expression of matrix metalloproteinase-9 in intrahepatic CCA (Shirabe et al, 1999). Over-expression
of aspartyl (asparaginyl)-hydroxylase is correlated with cell motility of CCA (Maeda et al, 2003) and cyclin D1 over-expression is more frequently observed in CCA with lymph node metastasis (Ito et al, 2001). Likewise, WISP1v expression is associated with lymphatic and perineural spread of CCA and poor clinical outcome (Tanaka et al, 2003).

CCA-RELATED GENES AND THEIR POTENTIAL ROLES

Several genes have been identified to be associated with CCA and each gene has its own unique potential benefit. Table 1 summarizes the study details of these genes which are categorized as diagnostic, therapeutic and prognostic roles.

Diagnostic role of CCA-related genes

Early diagnosis of CCA may provide better prognosis and survival. Srisomsap et al (2007) compared the expression of membrane-associated and cytosolic proteins from human CCA (HuCCA-1) and hepatocellular carcinoma cell lines (HCC-S102) by 2-DE and LC/MS/MS. The proteomic analysis showed that the cytoskeletal microfilament-associated proteins were highly expressed in both membrane-associated and cytosolic proteins of HuCCA-1 (40.9% in membrane and 26.2% in cytosol) than in HCC-S102 (3.0% in membrane and 9.1% in cytosol). In addition, ten membrane proteins including calgizzarin, ezrin, moesin, radixin, immunoglobulin kappa chain variable region, integrin alpha-6 precursor, cytochrome c oxidase polypeptide VIb isofrom 1, glycerol-3-phosphate dehydrogenase, hippocalcin-like protein 1, and MAPK/ERK kinase 2 found only in HuCCA-1, may be possible diagnostic markers for discrimination of CCA from HCC.

Therapeutic role of CCA-related genes

There are several potential genes that have been shown to be associated with a therapeutic role, such as human MutL homolog1 (hMLH1), methionine aminopeptidase 2 (MetAP2), galectin-3, C-X-C motif chemokine receptor 4 (CXCR4) and phosphoinositide-3-kinase (PI3K).

Limpaiiaihoon et al (2005) studied genetic and epigenetic alterations of hMLH1 gene using polymerase chain reaction (PCR)-based microsatellite marker D3S1611 and methylation-specific PCR, respectively. hMLH1 is one of the major DNA mismatch repair genes. Hypermethylation of hMLH1 promoter occurs in 29 of 65 CCA cases (45%), while all normal tissues show unmethylation. Genetic alterations of hMLH1 shown by the loss of heterozygosity (LOH) was detected in 12 of 51 informative cases (23.5%) and microsatellite instability (MSI) was found in 5 of 65 cases (8%). Of 29 cases showing hypermethylation of hMLH1, 4 (14%) and 5 (17%) show MSI at D3S1611 and LOH, respectively. Even though hMLH1 promoter hypermethylation has no association with gross type, nerve invasion and lymphatic invasion of CCA, it is significantly related to the poorly differentiated type ($p = 0.013$). These findings suggest that both genetic and epigenetic mechanisms cause inactivation of hMLH1. Since gene silencing by methylation in an early event in carcinogenesis, promoter hypermethylation of hMLH1 may be a target of therapy and prevention of liver fluke-related CCA.

Methionine aminopeptidases 2 (MetAP2), a metallopeptidase that selectively catalyzes the removal of the N-terminal methionine from newly synthesized protein, has been reported to be related with tumor growth in several cancers (Datta and Datta, 1999; Catalano et al, 2001; Selvakumar et al, 2006). There has been only one study of MetAP2 in CCA (Sawanyawisuth et al, 2007). Immunohistochemistry of MetAP2 was evaluated in 59, 52 and 82 liver specimens with nonneoplasia, dysplasia and CCA, respectively. Fifty percent of the normal bile duct
is negative for MetAP2 and the remaining expresses weak staining. However, hyperplastic/dysplastic (62%) and primary tumor (73%) of biliary epithelial cells are uniformly stained with moderate to strong intensity. The expression level of MetAP2 is significantly increased in dysplastic and tumor bile duct than in normal bile duct. Similar to the dysplastic and CCA bile duct epithelia, MetAP2 is constantly expressed in metastatic tumors with moderate to high intensity (Sawanyawisuth et al, 2007). However, there is no significant association of MetAP2 expression and pathological and clinical features (tumor grade, tumor stage, tumor size and tumor invasion).

Another treatment option to control metastatic CCA has been shown by Junking et al (2008). Galectin-3 is significantly less expressed in the poorly-differentiated CCA than in the papillary, well- to moderately-differentiated type ($p = 0.012$). In addition, low galectin-3 expression is significantly associated with lymphatic invasion ($p = 0.002$). Therefore, regulation of galectin-3 expression may be an alternative therapeutic approach to control metastasis of CCA.

