Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.3329

World J Gastroenterol 2009 July 21; 15(27): 3329-3340
World Journal of Gastroenterology ISSN 1007-9327

EDITORIAL

Bile acids as endogenous etiologic agents in gastrointestinal cancer

Harris Bernstein, Carol Bernstein, Claire M Payne, Katerina Dvorak

Harris Bernstein, Claire M Payne, Department of Cell Biology and Anatomy, College of Medicine, and Arizona Cancer Center, University of Arizona, Tucson Arizona 85724, United States
Carol Bernstein, Department of Cell Biology and Anatomy, College of Medicine, University of Arizona, Tucson Arizona 85724, United States; Hematology/Oncology Southern Arizona Veterans Affairs Health Care System, Tucson Arizona 85723, United States
Katerina Dvorak, Department of Cell Biology and Anatomy, College of Medicine, and Arizona Cancer Center, University of Arizona, Tucson Arizona 85724, United States; Hematology/Oncology Southern Arizona Veterans Affairs Health Care System, Tucson Arizona 85723, United States
Author contributions: Bernstein H, Bernstein C, Payne CM and Dvorak K contributed equally to this work.
Supported by Grants from the NIH (R21CA111513-01A1, 5 RO1 CA119087, and SPORE Grant 1 P50CA95060); grants from the Arizona Biomedical Research Commission (#0012 & #0803), by Biomedical Diagnostics & Research In., Tucson Arizona, and by a VA Merit Review Grant
Correspondence to: Katerina Dvorak, PhD, Research Associate Professor, Department of Cell Biology and Anatomy, College of Medicine, and Arizona Cancer Center, University of Arizona, 1501 N. Campbell Avenue, PO Box 245044, Tucson Arizona 85724, United States. kdvorak@email.arizona.edu
Telephone: +1-520-6263934 Fax: +1-520-6262097
Received: May 4, 2009 Revised: June 16, 2009
Accepted: June 23, 2009
Published online: July 21, 2009

Abstract

Bile acids are implicated as etiologic agents in cancer of the gastrointestinal (GI) tract, including cancer of the esophagus, stomach, small intestine, liver, biliary tract, pancreas and colon/rectum. Deleterious effects of bile acid exposure, likely related to carcinogenesis, include: induction of reactive oxygen and reactive nitrogen species; induction of DNA damage; stimulation of mutation; induction of apoptosis in the short term, and selection for apoptosis resistance in the long term. These deleterious effects have, so far, been reported most consistently in relation to esophageal and colorectal cancer, but also to some extent in relation to cancer of other organs. In addition, evidence is reviewed for an association of increased bile acid exposure with cancer risk in human populations, in specific human genetic conditions, and in animal experiments. A model for the role of bile acids in GI carcinogenesis is presented from a Darwinian perspective that offers an explanation for how the observed effects of bile acids on cells contribute to cancer development.

Key words: Bile acids; Cancer; Adenocarcinoma; Esophagus; Stomach; Small intestine; Pancreas; Colon; Apoptosis; DNA damage

Peer reviewers: Li-Qing Yu, MD, PhD, Assistant Professor, Department of Pathology, Lipid Sciences, Director of Transgenic Mouse Core Facility Wake Forest University School of Medicine Medical Center Blvd Winston-Salem, NC 27157-1040, United States; Wen Xie, MD, PhD, Assistant Professor, Center for Pharmacogenetics, University of Pittsburgh School of Pharmacy, 656 Salk Hall, 3501Terrace Street, Pittsburgh, PA 15261, United States

Bernstein H, Bernstein C, Payne CM, Dvorak K. Bile acids as endogenous etiologic agents in gastrointestinal cancer. *World J Gastroenterol* 2009; 15(27): 3329-3340 Available from: URL: http://www.wjgnet.com/1007-9327/15/3329.asp DOI: http://dx.doi.org/10.3748/wjg.15.3329

INTRODUCTION

Although it was proposed that bile acids are carcinogens as early as 1939 and 1940, there was little evidence at that early time that bile acids act as carcinogens in the gastrointestinal (GI) tract (reviewed in[1]). Since then, however, evidence has accumulated that exposure of cells of the GI tract to repeated high physiologic levels of bile acids is an important risk factor for GI cancer. Here we review the substantial evidence, much of it obtained in the last few years, for a role of bile acids in cancers of the esophagus, stomach, small intestine, liver, biliary tract, pancreas and colon/rectum. High exposure to bile acids may occur in a number of settings, but, most importantly, is prevalent among individuals who have a high dietary fat intake[2]. A rapid effect on cells of high bile acid exposure is the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS). Increased production of ROS/RNS, can lead to increased DNA damage and then increased mutation. The production of ROS/RNS following bile acid exposure likely occurs through multiple pathways involving disruptions of the cell membrane and mitochondria[1]. For each organ of the GI tract, we

review evidence, where available, on deleterious effects of bile acids, including the induction of ROS/RNS, induction of DNA damage, mutation and apoptosis, and the development of reduced apoptosis capability upon chronic exposure. Reduced ability to undergo apoptosis is important because apoptosis is a beneficial process that rids the body of cells with unrepaired DNA damage that can cause mutation. Reduced apoptosis capability has been linked to increased mutagenesis[3-5]. We also review epidemiologic evidence and results of animal experiments indicating that long-term exposure to elevated levels of bile acids increases GI cancer risk.

The annual world-wide number of deaths due to cancer is about 7.6 million, and among these about 2.8 million (36%) are due to cancers of the GI tract[6]. A recent prospective study was carried out on red and processed meat in relation to cancer incidence in a cohort of approximately half a million men and women[7]. Individuals in the highest quintile of red meat intake, compared with those in the lowest, had a statistically significant elevated risk of esophageal, colorectal and liver cancer. Also, for processed meat, the risk of colorectal cancer was elevated. Both types of meat are sources of saturated fat and iron, which have independently been associated with carcinogenesis. In addition, processed meats contain nitrates and nitrites, precursors of N-nitroso mutagenic compounds.

ESOPHAGUS

The estimated yearly number of deaths world-wide from esophageal cancer is 300 034 for men and 142 228 for women[6], making it the sixth leading cause of cancer deaths among men and women combined. There are two principal histologic types of esophageal cancer, adenocarcinoma and squamous cell carcinoma. In the United States, the incidence of adenocarcinoma has increased four-fold between 1973 and 2002, whereas squamous cell carcinoma has declined 30% over the same period, making adenocarcinoma the predominant form of esophageal cancer[8]. Barrett's metaplasia of the esophagus is an important predisposing condition for the development of esophageal adenocarcinoma[9]. Barrett's esophagus (BE) is a metaplastic lesion of the distal esophagus, characterized by the replacement of the normal squamous epithelium by columnar intestinal epithelium containing goblet cells. BE is associated with increased duodeno-gastro-esophageal reflux[10,11], which causes increased exposure of the esophagus to bile acids from the duodenum and acidity (gastric acidity) from the stomach. Individuals with esophageal adenocarcinoma experience even greater exposure to bile than persons with uncomplicated BE[12]. Expression of bile acid transporter proteins is increased in BE tissues, suggesting that the development of BE metaplasia may be an adaptation to protect cells from bile acids[13]. Thus progression to BE and to adenocarcinoma may be strongly influenced by bile acid exposure. As discussed next, evidence indicates that short-term exposure of esophageal cells to bile acids induces oxidative stress, DNA damage, mutation and apoptosis; and among surviving cells selects over the long-run for resistance to apoptosis and ultimately cancer.

