

Hilar cholangiocarcinoma: Pathology and tumor biology

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Abstract Hilar cholangiocarcinoma, first described by Klatskin in 1965, is a relatively rare tumor arising from the bile ducts. The histomorphological features of hilar cholangiocarcinoma are identical with other extra- and intra-hepatic bile duct carcinomas. The most common disease associated with cholangiocarcinoma is primary sclerosing cholangitis. The development of cholangiocarcinoma is a multistep process associated with several mutations in oncogenes and tumor-suppressor genes. Based on macroscopic appearance, three distinct subtypes have been described: sclerosing, nodular, and papillary. Microscopically, more than 95% of tumors are adenocarcinomas. Hilar cholangiocarcinoma is a slowly growing tumor and tends to spread longitudinally along the bile ducts with neural, perineural, and subepithelial extension. Lymph node invasion can be found in 30%–50% patients at the time of diagnosis, but blood-born metastases are rare and usually occur at late stages.

Keywords hilar cholangiocarcinoma; morphology; primary sclerosing cholangitis; metastasis; growth

1 Introduction

Cholangiocarcinoma is an uncommon adenocarcinoma that arises from the epithelial cells of bile ducts anywhere along the intrahepatic and extrahepatic biliary tree, excluding the papilla of Vater and the gall bladder. Cholangiocarcinoma comprises less than 10% of primary hepatic malignancies [1–5]. Although these tumors can occur at any level of the biliary tree, 67% occur at the bifurcation of the bile duct (hilar cholangiocarcinoma) [6], where they are often referred to as Klatskin tumors, a particular entity of biliary carcinomas [7].

The histomorphological features of hilar cholangiocarcinoma are identical with other extra- and intra-hepatic bile duct carcinomas. This particular localization leads to early clinical manifestation of this tumor, which contributes to better prognosis compared with intrahepatic cholangiocarcinoma. Lymph nodes are involved in 30%–50%, while distant metastases are observed in only 10% of patients at the time of surgery [8–12]. Unlike intrahepatic or distal cholangiocarcinoma, which can be treated with hepatic resection or pancreaticoduodenectomy, respectively, surgical management of hilar cholangiocarcinoma has evolved since its original description. Due to the particular localization of this tumor and its propensity to infiltrate the hilar region, hilar cholangiocarcinoma remains a challenge for surgeons, internists, and radiologists.

2 Etiology and risk factors

Certain disorders have been associated with an increased incidence of cholangiocarcinoma. The most common disease associated with cholangiocarcinoma is primary sclerosing cholangitis (PSC), an autoimmune disorder characterized by multifocal strictures of the intrahepatic and extrahepatic bile ducts [13]. In a large Swedish trial, 8% of patients with PSC developed the tumor within a mean follow-up period of 5 years. Occult cholangiocarcinoma has been identified in up to 40% of autopsy specimens and in 9%–36% of liver explants after liver transplantation in patients with PSC. A recent US study confirmed a similar risk of developing cholangiocarcinoma in PSC patients [14]. A European multicenter study [15], including 394 PSC patients from five European countries with a median follow-up of 18 years, has demonstrated that the majority of cholangiocarcinoma cases (50%) were diagnosed within the first year after diagnosis of PSC and in 27% at intended liver transplantation. Therefore, patients who present with a primary diagnosis of PSC should be carefully screened and regularly followed-up for

cholangiocarcinoma development mainly during the first 2 years after PSC diagnosis [16,17]. Unlike most cases of sporadic cholangiocarcinoma of the extrahepatic biliary tree, cholangiocarcinoma in PSC patients are frequently multifocal and may not be amenable to resection. Moreover, 70%–80% of patients with PSC also suffer from inflammatory bowel disease, mainly in the form of chronic ulcerative colitis.

Another well-known risk factor in the young is congenital biliary cystic disease in the form of choledochal cysts or Caroli's disease. In patients with untreated cysts, the risk of tumor occurrence increases to 15%–20% until adulthood [18]. This kind of cancer tends to present at an earlier age than the sporadic form. The reason for the increased risk of cholangiocarcinoma in patients with congenital biliary cysts is not clear, but is thought to be related to an abnormal choledochopancreatic duct junction, resulting in reflux of pancreatic secretions into the biliary tree, chronic inflammation, and bacterial contamination [19–22].

In Japan and Southeast Asia, the most important risk factor for the development of cholangiocarcinoma is biliary infection with liver flukes (e.g. *Opisthorchis viverrini*, *Clonorchis sinensis*) [23–25]. These liver flukes gain entry to the host through the duodenum and reside in the biliary system, thus resulting in chronic biliary obstruction and inflammatory response.

