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Review

Mechanisms of biliary carcinogenesis: A pathogenetic multi-stage cascade towards cholangiocarcinoma

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Summary

Carcinomas of the biliary tract are rare cancers developing from the epithelial or blast-like cells lining the bile ducts. A variety of known predisposing factors and recent experimental models of biliary carcinogenesis (e.g., infection with the liver fluke *Opisthorchis viverrini*, models of chemically induced carcinogenesis and experimental models of pancreaticobiliary maljunction) have elucidated different stages of this complex system of biliary tumorigenesis. Chronic inflammatory processes, generation of active oxygen radicals, altered cellular detoxification mechanisms, activation of oncogenes, functional loss of tumor-suppressor genes and dysregulation of cell proliferation and cell apoptotic mechanisms have been identified as important contributors in the development of cholangiocarcinomas. In this review, the known mechanisms involved in the carcinogenesis of biliary epithelium are addressed. We will divide the topic into four stages: 1) Predisposition and risk factors of biliary cancer. 2) Genotoxic events and alterations leading to specific DNA damage and mutation patterns. 3) Dysregulation of DNA repair mechanisms and apoptosis, permitting survival of mutated cells and 4) Morphological evolution from premalignant biliary lesions to cholangiocarcinoma. Finally, established and hypothetical future therapeutic strategies directed towards specific pathogenetic events during biliary carcinogenesis will be addressed.

Key words: biliary carcinogenesis, risk factors, genotoxicity, apoptosis, cholangiocarcinoma, therapeutic strategies

Introduction

Carcinomas of the biliary tract arise from either bile duct epithelial (oval) cells termed cholangiocytes or non-epithelial blast-like cells distributed among the oval/bile ductule epithelial cells. Cholangiocarcinoma (CC) represents a relatively rare cancer and its prevalence in autopsy series is reported as 0.01%-0.5% [1]. Despite its rarity, a variety of predisposing factors have been identified in epidemiological and clinical series during the past decades. Consequently, various biliary cancer models were established, that investigate the initiating mechanisms involved in biliary carcinogenesis. Such experimental and clinical tumor models are the liver fluke infection with *Opisthorchis viverrini (OV)* [2-4], models of anomalous arrangement of the pancreaticobiliary ducts (APBD) and bilioenterostomy [5,6], clinical series of patients with congenital bile duct cysts (CBDC) and pancreaticobiliary maljunctions (PBM) [7-9] and tumor induction models that use chemical hepatobiliary carcinogens such as N-nitrosobis(2-oxopropyl)amine (BOP) [6,10] or diethylnitrosamine (DEN) [11]. With the introduction of translational research methods (e.g. polymerase chain reaction (PCR)), a number of oncogenes, tumor-suppressor genes and apoptotic proteins involved in biliary carcinogenesis have been identified [12-14]. Finally, histomorphological studies have provided some evidence, that at least in the gallbladder [15,16] and ampulla of Vater the sequence "intestinal metaplasia-dysplasia-carcinoma" is significant in biliary carcinogenesis [17]. However, despite the recent insights into the mechanisms involved in biliary carcinogenesis, the key events and specific links in this multi-stage cascade leading to transformation of cholangiocytes into malignant cells remain unknown and will need further investigations.

Stage I: Predisposition to biliary carcinogenesis

Table 1 summarizes the identified risk-factors of biliary carcinogenesis and for some of the predisposing conditions, the relative risk to develop CC compared to the general population has been estimated (Table 1). The common denominator for biliary metaplasia, dysplastic epithelial transformation, and epithelial proliferation seems to be chronic inflammation through chemical and/or mechanical irritation and cholestasis with activation of injurious pancreatico-biliary compounds and carcinogens.

Anatomic Anomalies:

Congenital bile duct cysts (CBDC), pancreaticobiliary maljunction (PBM) and Caroli's disease have been clearly identified to be precursors of biliary cancer. It has been suggested, that there is an etiological link between congenital bile duct cyst formation and PBM, since several clinical series report coincidence of these two conditions

Table 1. Predisposition to biliary carcinogenesis and relative risk (rR) to develop cholangiocarcinoma compared to the general population

	Predisposition	rR
1. Anatomic Anomalies:	- Congenital bile duct cysts	86x
	- Caroli's disease	NA
	- PBM	32x
2. Chronic inflammatory conditions:	- PSC	30x
	- typhoid carriers (gallbladder)	6x
	- porcelain gallbladder	25x
	- Parasites:	
	- Opisthorchis viverrini (OV)	5x
	- Clonorchis sinensis (CS)	5x
3. Biliary calculi	- cholecystolithiasis	NA
	- hepatolithiasis	NA
4. Carcinogenes:	- Nitrosamines (BOP, DEN)	NA
	- Thorotrast	303x
	- betel nut	NA
5. Autoimmune diseases:	- PSC	30x
	- PBC	30x
	- CUC	75x