Five studies have shown that bile acids cause increased production of ROS in esophageal cells, including those from BE metaplasia. A cocktail of five bile acids designed to mimic the bile acids present in gastroesophageal reflux was used to test whether reflux induces ROS[14]. The five bile acids were glycocholic acid (GCA), taurocholic acid (TCA), glycodeoxycholic acid (GDCA), glycochenodeoxycholic acid (GCDCA) and deoxycholic acid (DCA). This cocktail induced ROS in biopsies from human BE metaplastic tissue. The bile acid cocktail also induced ROS in cultured SV40-transformed squamous esophageal epithelial cells (HET1-A). DCA induced ROS in cultured human esophageal adenocarcinoma cells (OE33) and squamous cell carcinoma cells (KYSE-30)[15]. GCDCA in acidic media induced ROS in cultured esophageal squamous cell lines derived from patients with gastroesophageal reflux disease (GERD) with BE, or without BE[16]. When mice were fed a zinc deficient diet containing a DCA supplement, ROS production was increased and BE-like lesions developed[17].

Six studies showed that bile acids induce DNA damage in esophageal cells (Table 1), and five of these reported evidence for oxidative DNA damage.

The findings that bile acids induce DNA damage suggest that bile acids may also increase the frequency of mutation, since replication of a damaged DNA template strand often results in a replication error and thus a mutation.

Esophagoduodenostomies were performed on Big Blue F1 *lac*I transgenic rats to surgically increase duodeno-gastro-esophageal reflux[21]. The frequency of *lac*I mutant cells proved to be significantly higher in the esophageal mucosa of the surgically altered rats than in the unaltered control rats, indicating that components of refluxate, such as bile acids, increase mutation. Forty-six percent of the mutant cells were altered at CpG dinucleotide sites, and the majority of these mutations (61%) were C to T or G to A transitions. This pattern of mutation is similar to that in human esophageal adenocarcinoma, suggesting that reflux is not only mutagenic, but also carcinogenic. Consistent with these findings, it was found that DCA treatment of cultured esophageal cells cause an increase in the frequency of GC to AT mutations in the *p53* gene[15]. In addition, increased duodeno-gastro-esophageal reflux was observed to increase mutagenesis using a surgical model in Big Blue mice (rather than rats)[22].

Bile acids induce apoptosis in esophageal cells, perhaps through the mediation of damaging ROS. DCA induced apoptosis in esophageal biopsies from normal human squamous epithelium[23]. Also, five different bile acids [GCDCA, GDCA, TCA, taurochenodeoxycholic acid (TCDCA) and taurodeoxycholic acid (TDCA)] individually, and also in a mixture, induced apoptosis of cultured human normal esophageal mucosal epithelial cells[24].

Although a short-term effect of high bile acid exposure is induction of apoptosis, a longer-term effect of repeated high exposure to apoptosis-inducing agents, such as bile acids, appears to be selection for apoptosis resistant cells. When tissue samples from patients with normal esophagus, esophagitis, BE lesions and

Table 1 Bile acids induce DNA damage in cells of the esophagus

Cells/tissues	Bile acids that induce DNA damage	Assay for damage	Ref.
Cultured SV40-transformed, squamous esophageal epithelial cells (HET1-A) and Barrett's associated adenocarcinoma cells (FLO-1)	DCA; also cocktail containing GCA, TCA, TCDCA	Comet assay[1] for strand breaks	[18]
Cultured SV40-transformed, squamous esophageal epithelial cells (HET1-A)	DCA	Comet assay for strand breaks; evidence for oxidative mechanism involving nitric oxide	[19]
Cultured human adenocarcinoma cells (OE33)	DCA	Micronuclei assay; induction of micronuclei by DCA, reduced by antioxidants	[15,20]
Biopsies from human Barrett's esophageal metaplastic tissue	Cocktail containing DCA, GCA, TCA, GDCA, GCDCA	8-OHdG, an oxidized form of the DNA base guanine; assayed by IHC	[14]
Mouse model of esophagitis and Barrett's esophagus	DCA (as dietary supplement; also zinc deficiency)	8-OHdG assayed by IHC	[17]

[1]Comet assay, also known as the single cell gel electrophoresis assay; 8-OHdG: 8-hydroxydeoxyguanosine; IHC: Immunohistochemical assay.

adenocarcinomas were studied for apoptosis capability, it was found that apoptosis is inhibited early in the dysplasia-carcinoma sequence of BE by over-expression of the anti-apoptotic protein, Bcl-2[25], presumably as a result of chronic gastroesophageal reflux containing bile acids. BE cells have high levels of the anti-apoptotic proteins IL-6, Bcl-xL and Mcl-1[26]. Studies of tissues obtained from patient biopsies, indicated that BE cells are resistant to apoptosis induction by DCA compared to esophageal squamous epithelium and normal colon epithelium[23]. Reduced apoptosis competence may arise by mutation in genes encoding proteins necessary for apoptosis. Since cells resistant to apoptosis have a growth advantage in the presence of agents that ordinarily induce apoptosis, such as bile acids, these cells will tend to proliferate to form a field of apoptosis resistant cells[27]. Within such a defective field, repeated encounters with bile acids in reflux would cause further DNA damage. Such DNA damage, leading to further mutation, may give rise to malignancy.

Considerable evidence indicates an association of bile acid exposure with esophageal cancer. In rats, reflux of duodenal or gastro-duodenal contents, that include bile acids, induced esophageal carcinoma in the absence of exogenous carcinogen[28]. Rat surgical models with increased duodenal reflux into the esophagus, but without added carcinogen, caused esophagitis, BE-like lesions and adenocarcinomas[29-32]. Persons with BE were found to have increased duodenoesophageal reflux and increased exposure to bile acids in their refluxate, suggesting that the BE premalignant lesion is linked to bile acid exposure[10,11]. In a rat duodenal-contents reflux model, a high animal-fat intake changed the bile acid composition of bile juice and increased the development of BE and esophageal adenocarcinoma[33].

In summary, evidence indicates that, in esophageal cells and tissues, bile acids have the short-term effect of inducing oxidative stress, oxidative DNA damage, mutation and apoptosis. Over a longer period, bile acids are implicated in the development of apoptosis resistance and eventually the development of adenocarcinoma.