Additional risk factors for tumor occurrence include exposure to several chemical agents and radionuclides (e.g. nitrosamines, dioxin, asbestos, thorotrast, radon) [26–28], obesity [29], alcohol [30], and tobacco smoking [31].

In contrast to hepatocellular carcinoma, hilar cholangiocarcinoma does not appear to be associated with hepatitis B virus (HBV) or hepatitis C virus (HCV) infection and is not related to alcoholic liver disease either. However, the incidence of cirrhosis in hilar cholangiocarcinoma is reported to be as high as 38%. Although an association between cirrhosis and cholangiocarcinoma has not been clearly shown, alteration in hormonal levels, impaired metabolism of carcinogens, and changes in immunological status may be contributing factors for the development of cholangiocarcinoma in patients with cirrhosis [32–36].

Although gall stones have been reported in one-third of patients with bile duct cancer, a cause-and-effect relationship has not been established. There is no strong link with the presence of gall stones although in one series, 37% of 94 cases had coincident cholelithiasis. Similarly, 50% of 109 patients with bile duct cancer seen at Lahey Clinic had a history of previous cholecystectomy, while 25% had other types of previous biliary tract surgery.

Patients with ulcerative colitis and cholangiocarcinoma have cancers diagnosed at a mean age of 45–55 years, approximately two decades earlier than in patients without ulcerative colitis. The medical and surgical treatment of ulcerative colitis does not appear to influence the subsequent development of biliary tract cancer.

3 Pathogenesis

Most cases of cholangiocarcinoma occur sporadically and the exact etiology remains obscure. However, certain pathological conditions that result in either acute or chronic biliary epithelial injury appear to predispose to its development. Nowadays, it is evident that pericholangitis, PSC, and cholangiocarcinoma may represent a spectrum of the same disease process. A common and important contributor to the malignant transformation of cholangiocytes is chronic inflammation, often coupled with injury of bile duct epithelium and obstruction of bile flow, which all increase cholangiocyte turnover [28,37]. Persistent inflammation is thought to promote carcinogenesis by causing damage in DNA mismatch repair genes/proteins, proto-oncogenes, and tumor suppressor genes [38]. In addition, inflammation creates a local environment enriched with cytokines and other growth factors capable of accelerating the cell cycle and that favor accumulation of somatic mutations [39,40].

Chronic inflammation in general and in PSC leads to activation of the NF- κ B family. In resting cells, NF- κ B dimers are sequestered in the cytoplasm. When activated during inflammation, these transcription factors move to the nucleus for regulation of inflammation, immunity, and other cell functions. This leads to enhanced tumor necrosis factor- α (TNF α) via the I κ B kinase-dependent pathway, and eventually interleukin-6 (IL-6) increase. Several growth factors and other cytokines produced bring changes in the microenvironment, which play a role in tumor promotion. Toxic agents and cytokines released in the biliary microenvironment during the process of inflammation result in DNA damage of the biliary epithelial cells, which in turn leads to malignant transformation.

Cytokines, such as IL-6, appear to play an important role in evasion of apoptosis, a mechanism that normally eliminates dysfunctional cells with accumulating DNA damage. Cholangiocarcinoma cells have been shown to secrete IL-6 that activates the pro-survival p38 mitogen activated protein kinase in an autocrine fashion [41,42]. In addition, IL-6 up-regulates the expression of myeloid cell leukemia-1 (Mcl-1) through signal transducer and activator of transcription 3 (STAT3) and serine/threonine protein kinase (AKT) related signaling pathways [43,44]. Mcl-1 is an anti-apoptotic protein belonging to the Bcl-2 family of apoptotic proteins.

The development of cholangiocarcinoma is a multistep process. Several mutations in oncogenes and tumor-suppressor genes have been identified in cholangiocellular cancer. Mutations identified include the k-Ras, c-Myc, c-Neu, c-ErbB2, c-Met, p53, and Bcl-2 genes. In a recent report, 94% of resected specimens from patients with cholangiocarcinoma stained positive for the tumor suppressor p53 gene, and 100% stained positive for proliferating cell nuclear antigen (PCNA) [45]. ErbB-2, which is overexpressed in cholangiocarcinoma, is involved

in cholangiocarcinoma carcinogenesis and progression [43,44]. ErbB-2 is an epidermal growth factor receptor (EGFR) homologue able to homodimerize or heterodimerize with other members of the EGF superfamily, resulting in activation of the Raf/mitogen activated protein kinase (Raf/MAPK) pathway [46,47]. Constitutive overexpression of v-ErbB2 erythroblastic leukemia viral oncogene homolog 2 ErbB2 and/or ErbB1 in malignant cholangiocytes has been documented in more than 50% of cholangiocarcinoma [48,49].

