Review Article

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Mechanisms of Biliary Carcinogenesis and Preneoplastic Lesions

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Biliary cancer
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Prevention strategies

Abstract
Carcinoma of the intra- and extrahepatic biliary tract is a relatively rare cancer. In most cases, the etiology of these cancers is rather obscure. Depending on the location of the carcinoma there are some risk factors identified such as hepatolithiasis and biliary calculi, congenital cystic and dysplastic lesions of the biliary tract, inflammatory bowel disease, thorotrast application, activated biliary bile acids, reflux and stasis of pancreatic juice, infection with the liver flukes *Clonorchis sinensis* (CL) and *Opisthorchis viverrini* (OV) and long-standing cholangitis. Multiple studies suggest that carcinogenesis in biliary epithelia is a multiple and multistage process through hyperplasia, metaplasia, adenoma, dysplasia, adenocarcinoma in situ to invasive adenocarcinoma in most cases of biliary cancer. Biliary carcinogenesis is caused by different mechanisms such as DNA damage produced by nitric oxide and by reactive oxygen species in infected and inflamed tissue, specific mutation in proto-oncogenes and tumor suppressor genes, mechanically damaged tissue by biliary calculi or parasites, reflux and stasis of pancreatic enzymes with activation of biliary acids and some other unidentified factors. Furthermore, some epidemiological facts and prevention strategies in relation to biliary cancer will be addressed based on a review of the literature.

Introduction

Carcinoma of the biliary tract should be subdivided into (1) cholangiocarcinoma; (2) carcinoma of the gallbladder; (3) carcinoma of the extrahepatic bile duct – divided into (a) proximal to the junction of the cystic duct including the hilar region (Klatskin tumors); (b) the lower mid-region with the cystic duct, and (c) the lower end of the common bile duct –, and (4) carcinoma of the papilla of Vater because of different incidence, clinical importance, therapeutic modalities and prognosis (table 1). Prognosis is considered worst for lesions affecting the confluence of the bile ducts (Klatskin tumors), and best for lesions near the papilla of Vater. Different histomorphological studies suggest that the biliary epithelium undergoes hyperplastic, adenomatous and dysplastic transformation by different risk factors and multistage carcinogenesis, and the adenomatous and dysplastic epithelium in turn transforms into noninvasive adenocarcinoma and finally to invasive adenocarcinoma [1–3]. Thus, the hyperplasia-metaplasia-adenoma-dysplasia-noninvasive and invasive carcinoma sequence may be operative in biliary carcinogenesis as is generally accepted for cancer development in the gastrointestinal tract (stomach and colon) [4]. In the following, we will discuss the epidemiological aspects of biliary cancer, the supposed risk factors for biliary carcinogenesis, some proposed mechanisms of carcinogenesis, the histomorphological findings, and, finally, some prevention strategies in order to prevent biliary cancer development.

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Epidemiological Aspects of Biliary Cancer

Biliary cancer is a relatively rare cancer (table 2). The frequency of cholangiocarcinomas, which strictly speaking are only tumors of the intrahepatic bile ducts, is reported to vary between 5 and 30% of all liver cancers showing an incidence rate of 0.1–0.5% with a very high incidence in northeast Thailand and Hong Kong because of the endemic presence of liver flukes in these regions [5]. It is estimated that worldwide there are 46,000 cases of cholangiocarcinoma each year. However, at least 80% of this world total is unrelated to opisthorchis (OV) infection [5]. Because of effective treatment of opisthorchiasis with Praziquantel, a reduction in the incidence of cholangiocarcinoma in the high-risk regions in Asia seems possible. A carcinoma of the gallbladder is known to be found in approximately 1–2% of all resected gallbladders giving risk/year for the development of a gallbladder carcinoma of 0.01% of patients with cholecystolithiasis [6]. Gallbladder cancer is the commonest form of biliary malignancy and represents the fifth most common gastrointestinal cancer. Extrahepatic bile duct cancer shows an incidence rate of 0.01–0.5% with the worst prognosis in the proximal (hilar) tumors [5]. Carcinoma of the papilla of Vater is found in 0.2–0.3% of general autopsy studies [7] and represents about 1% of all epithelial malignancies and about 5% of all gastrointestinal tumors. A neoplasm in the papilla of Vater can be diagnosed early and has a good prognosis after surgical therapy [1], compared with the prognosis of carcinoma of the gallbladder, the proximal bile duct and cholangiocarcinoma which are equally poor because of diagnostic delay in most cases [8].