Leelawat et al (2007, 2009), reported two potential mechanisms on treatment of CCA via C-X-C motif chemokine receptor 4 (CXCR4) and its signaling cascades, which is a key factor for cancer cell progression and metastasis in CCA cell lines. The activation of CXCR4 with CXC chemokine ligand-12 (CXCL12) trigs the signaling via extracellular signal-regulated kinase-1/2 (ERK1/2) and phosphoinositide 3-kinase (PI3K). To confirm this mechanism, the authors found no invasion activity when CCA cell lines (RMCCA1 and KKU100) are pretreated with a specific inhibitor of CXCR4 (AMD3100) and then treated with CXCL12. In addition, treatment with MEK1/2 inhibitor (U0126) or PI3K inhibitor (LY294002) also attenuates the effect of CXCL12-induced CCA cell invasion. These findings imply a potential role for the inhibition of CXCR4 or its signal cascades in the treatment of CCA.

In addition to the invasion effect, PI3K/Akt activation has been shown to be possible mechanism of chemotherapy-resistance of CCA (Leelawat et al, 2009). The oxabilplatin-treated CCA cell lines (RMCCA1 and KKU100) show high levels of Akt and mammalian target of rapamycin (mTOR), a downstream effector of PI3K/Akt activation. The combination of oxaliplatin with LY294001, an inhibitor of PI3K, results in a remarkable arrest of cell proliferation and a significant induction of apoptosis. Therefore, activation of PI3K might protect CCA cells from oxaliplatin regimen. Targeting the PI3K pathway may be a useful treatment to improve chemotherapeutic sensitivity of CCA.

**Prognostic role of CCA-related genes**

CCA-related genes that correlated with prognosis or survival have been reported mainly from Khon Kaen University, Khon Kaen, Thailand, the endemic area of CCA (Limpaipoon et al, 2006; Muenphon et al, 2006; Thanasai et al, 2006; Thuwajit et al, 2008; Chinnasri et al, 2009). Detection of specific genes may indicate the aggressiveness of CCA and also therapeutic interventions. Various study techniques and targeted molecules or chromosomal regions are shown in Table 1.

Ten polymorphic microsatellite markers were explored by LOH and MSI on chromosome region 1p36-pter in 90 CCA patients (Limpaipoon et al, 2006). Of those, 68 cases (75.6%) show LOH in one or more loci and the most frequent loci are D1S199 (40.0%), D1S507 (34.6%), D1S2845 (30.1%), and D1S2734 (30.1%). MSI was found in 34 cases (37.8%) at one or more loci. Patients with LOH at D1S234 and D1S2676 have significantly less and more lymphatic invasion, respectively ($p = 0.017$ and 0.031, respectively). LOH at D1S2845 shows a significant correlation with
<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>N</th>
<th>Source</th>
<th>Molecule/chromosomal region</th>
<th>Technique</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diagnostic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Srismbsap</td>
<td>2007</td>
<td>NA</td>
<td>HUCCA-1, HCC-S102</td>
<td>calgizzarin, ezrin, moesin, radixin, immunoglobulin kappa chain variable region, integrin</td>
<td>2-DE, LC/MS/MS and</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>alpha-6 precursor, cytochrome c oxidase polypeptide Vla isoform 1, glycerol-3-phosphate</td>
<td>Western blotting</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>dehydrogenase, hippocalcin-like protein 1, and MAPK/ERK kinase 2</td>
<td></td>
</tr>
<tr>
<td><strong>Therapeutic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limpaiiboon</td>
<td>2005</td>
<td>65</td>
<td>Intrahepatic CCA</td>
<td>Human mutL homolog1</td>
<td>PCR</td>
</tr>
<tr>
<td>Sawanyawisuth</td>
<td>2007</td>
<td>82</td>
<td>Intrahepatic CCA</td>
<td>Methionine aminopeptidase 2</td>
<td>Immunohistochemistry</td>
</tr>
<tr>
<td>Leelawat</td>
<td>2007</td>
<td>NA</td>
<td>RMCCA1, KKKU100</td>
<td>C-X-C motif chemokine receptor 4</td>
<td>Western blotting</td>
</tr>
<tr>
<td>Junking</td>
<td>2008</td>
<td>53</td>
<td>Intrahepatic CCA, KKKU 100,</td>
<td>Galectin-3</td>
<td>Immunohistochemistry</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>KKKU 214</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leelawat</td>
<td>2009</td>
<td>NA</td>
<td>RMCCA1, KKKU100</td>
<td>Phosphoinositide-3-kinase/Akt</td>
<td>Western blotting</td>
</tr>
<tr>
<td><strong>Prognostic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limpaiiboon</td>
<td>2006</td>
<td>90</td>
<td>Intrahepatic CCA</td>
<td>1p36-pter</td>
<td>PCR</td>
</tr>
<tr>
<td>Muenphon</td>
<td>2006</td>
<td>80</td>
<td>CCA</td>
<td>21q22-pter</td>
<td>Quantitative real-time PCR</td>
</tr>
<tr>
<td>Thanasai</td>
<td>2006</td>
<td>65</td>
<td>CCA</td>
<td>22q12-pter</td>
<td>Quantitative real-time PCR</td>
</tr>
<tr>
<td>Thuwajit</td>
<td>2008</td>
<td>51</td>
<td>CCA</td>
<td>Mucin6, trefoil-2 trefoil peptide</td>
<td>Immunohistochemistry</td>
</tr>
<tr>
<td>Chinnasri</td>
<td>2009</td>
<td>94</td>
<td>Intrahepatic CCA</td>
<td>p14ARF, p15INKb, p16INKa</td>
<td>PCR, immunohistochemistry</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N, numbers of subjects; NA, not applicable; HUCCA-1, human bile duct epithelial carcinoma cell line-1; HCC-S102, hepatocellular carcinoma cell line S102; CCA, cholangiocarcinoma patients; PCR, polymerase chain reaction
nerve invasion and MSI at D1S228 has a poor prognosis \((p = 0.029\) and \(0.0026, \) respectively). The authors concluded that allelic loss at chromosome 1p36 can be used as molecular prognostic indicators for CCA patients.