STOMACH

The estimated yearly number of deaths world-wide from gastric cancer is 511 549 for men and 288 681 for women[7], making it the second leading cause of cancer deaths among men and women combined. Infection by the bacterium *Helicobacter pylori* is the major etiologic risk factor in gastric carcinogenesis. However, gastroesophageal reflux appears to have an important role in the development of gastric cardia adenocarcinoma[34,35] which may have an etiology similar to that of esophageal adenocarcinoma[34].

Exposure of cultured gastric carcinoma cells (St23123) to TCDCA increased production of ROS[36]. DCA induced apoptosis in cultured human gastric epithelial cells[37]. In rats, TCA increased stomach tumorigenesis induced by the carcinogen N-methyl-N'-nitro-N-nitrosoguanidine[38]. Carcinoma in the gastric stump (generated in rats by surgical gastrectomy) was increased by dietary fat intake and increased bile acid output[39]. Gastric adenocarcinomas were found to develop in a rat surgical model of duodenal reflux[40]. Gastroesophageal reflux in humans is implicated in adenocarcinoma of the gastric cardia[34,35,41]. Thus, elevated bile acid exposure is associated with increased ROS, induction of apoptosis and increased development of cancer of the gastric cardia.

SMALL INTESTINE

Small intestinal cancer is relatively infrequent compared to other cancers of the GI tract. In the United States, only 0.2% of all cancer deaths are due to cancer of the small intestine. Elevated risk of carcinoid tumor of the small intestine is associated with saturated fat intake[42], consistent with an etiologic role of bile acids. Fifty-three percent of adenocarcinomas of the small intestine arise in the duodenum, although the length of the duodenum is only 4% of the entire length of the small intestine. In addition, 57% of these duodenal cancers arise in the 6-7 cm segment that includes the outlet (Ampulla of Vater) of the common bile duct where bile (including bile acids) and pancreatic secretions empty into the small intestine[43]. Most adenomas and carcinomas of the small intestine and extrahepatic bile ducts arise in the region of the Papilla of Vater (which includes the Ampulla of Vater)[44]. Patients who have undergone a cholecystectomy are at increased risk of cancer of the small intestine, a risk that declines with increasing distance from the common bile duct[45]. These findings indicate that exposure to high levels of bile might be the

Table 2 Bile acids induce apoptosis in liver cells

Cells/tissues	Bile acid(s) that induced apoptosis	Ref.
Isolated rat hepatocytes	GDCA	[64,65]
	GCDCA	[55,66]
	GCDCA, GCA	[57]
	GCDCA	[67]
Isolated rat and mouse hepatocytes	DCA	[68]
Liver tissue sections from rats fed DCA, and cultured human hepatocellular carcinoma cells (HuH-7)	DCA	[58]
Cultured rat hepatocyes (McNtcp.24 cells)	GCDC	[69,70]
Cultured human hepatocellular carcinoma cells (HuH-7)	GCDCA	[71]
Rat hepatocytes and human hepatoma carcinoma cells (HuH-7)	Taurolithocholate-3-sulfate	[59]

underlying cause of carcinomas of the small intestine.

Individuals with familial adenomatous polyposis (FAP) have an increased risk of developing adenomas and cancer of the small and large intestine. In the small intestine, these lesions arise mostly near the outlet of the common bile duct, where their distribution parallels bile acid exposure[46,47]. In a mouse model of FAP ($Apc^{min/+}$), higher dietary fat intake was associated with an increase in small intestinal tumors[48]. Administration of CDCA in this FAP mouse model increased duodenal tumors, suggesting that unconjugated bile acids contribute to periampullary tumor formation in the setting of an $Apc^{min/+}$ genotype[49].

The farnesoid X receptor (FXR) is a member of the nuclear receptor superfamily, and bile acids are endogenous ligands of FXR. FXR is necessary for maintaining bile acid homeostasis, and activation of FXR induces the expression of ileal bile acid binding protein (IBAB) and ileal bile acid transporters. In $Apc^{min/+}$ mice, FXR deficiency led to an increase in the size of small intestine adenocarcinomas[50]. Taken together, these results indicate that bile acids play a central role in cancer of the small intestine.

LIVER

The estimated yearly number of deaths world-wide from liver cancer is 474215 for men and 205656 for women[6], making it the third leading cause of cancer deaths among men and women combined. The majority of liver cancers world-wide arise as a result of chronic infection by hepatitis B or C virus, or from exposure to aflatoxin B1, a carcinogenic food contaminant. Excessive alcohol consumption is another risk factor. However, the risk of hepatocellular carcinoma is elevated in individuals with late stage primary biliary cirrhosis, a possible autoimmune disease[51]. Liver cancer can also arise in children with a defect in the bile acid export pump[52]. Thus bile acids are implicated in at least some cases of liver cancer.

Several studies have shown that bile acids induce ROS in cells of the liver. TCDCA induced ROS in isolated rat hepatocytes[53,54]. ROS were also induced in rat hepatocytes by GCDCA[55-57] and by DCA[58]. Taurolithocholate-3-sulfate induced ROS both in rat hepatocytes and a human hepatoma cell line (Huh7)[59].

Treatment of human hepatoma cells (HepG2) with DCA activated the *gadd153 promoter*[60]. This promoter is activated by DNA damage, suggesting that DCA induces DNA damage in hepatoma cells.

DCA is a promoter of preneoplastic lesions (hyperplastic nodules) in hepatocellular carcinogenesis[61,62]. Evidence has also been presented that DCA, given as a dietary supplement in rats, possess initiating activity for hepatocarcinogenesis[63]. At least 12 studies have shown that bile acids induce apoptosis in liver cells. These are listed in Table 2. Apoptosis induced in liver cells by hydrophobic bile acids is likely caused by oxidative stress[59].

Four studies indicated that bile acid-induced apoptosis in liver cells is mediated by ROS. A lazaroid antioxidant (U83836E) inhibited induction of apoptosis in isolated rat hepatocytes[55]. The antioxidants α-tocopherol, ebselen or idebenone (a coenzyme Q analogue) inhibited apoptosis of isolated rat hepatocytes by GCDCA and GCA[57]. Also in isolated rat hepatocytes, the antioxidants β-carotene and α-tocopherol inhibited GCDCA induced apoptosis[67]. LCA and CDCA activated the antioxidant responsive element Nrf2 in human hepatoma-derived cells (HepG2), mouse hepatoma-derived cells (Hepa1c1c7) and primary human hepatocytes[72]. Nrf2 activation inhibits apoptosis, and the target genes of activated Nrf2 include the genes that encode the rate-limiting enzyme in glutathione biosynthesis and thioredoxin reductase 1. The general finding that induction of apoptosis in liver cells by bile acids can be reduced by anti-oxidants implies that this induction is mediated by ROS.