Table 1. Classification of biliary cancer [35]

1	Cholangiocarcinoma (intrahepatic)
2	Carcinoma of the gallbladder
3	Bile duct cancer
	3.1 proximal (Klatskin)
	3.2 lower-mid region with cystic duct
	3.3 lower end
4	Carcinoma of the papilla of Vater

Table 2. Percent incidence of different biliary cancers in autopsy studies

1	Cholangiocarcinoma	0.1–0.5
2	Carcinoma of the gallbladder	1–2
3	Bile duct cancer	0.01–0.5
4	Carcinoma of the papilla of Vater	0.2–0.3

Table 3. Risk factors for biliary carcinogenesis

Proved	Possible
Hepatolithiasis	Cholecystolithiasis
Congenital cystic lesions	Choledocholithiasis
APBD	Nitrosamines
Cholecystitis	
Cholangitis (PSC)	
Chronic ulcerative colitis	
Familial adenomatous polyposis	
Thorotrast	
Parasites (OV and CL)	

Risk Factors for Biliary Carcinogenesis

There have been relatively few epidemiological studies of biliary cancer. Most of the information on risk factors (table 3) which are likely to be important in biliary carcinogenesis is derived from clinical series.

Congenital Cystic Lesions, Caroli's Disease and Anomalous Arrangement of the Pancreaticobiliary Ducts (APBD)

Congenital choledochal duct dilatation and cyst formation with often elevated amylase levels in the choledochal cysts as in APBD leads one to assume that the longstanding inflammation of the biliary tract caused by the reflux of pancreatic juice and formation of secondary bile acids (lithocholic acid) due to a lack of sphincter function often with bacterial degradation, might be one of the factors in carcinogenesis in the biliary tract, especially in the gallbladder and in poorly drained choledochal cysts [9, 10]. Nowadays, it is accepted that there is an etiological link between the formation of congenital choledochal cysts and APBD except in patients with Caroli's disease and choledochocele. Several clinical series have shown that congenital choledochal cysts are associated with APBD in over 90% with a distal common bile duct stenosis at the pancreaticobiliary junction [9]. Caroli's disease is also associated with the development of cholangiocarcinoma more frequently than in patients without this condition

[11]. The prevalence for biliary cancer in patients with congenital bile duct cysts and/or APBD is reported between 16 and 28% [9–11].

Biliary Calculi

Carcinoma of the gallbladder is well known to be a complication of cholecystolithiasis which is found in up to 98% in gallbladders with carcinoma. However, it appears in only 1% of cases with cholecystolithiasis [6]. A recent investigation concluded that gallstones may play a causative role in the development of extrahepatic bile duct cancer showing a significant decrease in the incidence of bile duct cancers 10 or more years after cholecystectomy [12]. The presence of impacted intrahepatic biliary stones (hepatolithiasis) is observed in an impressive proportion of cases of intrahepatic cholangiocarcinoma [2]. In up to 17% hepatolithiasis is complicated by development of cholangiocarcinoma largely around the stone-containing intrahepatic bile ducts (mostly by calcium bilirubinate stones) [2]. The hypothetical link between gallstones and carcinoma relates to the chronic trauma and inflammation of the mucosa produced by the stones leading to dysplastic changes and carcinoma.

Cholecystitis and Cholangitis

Histologically, cholecystitis is usually present in association with carcinoma of the gallbladder and when chronic cholecystitis has led to gallbladder calcifications, the risk of malignancy is much increased, as found in the porcelain gallbladder in 25% [13]. Also typhoid carriers, another chronic inflammatory state of the gallbladder, have a sixfold increased risk to develop gallbladder cancer in comparison to the general population [13]. Long-standing cholangitis, especially primary sclerosing cholangitis (PSC), is associated with an increased risk of developing biliary cancer. There is pathological evidence that cholangiocarcinoma may arise in the setting of PSC. In autopsy studies cholangiocarcinoma is present in 33–42% of patients with PSC [14]. Because PSC often occurs in patients with chronic ulcerative colitis (CUC), similar mechanisms for carcinogenesis for colonic cancer with CUC and bile duct cancer with PSC are assumed [14]. Rosen et al. [14] found a link between PSC and CUC in 83% of their patients. Interestingly, identical *ras* gene point mutations were found in both groups which could explain the coincident carcinogenic mechanism in the colon as well as in the biliary tract. At the same time, cholestasis with obliterative inflammation and bacterial bile salt degradation with formation of secondary bile acids complete the multistage process of carcinogenesis in these patients.