4 Classification and histological types

Hilar cholangiocarcinoma may be categorized using the Bismuth classification into 4 types: type I when the tumor is below the convergence and there is still a free communication between left and right ducts; type II when the tumor interrupts left and right duct communication but without involvement of the main bile ducts; type IIIa when the tumor interrupts the convergence and has spread to the secondary branches of the right duct; type IIIb when the tumor interrupts the convergence and has spread to the to the secondary branches of the left duct; and type IV when the tumor interrupts the convergence and has spread to both main bile ducts up to secondary ducts (Fig. 1). This classification is based on the anatomic location of the tumor and their extension into the hepatic ductal system. Although this system provides an anatomic classification that can guide therapy (either resectional or palliative), it does little to describe patients who are surgical candidates or to provide prognostic information about each subset.

Based on the macroscopic appearance, three distinct subtypes have been described: sclerosing (70%), nodular (20%), and papillary (< 5%) [50]. The TNM staging of hilar cholangiocarcinoma is according to the American Joint Commission on Cancer [51] (Table 1).

Cancer of the extrahepatic bile duct is divided into distal and proximal types to facilitate staging. Distal cholangiocarcinoma may have similar clinical features and management as pancreatic cancer, while proximal cholangiocarcinoma represents a more complicated problem from the

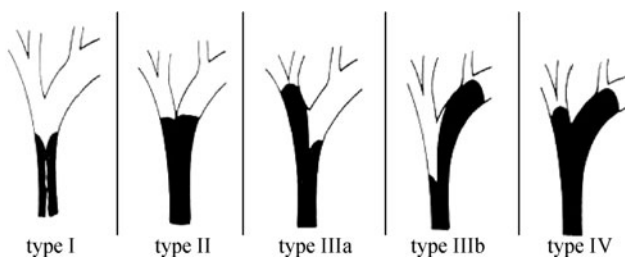


Fig. 1 Modified Bismuth-Corlette classification of hilar cholangiocarcinoma

Table 1 TNM classification from the American Joint Commission on Cancer (AJCC) for all types of cholangiocarcinoma

stage	T	N	M
stage 0	T _{is}	N ₀	M ₀
stage I	T ₁	N ₀	M ₀
stage II	T ₂	N ₀	M ₀
stage III	T ₁₋₂	N ₁₋₂	M ₀
stage IVA	T ₃	any N	M ₀
stage IVB	any T	any N	M ₁

T: T_{is}: carcinoma *in situ*. T₁: Tumor invasion into fibromuscular layer; T₂: Tumor invasion into perifibromuscular connective tissue; T₃: Tumor invasion into adjacent structures, such as liver, pancreases, duodenum, gallbladder, colon, and stomach.

N: N₀: No metastasis to lymph nodes; N₁: Metastasis to cystic duct, pericholedochal, and/or hilar lymph nodes; N₂: Metastasis to periduodenal, periportal, celiac, and/or superior mesenteric, peripancreatic, or posterior pancreaticoduodenal lymph nodes.

M: M₀: No distant metastasis; M₁: Distant metastasis, peritoneal dissemination, hepatic metastasis, or involvement of perigastric lymph nodes.

standpoint of endoscopic and surgical management. The definition of a T₃ proximal bile duct cancer, the so-called Klatskin tumor, is problematic in several aspects: a T₃ cancer invading the gallbladder along the cystic duct should be actually defined as T₂ cancer, while transmural tumor invasion of a Klatskin tumor into the gallbladder—direct tumor invasion through the bile duct wall of the gallbladder—should be defined as T₃ cancer.

The American Joint Commission on Cancer (AJCC) staging system incorporates the extent of tumor invasion into the wall of the bile duct, nodal metastasis, and distant metastasis. It is used for all cholangiocarcinoma, and therefore may not accurately differentiate between different patterns of tumor growth, e.g. hilar cholangiocarcinoma *versus* distal cholangiocarcinoma. This system is helpful in identifying prognostic subsets, but is applicable only to the minority of patients who undergo surgical resection because T staging requires transmural histopathologic evaluation of the tumor.