The bile salt export pump conveys bile acids from the hepatocyte cytoplasm into bile canaliculi. Mutations in the *ABCB11* gene cause a deficiency in the bile salt export pump, leading to intrahepatic accumulation of toxic bile salts. Children with such mutations have an increased incidence of hepatocellular carcinoma[52,73]. Mice lacking the farnesoid X receptor, which controls the synthesis and export of bile acids, have increased hepatic bile acids. These mice have a high incidence of liver tumors[74,75]. Such findings led to the suggestion that in cholestatic liver disease, chronic exposure to bile acids may play an important role in hepatocellular carcinogenesis[51].

BILIARY TRACT

Cholangiocarcinoma (CC) is an adenocarcinoma that arises from the bile duct epithelium. The CCs that occur within the liver are referred to as intrahepatic CCs. Those that occur at the confluence of the left and right hepatic duct are termed hilar CCs. The CCs that arise between the hepatic hilum and the duodenal papilla (or

Ampulla of Vater) are called extra hepatic CCs[76].

The gallbladder and bile duct are exposed to high concentrations of bile acids. The bile acids excreted from the liver into the gall bladder are at a concentration of approximately 100 mmol/L[77]. The lifetime risk for developing cholangiocarcinoma in patients with primary sclerosing cholangitis is estimated at 7%-13%[78], and it was suggested that chronic exposure to bile acids may play an important role in cholangiocellular carcinogenesis[51]. Two children with progressive familial intrahepatic cholestasis and cholangiocarcinoma were found to have an absence of bile salt export pump expression and mutations in the *ABCB11* gene[79]. Loss of a functional bile salt export pump may cause cholangiocarcinoma through intracellular accumulation of bile acids. Incubation of immortalized mouse cholangiocytes with GCDC resulted in the generation of ROS and an increase the percentage of cells with oxidative DNA damage (8-OHdG), suggesting that the a long-term effect of excessive exposure of the biliary tract to GCDC may be carcinogenesis[80].

PANCREAS

The estimated yearly number of deaths world-wide from pancreatic cancer is 137 206 for men and 122 185 for women[6], making it the eighth leading cause of cancer deaths among men and women combined. Most adenocarcinomas of the pancreas occur in the head of the gland, which is in close proximity to bile[81]. In a hamster surgical model, bile reflux into the pancreatic duct was shown to induce development of intraductal papillary carcinomas of the pancreas[82], suggesting that bile acid may be an etiologic agent in pancreatic cancer. Consistent with this idea, epidemiological studies found a positive correlation between ingestion of a western style high fat diet and the incidence of pancreatic cancer[83-85]. Treatment of human pancreatic cancer cell lines with bile acids (CDCA, DCA or TCDCA) induced cyclooxygenase-2 (COX-2) expression[81]. Since COX-2 is overexpressed in human pancreatic adenocarcinomas, these results also suggest a possible role for bile acids in pancreatic carcinogenesis.

COLON AND RECTUM

The estimated yearly number of deaths world-wide from cancer of the colon and rectum is 318 798 for men and 284 169 for women[6], making it the fourth leading cause of cancer deaths among men and women combined. Although both inherited mutations, environmental factors (e.g. smoking) and dietary factors are involved in colorectal cancer development, sporadic colorectal cancer appears to be caused predominantly by dietary factors.

The association of risk of colorectal cancer and consumption of red meat and processed meat was assessed in a meta-analysis of 15 prospective studies on red meat and 14 studies on processed meat[86]. The results showed consistent associations between high consumption of red and of processed meat and risk of colorectal cancer. In another recent study, a dose-dependent positive association between saturated fat intake and localized colorectal cancer was found in women, but not in men[87]. In earlier work, a positive association between dietary fat consumption and cancer incidence was reported[88-93]. Dietary total fat intake and saturated fat intake, but not polyunsaturated fat intake, are positively associated with colon cancer incidence[94]. In cancer prone *Apc*$^{min/+}$ mice, a high fat diet results in a significant increase in tumors[48]. A Western-style diet, containing elevated lipids and decreased calcium and vitamin D, induced colonic tumors in normal CB7Bl/6 mice[95-97]. Taken together, these studies implicate dietary fat (primarily from red and processed meat) in the etiology of human colorectal cancer.

Dietary intake of high-fat and high-beef foods results in a significantly higher excretion of fecal secondary bile acids, mainly DCA and LCA[98]. Presumably the increase in DCA and LCA reflects increased production of bile acids in order to emulsify the increased level of dietary fat. Epidemiologic studies have also found that fecal bile acid concentrations are increased in populations with a high incidence of colorectal cancer[99-106]. The most significant bile acids with respect to human colorectal cancer appear to be the secondary bile acids, DCA and LCA[99].

Although repeated exposure of the colorectal epithelium to high physiological concentrations of bile acids appears to be the major etiologic factor in colorectal carcinogenesis, other factors may also be significant. Intake of dietary heme iron is associated with increased risk of colorectal cancer[107], suggesting that iron catalyzed formation of ROS may play a role. The risk of colorectal cancer is also increased by smoking[108]. Bile acids and nicotine from smoking can interact synergistically in colon cells to increase oxidative stress and DNA damage[109].

Twelve studies have reported that bile acids induce production of ROS or RNS in colon cells (Table 3).

Fourteen studies showed that bile acids induce DNA damage in colon cells (Table 4), of which a component is likely oxidative DNA damage. Defective repair of oxidative DNA damage is linked to increased risk of colon cancer. The base excision repair pathway deals with oxidative damages in DNA caused by ROS. 8-OHdG is a major oxidative damage in DNA that can mispair with adenine causing G:C to T:A transversion mutations, unless the mispair is corrected. MUTYH is a mammalian DNA glycosylase that initiates base excision repair by excising adenine opposite 8-OHdG. Genetic

Table 3 Bile acids induce ROS/RNS in colon cells

Cells/tissues	Bile acid(s) that induced ROS/RNS	Ref.
Human colon surgical resections	DCA (RNS)	[110]
Cultured human adenocarcinoma cells (CACO-2)	DCA, LCA (ROS)	[111]
Cultured human adenocarcinoma cells (HT-29)	DCA, LCA (ROS)	[112]
	DCA (ROS)	[36,113]
	DCA (RNS)	[114]
Cultured human adenocarcinoma cells (HCT116)	DCA (ROS)	[109,115,116]
	DCA (RNS)	[117]
Rat colonic mucosa	DCA (ROS)	[118]
Mouse colonic mucosa	DCA (ROS, RNS)	[119]