Inflammatory Bowel Disease and Familial Adenomatous Polyposis

Carcinoma of the bile duct has been observed as a complication of chronic ulcerative colitis. Compared with the general United States population the relative risk of developing bile duct cancer in chronic ulcerative colitis patient was found to be 75 [15]. The lifetime risk for cholangiocarcinoma therefore is 1.4% in patients with CUC [15]. Total proctocolectomy does not protect against development of cholangiocarcinoma which usually appears 15–20 years after the onset of CUC. The risk of periampullary cancer is also 200-fold increased in familial adenomatous polyposis because of higher total biliary bile acid concentrations, specially of chenodeoxycholic acid and its metabolite lithocholic acid in these patients [16]. It is assumed that these bile acids abnormalities may be important in carcinogenesis of biliary cancer as well as in colorectal cancer [16].

Thorotrast

Thorotrast was widely used as a radiocontrast medium between 1930 and 1955. 10–12 years later, liver cancer, intra- and extrahepatic bile duct cancer and gallbladder tumors developed in a large number of patients exposed to Thorotrast. Based on autopsy findings the relative risk for developing cholangiocarcinoma in Thorotrast-exposed patients was found to be 303 [17].

Parasites

The association between the occurrence of cholangiocarcinoma and the presence of liver flukes (CL and OV) is very well established. Infection is acquired by eating raw fish which carry the infective stage. The flukes chronically infect the intrahepatic bile ducts. Opisthorchis viverrini (OV) is endemic in northeast Thailand (30% of population infected) where there is a high incidence of cholangiocarcinoma with a relative risk of 5 for persons with an antibody titer greater than 1:40 for OV [5]. This implies that at least two-thirds of cases of cholangiocarcinoma in Thailand are attributable to OV infection [5]. In contrast, infestation by *Fasciola hepatica* and *Schistosoma* does not appear to have similar carcinogenic effects on the biliary tract.

Nitrosamine Contaminated Food and Water

Multiple animal sudies have demonstrated that nitrosamine and its derivatives are potent carcinogens, inducing tumors in a range of organs (especially liver and bile tract) in many species, and are suspected to play also a role in human carcinogenesis [18].

Unidentified Risk Factors for Biliary Carcinogenesis

No significant association with biliary cancer was found with chronic carriage of hepatitis B, aflatoxin intake, tobacco and alcohol intake and use of oral contraceptives [19, 20].

Mechanisms of Carcinogenesis

It is generally agreed that chronic infections and inflammatory processes initiate or enhance carcinogenesis in humans (table 4). Some changes develop as an effect of obstruction of the biliary system. Therefore, inflammation of the biliary tract caused by the reflux of pancreatic juice into choledochal cystic lesions and APBD with stasis and activation of pancreatic enzymes, bile acids and some kinds of mutagenic substances can produce pathological epithelial changes which eventually lead to biliary carcinoma [9, 21] by repeated irritation, ulceration and inflammation. Also mechanically damaged tissue by biliary intra- or extrahepatic calculi (hepatolithiasis, cholecysto- and choledocholithiasis) often followed by cholangitis results in chronic irritation of the epithelium with increased cell proliferation and elevated spontaneous mutation at a later modulation stage of carcinogenesis [22]. There is currently great interest in the relationship between specific mutation spectra in protooncogenes (such as *ras*) and tumor suppressor genes (such as *p53*) and exposure to different etiological factors [23]. Recently, Tada et al. [23] observed point mutations in cholangiocarcinoma in the ras gene very similar to that of colonic cancer, suggesting an etiological link which could explain the high risk of both cancers in ulcerative colitis patients, as mentioned earlier. Because of the well known relationship between liver fluke infection (OV and CL) and increased risk of cholangiocarcinoma the parasitic infection provides a useful model to investigate the link between inflammation and carciongenesis [5, 24, 25]. It has been proposed that active oxygen species generated in inflamed tissues can cause injury to target cells and also damage DNA and adduct formation, increasing cell proliferation and fibrosis; all of which may facilitate activation of oncogenes or damage to tumor suppressor genes. There is now increasing evidence to suggest that nitric oxid (NO) and its derivatives produced by activated phagocytes may also play a role in the multiple-stage carcinogenesis process [25]. Endogenous NO is an essential signalling molecule mediating various cell functions, but it also induces cytotoxic and mutagenic effects when present in excess in combination with an immunosuppressive activity [26].