Bismuth-Corlette classification for hilar cholangiocarcinoma is useful for describing tumor location and its spread within the biliary tree, but it is not predictive of resectability. The Memorial Sloan-Kettering Cancer Center (MSKCC) has proposed the T-stage criterium, a modification to the AJCC system that takes into account of biological factors related to hilar cholangiocarcinoma [52] (Table 2). The MSKCC staging system is based on the location and extent of ductal involvement, the presence or absence of portal vein invasion, and the presence or absence of hepatic lobar atrophy irrespective of metastases or lymph node status. Long-term survival in patients with hilar cholangiocarcinoma depends critically on complete tumor resection. Achieving complete resection requires examination of all factors related to local tumor extent, which has increasingly become possible with non-invasive imaging studies. The MSKCC staging system for hilar

Table 2 T-stage criteria for hilar cholangiocarcinoma

	biliary involvement	PoV involment	lobar atrophy
T ₁	hilus±unilateral sectional bile ducts	no	no
T ₂	hilus±unilateral sectional bile ducts	+ ipsilateral	±ipsilateral
T ₃	hilus + bilateral sectional bile ducts	yes/no	yes/no
	hilus + unilateral sectional bile ducts	+ contralateral	yes/no
	hilus + unilateral sectional bile ducts	yes/no	+ contralateral
	hilus±unilateral sectional bile ducts	bilateral	yes/no

Sectional bile ducts: right anterior, right posterior, left medial, left lateral; PoV: portal vein.

cholangiocarcinoma correlates with resectability and survival, as 59% of T₁ lesions are resectable with a median survival of 20 months compared with 0% resectability for T₃ lesions with a median survival of only 8 months. However, this system is limited by its absolute dependence on accurate preoperative identification of the extent of the tumor and on correct interpretation of preoperative radiologic imaging, which may not be available to all physicians involved in management of hilar cholangiocarcinoma.

Microscopically, adenocarcinoma is the most common histologic subtype of hilar cholangiocarcinoma. More than 95% of tumors are adenocarcinomas ranging from well to poorly differentiated varieties. Over 90% of hilar cholangiocarcinoma are mucin-producing adenocarcinomas, and intracellular mucin can often be demonstrated. Immunohistochemical staining for epithelial membrane antigen and tissue polypeptide antigen may be useful in confirming the diagnosis of cholangiocarcinoma. Other histologic types, such as squamous cell carcinoma, small cell (oat cell) carcinoma, undifferentiated carcinoma, papillomatosis, papillary carcinoma, leiomyosarcoma, embryonal rhabdomyosarcoma, and cystadenocarcinoma, are rare.

Mixed tumors composed of hepatocellular carcinoma and cholangiocellular carcinoma are characterized by an intimate intermingling of elements from both types of tumor. Mixed type was found to comprise 1% of primary liver cancer in Japan. Combined hepatocellular and cholangiocellular carcinoma can be the result of incidental coexistence of elements, maturation of a common malignant stem cell into 2 mature forms, or transformation of hepatocellular into cholangiocellular or of cholangiocellular into hepatocellular carcinoma.

5 Tumor biology

The specific growth patterns of hilar cholangiocarcinoma include (1) transmural invasion of the bile ducts and extension into periductal tissues and adjacent structures, and (2) longitudinal extension along the bile ducts in the submucosa [28]. There are some morphologic differences between different subtypes.

The sclerosing variety is the most common type at the hilum. Histologically, this appears as annular thickening of the duct wall with both longitudinal and radial tumor infiltrations. These tumors can be locally invasive and tend to invade periductal neural tissues as well as major vascular structures of the hilum, resulting in marked fibrosis and infiltration of inflammatory cells in the hilar region. The nodular variety is characterized by irregular intraluminal nodules along the bile ducts. These tumors occur most commonly in the upper and mid bile duct and generally presents as a fibrotic mass with intraductal projections. Features of both sclerosing and nodular types may coexist; when features of both are present, the tumor is described as nodular-sclerosing [53]. The papillary variant is a soft and often friable tumor most common in the mid to distal bile duct. It has a predominantly intraluminal growth pattern with late transmural extension. These tumors grow primarily as an intraluminal soft, polypoid tumor with a limited propensity for transmural growth. These are less likely to cause periductal fibrosis or invade adjacent structures, thus leading to a more favorable prognosis [54].

Extensive subepithelial tumor spread beyond the gross tumor margin is common; in fact, this is an important feature of cholangiocarcinoma. This subepithelial spread emphasizes the importance of wider resections and confirmation of negative margins by frozen section during the resection. Longitudinal spread may extend up to 15–20 mm proximally and 5–10 mm distally, depending on tumor type [55]. An extensive pathologic analysis showed that the mean distance of microscopic invasion beyond the gross margin was 16.8 mm toward the liver and 6.5 mm toward the duodenum, thus resulting in difficulty in obtaining a complete resection.

The tendency to metastasis of hilar cholangiocarcinoma is limited. Hilar cholangiocarcinoma is often slow growing, although rapid progression has been seen in some patients. Spread by direct invasion to periductal hilar tissues and adjacent liver tissue are common. In addition to extension along the bile ducts, cholangiocarcinoma also frequently metastasize via the lymphatics; regional lymph node involvement is present in 30%–50% of cases. In general, hematogenous spread of hilar cholangiocarcinoma is rare, whereas nodal metastases may be present in up to 1/3 of cases.