Table 4 Bile acids induce DNA damage in colon cells

Cells/tissues	Bile acid(s)	Assay for DNA damage	Ref.
Isolated mouse colon crypt cells	LCA	Nucleoid sedimentation for strand breaks	[122]
Isolated human and rat colon cells LCA	LCA	Comet assay for strand breaks	[123]
Isolated rat colon cells	DCA	Immunostaining for poly (ADP-ribose) an indicator of DNA damage	[124]
Freshly isolated normal human colonocytes	DCA, CDCA	Comet assay for strand breaks	[125]
Cultured human adenocarcinoma cells (HT-29)	DCA, CDCA	Comet assay for strand breaks and modified comet assay for oxidative DNA damage	
Cultured human adenocarcinoma cells (HT-29)	DCA, LCA	Comet assay for strand breaks	[112,126]
Cultured human adenocarcinoma cells (CACO-2)	DCA, LCA	Comet assay for oxidative DNA damage	[111]
Cultured human colon adenocarcinoma cells (HCT-116 & HCT-15)	DCA	Comet assay for strand breaks	[127]
Cultured human colon adenocarcinoma cells (HCT-116 & HT-29)	DCA	Comet assay for strand breaks	[128]
Cultured human colon adenocarcinoma cells (HCT-116)	DCA	Induction of the DNA repair protein BRCA-1	[129]
		Induction of DNA damage inducible gene GADD153	[130]
		Comet assay	[116]
Cultured human colon adenocarcinoma cells (HCT-116 and HCT-15)	DCA	Induction of DNA damage inducible genes GADD34, GADD45, GADD153	[131]
Colon samples from mouse dietary colitis model	DCA	Oxidative DNA damage: 8-OHdG assayed by immunohistochemistry	[119]

Table 5 Bile acids induce apoptosis in colon cells

Cells/tissues	Bile acid(s) that induced apoptosis	Ref.
Biopsies from normal human colonic mucosa	DCA	[135-139]
Colon adenoma cell lines (AA/C1 and RG/C2), and carcinoma cell line (PC/JW/F1)	DCA	[140]
Cultured human adenocarcinoma cells (HT-29 and CaCo-2)	DCA	[141,142]
Cultured human adenocarcinoma cells (HCT-116)	DCA, CDCA	[130,143-146]
	DCA	[116,147-149]
Cultured human adenocarcinoma cells (HT-29)	DCA	[114]
Cultured human adenocarcinoma cells (HT-29 and HCT-116)	DCA	[150]
	DCA	[128]
	DCA, LCA, CDCA	[151]
Cultured human adenocarcinoma cells (HT-29) and human fetal colonic mucosal cells (FHC)	DCA, LCA, CA, CDCA	[152]
Cultured human adenocarcinoma cells (HT-29, SW480, SW620)	DCA, CDCA	[153,154]
Cultured human adenocarcinoma cells [HCT-116 ($p53^+$) and HCT-15 ($p53^-$)]	DCA	[127]
Cultured human adenocarcinoma cells (HCT-116SA apoptosis-sensitive and HCT-116RB, HCT-116RC and HCT-116RD apoptosis resistant)	DCA	[155]
Human colonic mucosal samples from surgical resections	DCA	[156]

defects in MUTYH cause multiple polyps[120] and greatly increased risk of colorectal cancer[121] in humans.

The numerous studies showing that bile acids induce DNA damage in colon cells suggest that bile acids may also induce mutation and genomic instability. In a model system for inducing tumors in the rat using the carcinogen azoxymethane, DCA not only increased the incidence of colon tumors, but also increased the incidence of tumors with *K-ras* point mutations[132], suggesting that DCA may induce *K-ras* mutations. Hydrophobic bile acids cause aneuploidy and micronuclei formation, indicators of genomic instability, in a variety of cell types including colon epithelial cells[133]. Persistent exposure of cultured colon epithelial cells to DCA results in alterations in expression of chromosomal maintenance/mitosis-related genes that might give rise to the observed genomic instability[133].

The 27 studies listed in Table 5 indicate that hydrophobic bile acids induce apoptosis in colon cells. Exposure of colon epithelial cells to DCA causes induction of growth arrest and DNA damage-inducible genes *GADD34*, *GADD45* and *GADD153*, probably in response to the DNA damage caused by DCA[131]. DCA induced expression of *GADD153* is essential for DCA induction of apoptosis[130]. These findings suggest that induction of DNA damage by DCA results in apoptosis. Induction of apoptosis by DCA may protect against the survival of cells with damaged template DNA that upon replication might undergo mutation leading to cancer[134].

Repeated long-term exposure of colonic epithelial cells to high physiologic concentrations of bile acids appears to select for cells that are resistant to induction of apoptosis by bile acids. Such apoptosis-resistant cells might arise and clonally expand through the processes of mutation (or epimutation) and natural selection. Several studies of colon cancer patients have shown that epithelial cells in areas of the colonic mucosa that do not contain the cancer itself have increased resistance to induction of apoptosis by DCA[115,135,137-139]. The expression of anti-apoptotic protein Bcl-xL is elevated in the colorectal mucosa adjacent to colorectal adenocarcinomas[157]. These findings suggest that tumors may often arise in a field of apoptosis-resistant epithelial cells. A variant of ileal bile acid binding protein (IBABP), termed IBABP-L,

is upregulated in colorectal cancer and is necessary for survival of HCT116 colon cancer cells in the presence of physiologic levels of hydrophobic bile acid[158]. This finding suggests that IBABP-L is a key factor in the development of resistance to bile acids in colon cancer cells. Furthermore, repeated long-term exposure of HCT-116 human colonic epithelial cells in culture to sublethal concentrations of DCA selects for cells that have further increased resistance to DCA-induced apoptosis[159]. These observations suggest a link between development of resistance to bile acid-induced apoptosis and colon cancer.

In summary, evidence indicates that, in colonic epithelial cells and tissues, bile acids have the short-term effect of inducing oxidative stress that causes DNA damage leading to mutation and apoptosis. Over a longer period, repeated exposure to high levels of bile acid may select for the development of apoptosis resistant fields of cells and eventually to the development of adenocarcinoma.

DNA DAMAGE COUPLED WITH RESISTANCE TO CELL DEATH DRIVES TUMORIGENESIS

We have emphasized, above, the role of bile acids in inducing ROS/RNS and DNA damage in cells of the GI tract. These stresses, if excessive, can overwhelm cellular defenses resulting in cell death[139,160-163]. However, we have also shown that bile acids can activate two major cell survival pathways, NF-κB[115,124] and autophagy[164] (Figure 1). Both of the pathways are known to be activated by ROS[165,166]. Results from our laboratory indicate that the activation of both pathways by DCA can be attenuated by the use of antioxidants[113,115,124,164]. We have also shown that the NF-κB and autophagy pathways contribute to the stable apoptosis resistance that characterizes cell lines persistently exposed to DCA[159,164]. The sensitization to DOC-induced cell death after interfering with these pathways was documented using antisense oligonucleotides against the p65 subunit of NF-κB[159] and pharmacologically through the use of 3-methyladenine[164], an inhibitor of autophagy.

The induction of persistent DNA damage in apoptosis-resistant cells is a dangerous situation that can lead to further mutation and ultimately cancer (Figure 1). An increase in Bcl-2 (an anti-apoptotic protein), for example, may also downregulate Ku DNA binding activity, thereby further amplifying genomic instability through interference with the non-homologous end-joining pathway of DNA repair[167]. The cross-talk between anti-apoptotic proteins and DNA repair proteins is a current area of investigation.