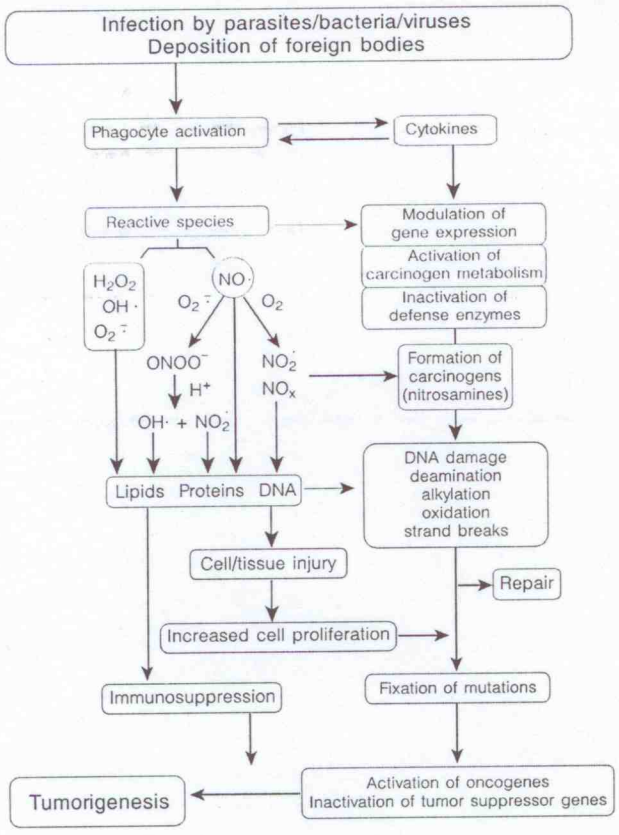
Table 4. Mechanisms of biliary carcinogenesis

1	Chronic infection
	Biliary obstruction with long-standing cholangitis
	Infected choledochal cysts
	Chronic cholecystitis
	Parasites (OV and CL)
2	Chronic Inflammation
	PSC
	Reflux of pancreatic juice
	Stasis of bile salts
3	Mechanically damaged epithelium
	Cholelithiasis
4	Activation and mutation of oncogens and mediators
	Proto-oncogenes (ras)
	Tumor suppressor genes (p53)
	Active oxygen species (NO)
	Cytokines (IL-1, tumor necrosis factor)

Infection with liver flukes increases the generation of endogenous NO, as estimated by salivary nitrite and urinary and plasma nitrate levels in infected persons [24]. Furthermore, the presence of parasites may stimulate release by macrophages and other cell types of cytokines such as IL-1 and tumor necrosis factor, which may be involved in tumor enhancement by stimulated angiogenesis [27]. A proposed hypothetical scheme for the role of NO in the multi-stage carcinogenesis process triggered by chronic infection or inflammation is shown in figure 1 [25]. However, the oncogenes and tumor suppressor genes involved in fluke-associated cholangiocarcinoma have still to be identified. In addition, human cancer development in general, as biliary carcinogenesis in special is clearly a complex phenomenon with susceptibility to cancer in many additional ways, including chronic cell proliferation by mechanical factors, bile and pancreatic juice stasis and endogenous generation of carcinogens by inflammatory and chronic infective processes.