6 Animal model and cholangiocarcinoma

Increasing evidence suggests that human cholangiocarcinoma develops through a multi-step process, and that invasive cholangiocarcinoma is preceded by dysplasia in the biliary epithelium. The molecular and genetic alterations involved in cholangiocarcinoma tumorigenesis have not been well investigated. The development of a reproducible animal model for cholangiocarcinoma, particularly one that recapitulates the dysplasia-carcinoma sequence of human cholangiocarcinoma, would not only enhance our understanding of genetic changes underlying cholangiocellular neoplasia, but also facilitate the development of pre-clinical chemoprevention and therapeutic trials.

Several environmental carcinogenesis models of cholangiocarcinoma have been established in animals. For example, Syrian hamsters treated with *Clonorchis sinensis* or *Opisthorchis viverrini* followed by dimethylnitrosamine (DMN) resulted in the development of cholangiocarcinoma [56–58]. Similarly, Syrian hamsters treated with DMN followed by bile duct ligation also developed cholangiocarcinoma. In these models, cholangiocarcinoma develops about 24 weeks after exposure, with a yield rate of only 10%. One of the better characterized rat models of cholangiocarcinoma has been the Furan model described by Sirica *et al.*, which led to the development of intestinal-type cholangiocarcinoma in the caudate lobe of the liver. Several studies had demonstrated the relationship between thioacetamide (TAA) administration and cholangiocarcinoma. Chun [59] had demonstrated that oral administration of TAA in drinking water to male SD rats results in a multi-step model of biliary dysplasia and invasive cholangiocarcinoma that closely mimics human disease. Similar to preneoplastic lesions described in human cholangiocarcinoma, rat cholangiolar epithelium displays a phase of progressive biliary dysplasia preceding invasive cancer. In addition, both precancerous and neoplastic biliary epithelia demonstrate foci of intestinal metaplasia (goblet cells), another well-known feature of the human counterpart.

7 Summary

Cholangiocarcinoma is a rare cancer originating from the neoplastic transformation of epithelial cells (i.e. cholangiocytes) that line the biliary tract, accounting for approximately 2% of all diagnosed cancers. Upper third or perihilar (Klatskin) tumor accounts for 30%–50% of cases. It has been suggested that chronic inflammation and biliary duct cell injury are two of the main conditions responsible for the development of cholangiocarcinoma. The most common disease associated with cholangiocarcinoma is PSC. Cholangiocarcinomas are adenocarcinomas that arise from the neoplastic transformation of

cholangiocytes. During the development of tumor, several oncogenes and tumor-suppressor genes are involved, including k-Ras, c-Myc, c-Neu, c-ErbB2, c-Met, p53, and Bcl-2. Hilar cholangiocarcinoma may be classified into 3 subtypes: sclerosing (70%), nodular (20%), and papillary (<5%) according to macroscopic appearance. Hilar cholangiocarcinomas are slow growing, metastasize late during progression, and present with symptoms of cholestasis due to blockage of the bile duct by tumor growth. In general, cholangiocarcinoma frequently metastasize via the lymphatics, while hematogenous spread is rare.

References

1. Blehacz B, Gores G J. Cholangiocarcinoma: advances in pathogenesis, diagnosis, and treatment. *Hepatology*, 2008, 48(1): 308–321
2. Welzel T M, McGlynn K A, Hsing A W, O'Brien T R, Pfeiffer R M. Impact of classification of hilar cholangiocarcinomas (Klatskin tumors) on the incidence of intra- and extrahepatic cholangiocarcinoma in the United States. *J Natl Cancer Inst*, 2006, 98(12): 873–875
3. Ustundag Y, Bayraktar Y. Cholangiocarcinoma: a compact review of the literature. *World J Gastroenterol*, 2008, 14(42): 6458–6466
4. Mosconi S, Beretta G D, Labianca R, Zampino M G, Gatta G, Heinemann V. Cholangiocarcinoma. *Crit Rev Oncol Hematol*, 2009, 69(3): 259–270
5. Khan S A, Thomas H C, Davidson B R, Taylor-Robinson S D. Cholangiocarcinoma. *Lancet*, 2005, 366(9493): 1303–1314
6. Yachimski P, Pratt D S. Cholangiocarcinoma: natural history, treatment, and strategies for surveillance in high-risk patients. *J Clin Gastroenterol*, 2008, 42(2): 178–190
7. Klatskin G. Adenocarcinoma of the hepatic duct at its bifurcation within the porta hepatis. *Am J Med*, 1965, 38: 241–256
8. Khan S A, Miras A, Pelling M, Taylor-Robinson S D. Cholangiocarcinoma and its management. *Gut*, 2007, 56(12): 1755–1756
9. Yang J, Yan L N. Current status of intrahepatic cholangiocarcinoma. *World J Gastroenterol*, 2008, 14(41): 6289–6297
10. Hammill C W, Wong L L. Intrahepatic cholangiocarcinoma: a malignancy of increasing importance. *J Am Coll Surg*, 2008, 207(4): 594–603
11. Aljiffry M, Walsh M J, Molinari M. Advances in diagnosis, treatment and palliation of cholangiocarcinoma: 1990–2009. *World J Gastroenterol*, 2009, 15(34): 4240–4262
12. McLean L, Patel T. Racial and ethnic variations in the epidemiology of intrahepatic cholangiocarcinoma in the United States. *Liver Int*, 2006, 26(9): 1047–1053
13. Abbas G, Lindor K D. Cholangiocarcinoma in primary sclerosing cholangitis. *J Gastrointest Cancer*, 2009, 40(1–2): 19–25
14. Burak K, Angulo P, Pasha T M, Egan K, Petz J, Lindor K D. Incidence and risk factors for cholangiocarcinoma in primary sclerosing cholangitis. *Am J Gastroenterol*, 2004, 99(3): 523–526
15. Boberg K M, Bergquist A, Mitchell S, Pares A, Rosina F, Broomé