NUCLEAR BILE ACID RECEPTORS FXR, VDR AND PXR/SXR

Recently, it has become apparent that nuclear bile acid receptors FXR, VDR and PXR/SXR play an important role in protecting against carcinogenic effects of bile acids. FXR, a member of the nuclear receptor superfamily, responds to bile acids as physiological ligands[168-170]. FXR has a key role in activating pathways that maintain bile acid homeostasis[50]. FXR protects against intestinal tumorigenesis, possibly by a mechanism involving induction of apoptosis[50,171].

The vitamin D receptor (VDR) functions as a receptor for the secondary bile acid lithocholic acid, and has a key role in activating a pathway that detoxifies lithocholic acid[172]. Similarly, the human xenobiotic receptor SXR (steroid xenobiotic receptor) and its rodent homolog PXR (pregnane X receptor) are bile acid receptors that, when activated, induce a response that detoxifies bile acids[173,174]. PXR promotes bile acid detoxification by activating bile acid metabolizing enzymes and transporters. In both human colon cancer cells and normal mouse colon epithelium PXR/SXR protects against bile acid induced apoptosis[149].

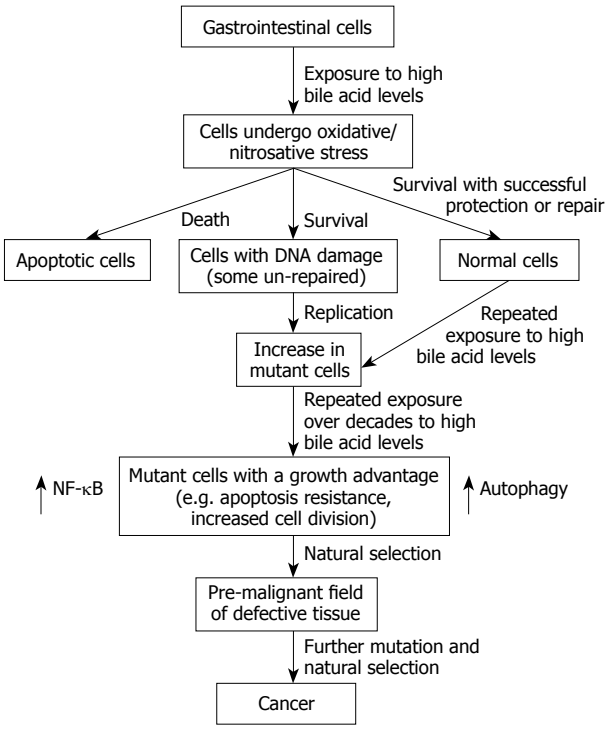

Figure 1 The role of bile acids in the sequence of events leading to gastrointestinal cancer.

CONCLUSION

In Figure 1, we suggest a possible general pathway for bile acid induced carcinogenesis based on evidence reviewed above. An immediate effect on cells of the GI tract to exposure to a high physiologic level of bile acids is the induction of ROS/RNS. This can lead to DNA damage and apoptosis in some cells. Among surviving cells, some may remain normal by successfully employing protective and repair mechanisms. Other surviving cells, however, may retain unrepaired DNA damage. When such cells undergo DNA replication using a damaged strand as template, mutations will likely arise. Over years of frequently repeated exposure to high

levels of bile acids many mutations will occur, and some of these mutations may provide a growth advantage to the cell in which they occur. The growth advantage may involve apoptosis resistance, and increased and/or aberrant proliferation. Such cells will tend to expand clonally at the expense of neighboring cells to form a field of defective cells. Further repeated exposure to high levels of bile acids will lead to additional mutations. Should some of these mutations arise within a defective field and also provide additional growth advantages, a secondary field will spread within the first field by natural selection. Repetition of this "mutation-and-selection" process over many years, perhaps decades, will lead to a pre-malignant field and eventually to cancer.