Abbreviations: rR - relative risk; NA - not available; PBM - pancreaticobiliary maljunction; PSC - primary sclerosing cholangitits; BOP - N-nitrosobis(2-oxopropyl)amine; DEN - diethylnitrosamine; PBC - primary biliary cirrhosis; CUC - chronic ulcerative colitis

in over 90% of cases [8,18]. In CBDC with PBM chronic exposure of the biliary epithelium to a regurgitated mixture of bile and pancreatic juice is due to an impaired sphincter of Oddi function and induces chronic inflammation. In comparison to normal bile, an elevated amylase level, preactivation of trypsin, phospholipase A2 and elastase I, and deconjugated secondary bile acids (e.g. lithocholic acid, deoxycholic acid) are found in the bile of patients with CBDC and PBM [8,9,19,20]. Retention of these injurious and comutagenic substances in the dilated bile duct and/or gallbladder seems to initiate proliferative epithelial processes with possible activation of the carcinogenic cascade.

Chronic inflammatory conditions

Primary sclerosing cholangitis (PSC) is the most important risk-factor for CC in the Western countries. In autopsy studies CC is present in 33%-42% of patients with PSC [21]. In a series of 30 patients by Rosen and colleagues the diagnosis of PSC preceded diagnosis of CC by a mean of 52 months [21]. Since PSC mostly develops in patients with chronic ulcerative colitis (CUC), similar mechanisms for carcinogenesis of bile duct cancer in PSC and colon cancer in CUC have been assumed. In fact, identical K-*ras* gene point mutations were found in the biliary and colonic epithelial tumor cells of these patients.

Chronic cholangitis, the chronic infected gallbladder of typhoid carriers and the porcelain gallbladder (calcification of the gallbladder) represent endpoints of sustained chronic cholecystitis and all these conditions have an elevated risk of developing CC.

Parasites

The association between CC and the presence of liver flukes (*Opisthorchis viverrini* (OV) and *Clonorchis sinensis* (CS)) has been very well established [2,4]. Infection is acquired by eating raw or undercooked cypriniod fish contaminated with encysted metacercariae of the parasites. The flukes chronically infect the intrahepatic bile ducts, the gallbladder as well as the pancreatic duct. It has been suggested, that the parasite infestation by itself is not strongly carcinogenic but exerts a marked promoting influence on cholangiocellular tumor development via chronic irritation and increased cell turnover, rendering the cells susceptible to either exogenous or endogenous carcinogens [3]. Furthermore, Haswell-Elkins [2] and Oshima [4] have shown among others, that OV-infected subjects, as identified by the presence of OV eggs in the feces, had increased biosynthesis of nitrosamines and increased nitrosation potential of macrophages compared to uninfected subjects. Their data supports the hypothesis that infection with OV increases the generation of genotoxic nitric oxide (NO), as estimated by elevated salivary nitrite and urinary nitrate levels in infected subjects [2,4].

Biliary calculi

Gallstones are found in 70-98% of gallbladders resected for carcinoma [22]. The hypothetical link between gallstones and carcinoma of the gallbladder relates to the chronic mechanical irritation and inflammation of the biliary epithelium. A recent investigation by Ekblom and colleagues concluded, that gallstones may also play a causative role in the development of extrahepatic bile duct cancer, since a significant decrease in the incidence of cholangiocarcinoma 10 or more years after cholecystectomy for cholecystolithiasis was shown [23]. In analogy to cholecystolithiasis, hepatolithiasis, which is not uncommon in East Asia, has been observed in an impressive proportion (up to 17.5%) of registered cases of CC in these countries [24]. The liver containing intraductal hepatoliths (mostly calcium- bilirubinate) show features of chronic proliferative cholangitis in vicinity to stone-bearing ducts [25].

Carcinogens:

Nitrosamines are potent carcinogens experimentally inducing tumors in different organs and are suspected to play a role in human carcinogenesis. Exposure occurs through dietary intake of preformed nitrosamines, through tobacco use and through endogenous nitrosation of nitrogenous compounds via nitric oxide (NO) [4]. However, in vivo little is known about the carcinogenic concentration and the time of exposure necessary to induce biliary cancer.

Thorotrast, a radioactive α-particle emitter, was widely used as a radiocontrast medium between 1930 and 1955. 10-12 years after exposition, liver cancer and cholangiocarcinoma developed in a large number of Thorotrast treated patients. Proliferative and dysplastic changes of the bile duct epithelium were noted in the noncancerous areas of Thorotrast-induced cholangiocarcinomas which possibly represent precursor lesions of these cancers [26].

Autoimmune diseases:

Three autoimmune diseases, primary biliary cirrhosis (PBC), PSC and CUC, have been identified as predisposing conditions in the development of CC. As mentioned above, PSC and CUC are often linked, since 75%-83% of patients with PSC have evidence of CUC. The lifetime risk to develop CC in patients with CUC was estimated to be 1.4% [27]. Total proctocolectomy does not