- U, Chapman R, Fausa O, Egeland T, Rocca G, Schrumpf E. Cholangiocarcinoma in primary sclerosing cholangitis: risk factors and clinical presentation. *Scand J Gastroenterol*, 2002, 37(10): 1205–1211
16. Lazaridis K N, Gores G J. Primary sclerosing cholangitis and cholangiocarcinoma. *Semin Liver Dis*, 2006, 26(1): 42–51
 17. Charatcharoenwithaya P, Enders F B, Halling K C, Lindor K D. Utility of serum tumor markers, imaging, and biliary cytology for detecting cholangiocarcinoma in primary sclerosing cholangitis. *Hepatology*, 2008, 48(4): 1106–1117
 18. de Vries J S, de Vries S, Aronson D C, Bosman D K, Rauws E A, Bosma A, Heij H A, Gouma D J, van Gulik T M. Choledochal cysts: age of presentation, symptoms, and late complications related to Todani's classification. *J Pediatr Surg*, 2002, 37(11): 1568–1573
 19. Ishiguro S, Inoue M, Kurahashi N, Iwasaki M, Sasazuki S, Tsugane S. Risk factors of biliary tract cancer in a large-scale population-based cohort study in Japan (JPHC study); with special focus on cholelithiasis, body mass index, and their effect modification. *Cancer Causes Control*, 2008, 19(1): 33–41
 20. Miyazaki M, Takada T, Miyakawa S, Tsukada K, Nagino M, Kondo S, Furuse J, Saito H, Tsuyuguchi T, Chijiwa K, Kimura F, Yoshitomi H, Nozawa S, Yoshida M, Wada K, Amano H, Miura F. Risk factors for biliary tract and ampullary carcinomas and prophylactic surgery for these factors. *J Hepatobiliary Pancreat Surg*, 2008, 15(1): 15–24
 21. Strömberg C, Luo J, Enochsson L, Arnemo U, Nilsson M. Endoscopic sphincterotomy and risk of malignancy in the bile ducts, liver, and pancreas. *Clin Gastroenterol Hepatol*, 2008, 6(9): 1049–1053
 22. Mortensen F V, Jepsen P, Tarone R E, Funch-Jensen P, Jensen L S, Sørensen H T. Endoscopic sphincterotomy and long-term risk of cholangiocarcinoma: a population-based follow-up study. *J Natl Cancer Inst*, 2008, 100(10): 745–750
 23. Kim H G, Han J, Kim M H, Cho K H, Shin I H, Kim G H, Kim J S, Kim J B, Kim T N, Kim T H, Kim T H, Kim J W, Ryu J K, Moon Y S, Moon J H, Park S J, Park C G, Bang S J, Yang C H, Yoo K S, Yoo B M, Lee K T, Lee D K, Lee B S, Lee S S, Lee S O, Lee W J, Cho C M, Joo Y E, Cheon G J, Choi Y W, Chung J B, Yoon Y B. Prevalence of clonorchiasis in patients with gastrointestinal disease: a Korean nationwide multicenter survey. *World J Gastroenterol*, 2009, 15(1): 86–94
 24. Poomphakwae K, Promthet S, Kamsa-Ard S, Vatanasapt P, Chaveepojnkamjorn W, Klaewkla J, Sujirarat D, Pichainarong N. Risk factors for cholangiocarcinoma in Khon Kaen, Thailand: a nested case-control study. *Asian Pac J Cancer Prev*, 2009, 10(2): 251–258
 25. Andoh H, Yasui O, Kurokawa T, Sato T. Cholangiocarcinoma coincident with schistosomiasis japonica. *J Gastroenterol*, 2004, 39(1): 64–68
 26. Sahani D, Prasad S R, Tannabe K K, Hahn P F, Mueller P R, Saini S. Thorotrast-induced cholangiocarcinoma: case report. *Abdom Imaging*, 2003, 28(1): 72–74
 27. Lipshutz G S, Brennan T V, Warren R S. Thorotrast-induced liver neoplasia: a collective review. *J Am Coll Surg*, 2002, 195(5): 713–718
 28. Lazaridis K N, Gores G J. Cholangiocarcinoma. *Gastroenterology*, 2005, 128(6): 1655–1667