Aspects of Morphogenesis in Biliary Carcinogenesis

As in the colon and stomach, it appears that carcinomas of the biliary tract arise from precancerous lesions (fig. 2). This hypothesis was supported by Kozuka et al. [28] who performed histological analyses of 22 invasive carcinomas of the papilla of Vater and found a rate of 82% of vestigial adenomas. The same authors showed in a series of 1,605 cholecystectomies that the transition of benign adenoma to carcinoma is histologically traceable



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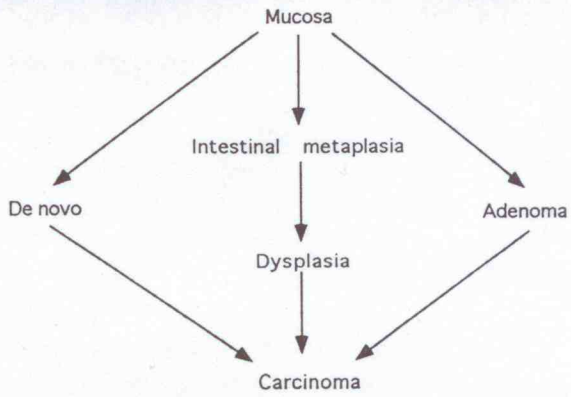

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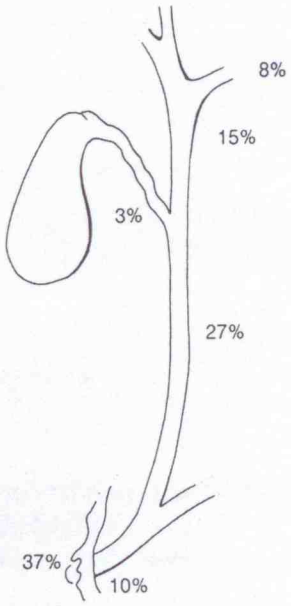

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Fig. 1. A proposed hypothetical scheme for the role of NO and other reactive oxygen/nitrogen species in the multiple-stage carcinogenesis process, triggered by chronic infection and inflammation. According to Ohshima and Bartsch [25].

Fig. 2. Morphogenesis in biliary carcinogenesis.

Fig. 3. Location and frequency of papillomas and adenomas reported from the hepatic duct confluence to the ampulla of Vater. According to Beazley and Blumgart [29].

[3]. Two-thirds of the benign neoplasms of the biliary tree fall into the category of polyp, adenomatous papilloma or adenoma. The anatomical distribution of papillomas or adenomatous lesions reported can be found in figure 3. The majority of them are found either in the ampulla of Vater or in close proximity to the Vaterian system (47%) while the common bile duct (27%) is the second most frequent site [29].

The retrospective analysis of our own case material confirms this hypothesis [30]. Seventy-two patients with tumors of the ampulla of Vater were examined histologically. In accordance with the WHO classification of intestinal tumors [31], the noncarcinomatous sections of the ampulla were examined for epithelial-dysplastic changes and the incidence of adenomatous structures. There was subdivision into micro-adenomas or mucosal adenomas and into macro-adenomas or adenomatous residues. Table 5 summarizes the results of the histological evaluation of the carcinoma of the ampulla.

We found in 82.8% of the examined surgical specimens moderate to severe epithelial dysplasias in the noncarcinomatous part of the ampulla. In 91.4% of the specimens adenoma structures were found. These results allow us to conclude that the morphogenesis of carcinoma of the ampulla of Vater occurs largely via premalignant precursors. Dysplasias and adenomas are developmental stages

in the neoplastic transformation of the epithelium. These findings are of interest to the clinician from the point of view of diagnosis and therapy of precursor lesions of the ampulla. Observation of mucosal changes in this location by endoscopy and biopsy is of great clinical value for the detection of precancerous lesions. Indirect support for this hypothesis was also derived from the findings of Yamagiwa and Tomiyama [32] who considered that the sequence of intestinal metaplasia-adenoma-dysplasia-carcinoma was significant in 1,000 cases of resected gallbladders. In addition, the recent study of Terada et al. [2] on neoplastic transformation in the intrahepatic biliary tree in hepatolithiasis confirm that carcinogenesis in bile duct epithelial cells progresses in a multistep manner, through hyperplasia, dysplasia, noninvasive adenocarcinoma to finally invasive adenocarcinoma. All cases showed mucosal hyperplasia in stone-bearing bile ducts. Interestingly, CEA was expressed on invasive adenocarcinoma cells and adenocarcinoma in situ cells in most cases and in dysplastic cells in about a half, while CEA was never present in hyperplastic epithelia [33]. These findings show that CEA is specific for dysplastic and neoplastic epithelia and that it appears in preneoplastic lesions and increases through malignant transformation of bile duct epithelia also suggesting multistage carcinogenesis. It appears that the use of CEA can help to identify early cancers in transformed bile duct epithelia. Immunocytochemical staining is positive for CEA but negative for AFP in about 50% of the instances. However, the determining factor(s) responsible for this multi-stage process remain(s) unidentified. Further study in this wide area of carcinogenesis is needed.