Researchers from the Liver Fluke and Cholangiocarcinoma Research Center, Khon Kaen University, Khon Kaen, Thailand also discovered other prognostic markers for CCA. D21S1893-D21S1890 region of chromosome 21q22-qter may carry candidate genes especially trefoil factor or serine protease family and might be involved in tumor invasion, metastasis and poor survival (Muenphon et al, 2006). On the other hand, D22S283 amplification on 22q12-qter chromosomal area is an independent indicator of favorable prognosis in liver fluke-related CCA (Thanasai et al, 2006).

Chinnasri et al (2009) showed a correlation between clinicopathological data and genetic-epigenetic alteration including loss of protein expressions of \(p14^{ARF}, p15^{INK4b},\) and \(p16^{INK4a}\) in 94 CCA samples. LOH, MSI and fine mapping of the chromosomal region 9p21-pter were performed using PCR-based microsatellite markers. Methylation and protein expression of \(p14^{ARF}, p15^{INK4b},\) and \(p16^{INK4a}\) were determined using methylation-specific PCR and immunohistochemistry, respectively. Region between D9S286 and D9S1752 of chromosome 9p21-pter was demonstrated to be a common loss. Methylation frequency for \(p14^{ARF}, p15^{INK4b},\) and \(p16^{INK4a}\) is 40.2, 48.9, 28.3%, respectively. Loss of these 3 proteins is 30.9, 58, and 81.5%, respectively. Poor prognosis is associated with only the loss of \(p16^{INK4a}\) by Kaplan-Meier survival analysis \((p = 0.026)\).

Additionally, mucin6 (MUC6), a mucin that is normally co-expressed with the trefoil factor family-2 (TFF2) trefoil peptide, has shown a good correlation with survival of CCA patients (Thuwajit et al, 2008). MUC6 and TFF2 levels were examined in 51 CCA patients by immunohistochemistry. The high expression levels of both proteins significantly correlate with prolonged post-operative survival time, but only a high expression of MUC6 is significantly related with a 5-year survival rate. Independent poor prognostic factors including a low expression of MUC6, high expression of TFF2, age of patients of more than 56 years, tumor size of more than 5 cm, and poorly-differentiated histological type were indicated by multivariate Cox regression analysis.

CONCLUSION

CCA is a slow growing cancer with poor prognosis. It is highly prevalent in Northeast Thailand where it is endemic. In this region, CCA is associated with OV infection. Several researchers aimed to improve the procedures of diagnosis and treatment by targeting molecule markers related to CCA. This review indicates the significance of CCA-related genes and their potential diagnostic, therapeutic and prognostic roles. Clinical application of these molecular markers may be useful for early detection and therapy of CCA in the future. However, further studies are still needed to provide prevention strategies and the best therapeutic outcome.

ACKNOWLEDGEMENTS

The author thanks Dr Kittisak Sawanyawisuth for his kind support and assistance.

REFERENCES


Anderson CD, Pinson CW, Berlin J, Charl RS. Diagnosis and treatment of cholangiocarcin-


Thuwajit C, Thuwajit P, Kaewkes S, *et al.* Increased cell proliferation of mouse fibroblast NIH-3T3 *in vitro* induced by excretory/secretory product(s) from *Opisthorchis viverrini*. *Parasitology* 2004; 129: 455-64.