 29. Okuda K, Nakanuma Y, Miyazaki M. Cholangiocarcinoma: recent progress. Part 1: epidemiology and etiology. *J Gastroenterol Hepatol*, 2002, 17(10): 1049–1055
 30. Wu T T, Levy M, Correa A M, Rosen C B, Abraham S C. Biliary intraepithelial neoplasia in patients without chronic biliary disease: analysis of liver explants with alcoholic cirrhosis, hepatitis C infection, and noncirrhotic liver diseases. *Cancer*, 2009, 115(19): 4564–4575
 31. Ben-Menachem T. Risk factors for cholangiocarcinoma. *Eur J Gastroenterol Hepatol*, 2007, 19(8): 615–617
 32. Chuang S C, La Vecchia C, Boffetta P. Liver cancer: descriptive epidemiology and risk factors other than HBV and HCV infection. *Cancer Lett*, 2009, 286(1): 9–14
 33. Lee C H, Chang C J, Lin Y J, Yeh C N, Chen M F, Hsieh S Y. Viral hepatitis-associated intrahepatic cholangiocarcinoma shares common disease processes with hepatocellular carcinoma. *Br J Cancer*, 2009, 100(11): 1765–1770
 34. Kobayashi M, Ikeda K, Saitoh S, Suzuki F, Tsubota A, Suzuki Y, Arase Y, Murashima N, Chayama K, Kumada H. Incidence of primary cholangiocellular carcinoma of the liver in Japanese patients with hepatitis C virus-related cirrhosis. *Cancer*, 2000, 88(11): 2471–2477
 35. Zhou Y M, Yin Z F, Yang J M, Li B, Shao W Y, Xu F, Wang Y L, Li D Q. Risk factors for intrahepatic cholangiocarcinoma: a case-control study in China. *World J Gastroenterol*, 2008, 14(4): 632–635
 36. El-Serag H B, Engels E A, Landgren O, Chiao E, Henderson L, Amarantunge H C, Giordano T P. Risk of hepatobiliary and pancreatic cancers after hepatitis C virus infection: A population-based study of U.S. veterans. *Hepatology*, 2009, 49(1): 116–123
 37. Jaiswal M, LaRusso N F, Burgart L J, Gores G J. Inflammatory cytokines induce DNA damage and inhibit DNA repair in cholangiocarcinoma cells by a nitric oxide-dependent mechanism. *Cancer Res*, 2000, 60(1): 184–190
 38. Jaiswal M, LaRusso N F, Gores G J. Nitric oxide in gastrointestinal epithelial cell carcinogenesis: linking inflammation to oncogenesis. *Am J Physiol Gastrointest Liver Physiol*, 2001, 281(3): G626–G634
 39. Schottenfeld D, Beebe-Dimmer J. Chronic inflammation: a common and important factor in the pathogenesis of neoplasia. *CA Cancer J Clin*, 2006, 56(2): 69–83
 40. Wise C, Pihanathanond M, Perry B F, Alpini G, McNeal M, Glaser S S. Mechanisms of biliary carcinogenesis and growth. *World J Gastroenterol*, 2008, 14(19): 2986–2989
 41. Prawan A, Buranrat B, Kukongviriyapan U, Sripan B, Kukongviriyapan V. Inflammatory cytokines suppress NAD(P)H:quinone oxidoreductase-1 and induce oxidative stress in cholangiocarcinoma cells. *J Cancer Res Clin Oncol*, 2009, 135(4): 515–522
 42. Mon N N, Kokuryo T, Hamaguchi M. Inflammation and tumor progression: a lesson from TNF-alpha-dependent FAK signaling in cholangiocarcinoma. *Methods Mol Biol*, 2009, 512: 279–293
 43. Han C, Wu T. Cyclooxygenase-2-derived prostaglandin E2 promotes human cholangiocarcinoma cell growth and invasion through EP1 receptor-mediated activation of the epidermal growth factor receptor and Akt. *J Biol Chem*, 2005, 280(25): 24053–24063
 44. Sirica A E. Role of ErbB family receptor tyrosine kinases in intrahepatic cholangiocarcinoma. *World J Gastroenterol*, 2008, 14(46): 7033–7058