Table 5. Histomorphologic alterations of the non-tumour-infiltrated ampulla in the immediate vicinity of the carcinoma (n = 58)

Epithelial dysplasia		Micro-adenomas of the mucosa, %	Macro-adenomas and adenomatous residues, %
stage	%		
I	17.2		
II	44.9	58.6	32.8
III	37.9		
II + III	82.8	adenoma structures (total) 91.4	

Prevention and Therapeutic Strategies

Because of the lack of characteristic symptoms and physical findings, benign biliary tumors and preneoplastic lesions are usually not diagnosed preoperatively. A benign adenomatous tumor in the proximal or mid-part of the bile duct is generally difficult to detect. This may be the reason why in comparison with the gallbladder and the papilla of Vater it is difficult to prove any association between the presence of pre-existing adenomas or papillomas and bile duct cancer.

Prevention strategies for biliary cancer depend on location, detection of precancerous lesions and etiological risk factors.

Primary prevention of cholangiocarcinoma in the high-risk areas where the tumors are associated with liver fluke infestation is available by the use of the drug Praziquantel which, administered in a single dose, can successfully eliminate parasites from infested individuals [34]. It is possible that if endogenous nitrosation is truly important in the etiological process of biliary carcinogenesis, administration of vitamin C may be effective in high-risk patients interacting with the NO synthase. The lack of clinical studies on this means this fact is still hypothetical.

If suspected precancerous lesions are found in the gallbladder, cholecystectomy is mandatory. Asymptomatic cholecystolithiasis is not regarded as a precancerous stage because of the very low risk of finding gallbladder cancer in only 1% approximately. Adenomas of the gallbladder over 1 cm in diameter should be removed because of their malignant potential [3]. The risk of cancer in the porcelain gallbladder is also very high and justifies prophylactic cholecystectomy.

Treatment of bile duct cancer should include total resection of the lesion. In the field of congenital choledochal cystic lesions early resection of the dilated choledochal cyst wall with reconstruction of the biliary duct should be done to halt the reflux and stasis of pancreatic juice into the biliary duct. In cases with anomalous arrangement of the pancreaticobiliary ducts (APBD) radical surgery including a complete excision of the extrahepatic biliary tract followed by reconstruction may decrease the likelihood of developing carcinoma. In tumors of the hilar bile ducts resection, including hepatic resection, may be carried out in approximately 20% of the patients. Better results are obtained in patients submitted to resection than those treated by nonresective procedures.

Best diagnostic (ERCP) and treatment modalities are found for tumors and precancerous lesions of the papilla of Vater because relatively small epithelial changes can easily obstruct the orifice or duct lumen giving clinical signs. It is also known that papillary ampullary tumors

usually have no evidence of venous invasion but more proximal sclerotic and nodular tumors show involvement of veins and nerves affecting prognosis of the disease [29]. In cases of histologically proven adenomatous changes of the mucosa or severe epithelial dysplasia, prophylactic surgical excision of the papilla of Vater is indicated. If mild or moderate epithelial dysplasia is detected, periodic endoscopic directed biopsies (evey 6 months) are advisable to prevent diagnostic delay.

In conclusion, carcinogenesis of the biliary tract is a widespread and multistage field which is still open for further research expecting new starting points from new branches of knowledge like molecular biology and tumor biology. Furthermore, it is important to recognize preneoplastic lesions, which in the biliary tract are extremely difficult to detect, to improve treatment of benign biliary lesions and therefore preventing development of biliary cancer.

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