REFERENCES

1 **Bernstein H**, Bernstein C, Payne CM, Dvorakova K, Garewal H. Bile acids as carcinogens in human gastrointestinal cancers. *Mutat Res* 2005; **589**: 47-65
2 **Reddy BS**. Diet and excretion of bile acids. *Cancer Res* 1981; **41**: 3766-3768
3 **Cherbonnel-Lasserre C**, Gauny S, Kronenberg A. Suppression of apoptosis by Bcl-2 or Bcl-xL promotes susceptibility to mutagenesis. *Oncogene* 1996; **13**: 1489-1497
4 **Cherbonnel-Lasserre C**, Dosanjh MK. Suppression of apoptosis by overexpression of Bcl-2 or Bcl-xL promotes survival and mutagenesis after oxidative damage. *Biochimie* 1997; **79**: 613-617
5 **Saintigny Y**, Dumay A, Lambert S, Lopez BS. A novel role for the Bcl-2 protein family: specific suppression of the RAD51 recombination pathway. *EMBO J* 2001; **20**: 2596-2607
6 **Garcia M**, Jemal A, Ward EM, Center MM, Hao Y, Siegel RL, Thun MJ. Global Cancer Facts & Figures 2007. Atlanta, GA: American Cancer Society, 2007: 25-26
7 **Cross AJ**, Leitzmann MF, Gail MH, Hollenbeck AR, Schatzkin A, Sinha R. A prospective study of red and processed meat intake in relation to cancer risk. *PLoS Med* 2007; **4**: e325
8 **Holmes RS**, Vaughan TL. Epidemiology and pathogenesis of esophageal cancer. *Semin Radiat Oncol* 2007; **17**: 2-9
9 **Falk GW**. Barrett's esophagus. *Gastroenterology* 2002; **122**: 1569-1591
10 **Nehra D**, Howell P, Williams CP, Pye JK, Beynon J. Toxic bile acids in gastro-oesophageal reflux disease: influence of gastric acidity. *Gut* 1999; **44**: 598-602
11 **Menges M**, Müller M, Zeitz M. Increased acid and bile reflux in Barrett's esophagus compared to reflux esophagitis, and effect of proton pump inhibitor therapy. *Am J Gastroenterol* 2001; **96**: 331-337
12 **Stein HJ**, Kauer WK, Feussner H, Siewert JR. Bile reflux in benign and malignant Barrett's esophagus: effect of medical acid suppression and nissen fundoplication. *J Gastrointest Surg* 1998; **2**: 333-341
13 **Dvorak K**, Watts GS, Ramsey L, Holubec H, Payne CM, Bernstein C, Jenkins GJ, Sampliner RE, Prasad A, Garewal HS, Bernstein H. Expression of bile acid transporting proteins in Barrett's esophagus and esophageal adenocarcinoma. *Am J Gastroenterol* 2009; **104**: 302-309
14 **Dvorak K**, Payne CM, Chavarria M, Ramsey L, Dvorakova B, Bernstein H, Holubec H, Sampliner RE, Guy N, Condon A, Bernstein C, Green SB, Prasad A, Garewal HS. Bile acids in combination with low pH induce oxidative stress and oxidative DNA damage: relevance to the pathogenesis of Barrett's oesophagus. *Gut* 2007; **56**: 763-771
15 **Jenkins GJ**, D'Souza FR, Suzen SH, Eltahir ZS, James SA, Parry JM, Griffiths PA, Baxter JN. Deoxycholic acid at neutral and acid pH, is genotoxic to oesophageal cells through the induction of ROS: The potential role of anti-oxidants in Barrett's oesophagus. *Carcinogenesis* 2007; **28**: 136-142
16 **Feagins LA**, Zhang HY, Zhang X, Hormi-Carver K, Thomas T, Terada LS, Spechler SJ, Souza RF. Mechanisms of oxidant production in esophageal squamous cell and Barrett's cell lines. *Am J Physiol Gastrointest Liver Physiol* 2008; **294**: G411-G417
17 **Guy NC**, Garewal H, Holubec H, Bernstein H, Payne CM, Bernstein C, Bhattacharyya AK, Dvorak K. A novel dietary-related model of esophagitis and Barrett's esophagus, a premalignant lesion. *Nutr Cancer* 2007; **59**: 217-227
18 **Jolly AJ**, Wild CP, Hardie LJ. Acid and bile salts induce DNA damage in human oesophageal cell lines. *Mutagenesis* 2004; **19**: 319-324
19 **Jolly AJ**, Wild CP, Hardie LJ. Sodium deoxycholate causes nitric oxide mediated DNA damage in oesophageal cells. *Free Radic Res* 2009; **43**: 234-240
20 **Jenkins GJ**, Cronin J, Alhamdani A, Rawat N, D'Souza F, Thomas T, Eltahir Z, Griffiths AP, Baxter JN. The bile acid deoxycholic acid has a non-linear dose response for DNA damage and possibly NF-kappaB activation in oesophageal cells, with a mechanism of action involving ROS. *Mutagenesis* 2008; **23**: 399-405
21 **Theisen J**, Peters JH, Fein M, Hughes M, Hagen JA, Demeester SR, Demeester TR, Laird PW. The mutagenic potential of duodenoesophageal reflux. *Ann Surg* 2005; **241**: 63-68
22 **Fein M**, Peters JH, DeMeester TR. Carcinogenesis in reflux disease--in search for bile-specific effects. *Microsurgery* 2007; **27**: 647-650
23 **Dvorakova K**, Payne CM, Ramsey L, Bernstein H, Holubec H, Chavarria M, Bernstein C, Sampliner RE, Riley C, Prasad A, Garewal H. Apoptosis resistance in Barrett's esophagus: ex vivo bioassay of live stressed tissues. *Am J Gastroenterol* 2005; **100**: 424-431
24 **Zhang R**, Gong J, Wang H, Wang L. Bile salts inhibit growth and induce apoptosis of culture human normal esophageal mucosal epithelial cells. *World J Gastroenterol* 2005; **11**: 6466-6471
25 **Katada N**, Hinder RA, Smyrk TC, Hirabayashi N, Perdikis G, Lund RJ, Woodward T, Klingler PJ. Apoptosis is inhibited early in the dysplasia-carcinoma sequence of Barrett esophagus. *Arch Surg* 1997; **132**: 728-733
26 **Dvorakova K**, Payne CM, Ramsey L, Holubec H, Sampliner R, Dominguez J, Dvorak B, Bernstein H, Bernstein C, Prasad A, Fass R, Cui H, Garewal H. Increased expression and secretion of interleukin-6 in patients with Barrett's esophagus. *Clin Cancer Res* 2004; **10**: 2020-2028
27 **Bernstein C**, Bernstein H, Payne CM, Dvorak K, Garewal H. Field defects in progression to gastrointestinal tract cancers. *Cancer Lett* 2008; **260**: 1-10
28 **Miwa K**, Sahara H, Segawa M, Kinami S, Sato T, Miyazaki I, Hattori T. Reflux of duodenal or gastro-duodenal contents induces esophageal carcinoma in rats. *Int J Cancer* 1996; **67**: 269-274
29 **Goldstein SR**, Yang GY, Curtis SK, Reuhl KR, Liu BC, Mirvish SS, Newmark HL, Yang CS. Development of esophageal metaplasia and adenocarcinoma in a rat surgical model without the use of a carcinogen. *Carcinogenesis* 1997; **18**: 2265-2270
30 **Fein M**, Peters JH, Chandrasoma P, Ireland AP, Oberg S, Ritter MP, Bremner CG, Hagen JA, DeMeester TR. Duodenoesophageal reflux induces esophageal adenocarcinoma without exogenous carcinogen. *J Gastrointest Surg* 1998; **2**: 260-268
31 **Chen X**, Yang G, Ding WY, Bondoc F, Curtis SK, Yang CS. An esophagogastroduodenal anastomosis model for esophageal adenocarcinogenesis in rats and enhancement by iron overload. *Carcinogenesis* 1999; **20**: 1801-1808
32 **Su Y**, Chen X, Klein M, Fang M, Wang S, Yang CS, Goyal RK. Phenotype of columnar-lined esophagus in rats with esophagogastroduodenal anastomosis: similarity to human