45. Fava G, Marzioni M, Benedetti A, Glaser S, DeMorrow S, Francis H, Alpini G. Molecular pathology of biliary tract cancers. *Cancer Lett*, 2007, 250(2): 155–167
46. Lai G H, Zhang Z, Shen X N, Ward D J, Dewitt J L, Holt S E, Rozich R A, Hixson D C, Sirica A E. erbB-2/neu transformed rat cholangiocytes recapitulate key cellular and molecular features of human bile duct cancer. *Gastroenterology*, 2005, 129(6): 2047–2057
47. Aishima S I, Taguchi K I, Sugimachi K, Shimada M, Sugimachi K, Tsuneyoshi M. c-erbB-2 and c-Met expression relates to cholangiocarcinogenesis and progression of intrahepatic cholangiocarcinoma. *Histopathology*, 2002, 40(3): 269–278
48. Yoon J H, Gwak G Y, Lee H S, Bronk S F, Werneburg N W, Gores G J. Enhanced epidermal growth factor receptor activation in human cholangiocarcinoma cells. *J Hepatol*, 2004, 41(5): 808–814
49. Choi H J, Kim H J, Choi J H. Expression of c-erbB-2 and cyclooxygenase-2 in intrahepatic cholangiocarcinoma. *Hepatogastroenterology*, 2009, 56(91-92): 606–609
50. Batheja N, Suriawinata A, Saxena R, Ionescu G, Schwartz M, Thung S N. Expression of p53 and PCNA in cholangiocarcinoma and primary sclerosing cholangitis. *Mod Pathol*, 2000, 13(12): 1265–1268
51. Greene F L, Page D L, Fleming I D. AJCC(American Joint Committee on Cancer), cancer staging manual. 6th ed. New York: Springer-Verlag, 2002
52. Nathan H, Aloia T A, Vauthey J N, Abdalla E K, Zhu A X, Schulick R D, Choti M A, Pawlik T M. A proposed staging system for intrahepatic cholangiocarcinoma. *Ann Surg Oncol*, 2009, 16(1): 14–22
53. Ito F, Agni R, Rettammel R J, Been M J, Cho C S, Mahvi D M, Rikkers L F, Weber S M. Resection of hilar cholangiocarcinoma: concomitant liver resection decreases hepatic recurrence. *Ann Surg*, 2008, 248(2): 273–279
54. Anderson C D, Rice M H, Pinson C W, Chapman W C, Chari R S, Delbeke D. Fluorodeoxyglucose PET imaging in the evaluation of gallbladder carcinoma and cholangiocarcinoma. *J Gastrointest Surg*, 2004, 8(1): 90–97
55. Jarnagin W R, Bowne W, Klimstra D S, Ben-Porat L, Roggin K, Cymes K, Fong Y, DeMatteo R P, D'Angelica M, Koea J, Blumgart L H. Papillary phenotype confers improved survival after resection of hilar cholangiocarcinoma. *Ann Surg*, 2005, 241(5): 703–712, discussion 712–714
56. Boonmars T, Wu Z, Boonjaruspinyo S, Pinlaor S, Nagano I, Takahashi Y, Kaewsamut B, Yongvanit P. Alterations of gene expression of RB pathway in *Opisthorchis viverrini* infection-induced cholangiocarcinoma. *Parasitol Res*, 2009, 105(5): 1273–1281
57. Songserm N, Prasongwattana J, Sithithaworn P, Sripa B, Pipitkool V. Cholangiocarcinoma in experimental hamsters with long-standing *Opisthorchis viverrini* infection. *Asian Pac J Cancer Prev*, 2009, 10(2): 299–302
58. Loilome W, Yongvanit P, Wongkham C, Tepsiri N, Sripa B, Sithithaworn P, Hanai S, Miwa M. Altered gene expression in *Opisthorchis viverrini*-associated cholangiocarcinoma in hamster model. *Mol Carcinog*, 2006, 45(5): 279–287
59. Yeh C N, Maitra A, Lee K F, Jan Y Y, Chen M F. Thioacetamide-induced intestinal-type cholangiocarcinoma in rat: an animal model recapitulating the multi-stage progression of human cholangiocarcinoma. *Carcinogenesis*, 2004, 25(4): 631–636