Barrett's esophagus. *Lab Invest* 2004; **84**: 753-765
33 **Chen KH**, Mukaisho K, Sugihara H, Araki Y, Yamamoto G, Hattori T. High animal-fat intake changes the bile-acid composition of bile juice and enhances the development of Barrett's esophagus and esophageal adenocarcinoma in a rat duodenal-contents reflux model. *Cancer Sci* 2007; **98**: 1683-1688
34 **Crew KD**, Neugut AI. Epidemiology of gastric cancer. *World J Gastroenterol* 2006; **12**: 354-362
35 **Ye W**, Chow WH, Lagergren J, Yin L, Nyrén O. Risk of adenocarcinomas of the esophagus and gastric cardia in patients with gastroesophageal reflux diseases and after antireflux surgery. *Gastroenterology* 2001; **121**: 1286-1293
36 **Lechner S**, Müller-Ladner U, Schlottmann K, Jung B, McClelland M, Rüschoff J, Welsh J, Schölmerich J, Kullmann F. Bile acids mimic oxidative stress induced upregulation of thioredoxin reductase in colon cancer cell lines. *Carcinogenesis* 2002; **23**: 1281-1288
37 **Redlak MJ**, Dennis MS, Miller TA. Apoptosis is a major mechanism of deoxycholate-induced gastric mucosal cell death. *Am J Physiol Gastrointest Liver Physiol* 2003; **285**: G870-G879
38 **Kobori O**, Shimizu T, Maeda M, Atomi Y, Watanabe J, Shoji M, Morioka Y. Enhancing effect of bile and bile acid on stomach tumorigenesis induced by N-methyl-N'-nitro-N-nitrosoguanidine in Wistar rats. *J Natl Cancer Inst* 1984; **73**: 853-861
39 **Miwa K**, Kinami S, Miyazaki I, Hattori T. Positive association between dietary fat intake and risk of gastric stump carcinoma in rats. *Carcinogenesis* 1996; **17**: 1885-1889
40 **Mukaisho K**, Miwa K, Kumagai H, Bamba M, Sugihara H, Hattori T. Gastric carcinogenesis by duodenal reflux through gut regenerative cell lineage. *Dig Dis Sci* 2003; **48**: 2153-2158
41 **Dixon MF**, Mapstone NP, Neville PM, Moayyedi P, Axon AT. Bile reflux gastritis and intestinal metaplasia at the cardia. *Gut* 2002; **51**: 351-355
42 **Cross AJ**, Leitzmann MF, Subar AF, Thompson FE, Hollenbeck AR, Schatzkin A. A prospective study of meat and fat intake in relation to small intestinal cancer. *Cancer Res* 2008; **68**: 9274-9279
43 **Ross RK**, Hartnett NM, Bernstein L, Henderson BE. Epidemiology of adenocarcinomas of the small intestine: is bile a small bowel carcinogen? *Br J Cancer* 1991; **63**: 143-145
44 **Fischer HP**, Zhou H. Pathogenesis of carcinoma of the papilla of Vater. *J Hepatobiliary Pancreat Surg* 2004; **11**: 301-309
45 **Lagergren J**, Ye W, Ekbom A. Intestinal cancer after cholecystectomy: is bile involved in carcinogenesis? *Gastroenterology* 2001; **121**: 542-547
46 **Jagelman DG**, DeCosse JJ, Bussey HJ. Upper gastrointestinal cancer in familial adenomatous polyposis. *Lancet* 1988; **1**: 1149-1151
47 **Spigelman AD**, Williams CB, Talbot IC, Domizio P, Phillips RK. Upper gastrointestinal cancer in patients with familial adenomatous polyposis. *Lancet* 1989; **2**: 783-785
48 **Wasan HS**, Novelli M, Bee J, Bodmer WF. Dietary fat influences on polyp phenotype in multiple intestinal neoplasia mice. *Proc Natl Acad Sci USA* 1997; **94**: 3308-3313
49 **Mahmoud NN**, Dannenberg AJ, Bilinski RT, Mestre JR, Chadburn A, Churchill M, Martucci C, Bertagnolli MM. Administration of an unconjugated bile acid increases duodenal tumors in a murine model of familial adenomatous polyposis. *Carcinogenesis* 1999; **20**: 299-303
50 **Maran RR**, Thomas A, Roth M, Sheng Z, Esterly N, Pinson D, Gao X, Zhang Y, Ganapathy V, Gonzalez FJ, Guo GL. Farnesoid X receptor deficiency in mice leads to increased intestinal epithelial cell proliferation and tumor development. *J Pharmacol Exp Ther* 2009; **328**: 469-477
51 **Jansen PL**. Endogenous bile acids as carcinogens. *J Hepatol* 2007; **47**: 434-435
52 **Knisely AS**, Strautnieks SS, Meier Y, Stieger B, Byrne JA, Portmann BC, Bull LN, Pawlikowska L, Bilezikçi B, Ozçay F, László A, Tiszlavicz L, Moore L, Raftos J, Arnell H, Fischler B, Németh A, Papadogiannakis N, Cielecka-Kuszyk J, Jankowska I, Pawłowska J, Melín-Aldana H, Emerick KM, Whitington PF, Mieli-Vergani G, Thompson RJ. Hepatocellular carcinoma in ten children under five years of age with bile salt export pump deficiency. *Hepatology* 2006; **44**: 478-486
53 **Sokol RJ**, Winklhofer-Roob BM, Devereaux MW, McKim JM Jr. Generation of hydroperoxides in isolated rat hepatocytes and hepatic mitochondria exposed to hydrophobic bile acids. *Gastroenterology* 1995; **109**: 1249-1256
54 **Sokol RJ**, McKim JM Jr, Goff MC, Ruyle SZ, Devereaux MW, Han D, Packer L, Everson G. Vitamin E reduces oxidant injury to mitochondria and the hepatotoxicity of taurochenodeoxycholic acid in the rat. *Gastroenterology* 1998; **114**: 164-174
55 **Patel T**, Gores GJ. Inhibition of bile-salt-induced hepatocyte apoptosis by the antioxidant lazaroid U83836E. *Toxicol Appl Pharmacol* 1997; **142**: 116-122
56 **Sokol RJ**, Straka MS, Dahl R, Devereaux MW, Yerushalmi B, Gumpricht E, Elkins N, Everson G. Role of oxidant stress in the permeability transition induced in rat hepatic mitochondria by hydrophobic bile acids. *Pediatr Res* 2001; **49**: 519-531
57 **Yerushalmi B**, Dahl R, Devereaux MW, Gumpricht E, Sokol RJ. Bile acid-induced rat hepatocyte apoptosis is inhibited by antioxidants and blockers of the mitochondrial permeability transition. *Hepatology* 2001; **33**: 616-626
58 **Rodrigues CM**, Fan G, Ma X, Kren BT, Steer CJ. A novel role for ursodeoxycholic acid in inhibiting apoptosis by modulating mitochondrial membrane perturbation. *J Clin Invest* 1998; **101**: 2790-2799
59 **Reinehr R**, Becker S, Keitel V, Eberle A, Grether-Beck S, Häussinger D. Bile salt-induced apoptosis involves NADPH oxidase isoform activation. *Gastroenterology* 2005; **129**: 2009-2031
60 **Bernstein H**, Payne CM, Bernstein C, Schneider J, Beard SE, Crowley CL. Activation of the promoters of genes associated with DNA damage, oxidative stress, ER stress and protein malfolding by the bile salt, deoxycholate. *Toxicol Lett* 1999; **108**: 37-46
61 **Cameron RG**, Imaida K, Tsuda H, Ito N. Promotive effects of steroids and bile acids on hepatocarcinogenesis initiated by diethylnitrosamine. *Cancer Res* 1982; **42**: 2426-2428
62 **Ohtaki Y**, Hida T, Hiramatsu K, Kanitani M, Ohshima T, Nomura M, Wakita H, Aburada M, Miyamoto KI. Deoxycholic acid as an endogenous risk factor for hepatocarcinogenesis and effects of gomisin A, a lignan component of Schizandra fruits. *Anticancer Res* 1996; **16**: 751-755
63 **Kitazawa S**, Denda A, Tsutsumi M, Tsujiuchi T, Hasegawa K, Tamura K, Maruyama H, Konishi Y. Enhanced preneoplastic liver lesion development under 'selection pressure' conditions after administration of deoxycholic or lithocholic acid in the initiation phase in rats. *Carcinogenesis* 1990; **11**: 1323-1328
64 **Patel T**, Bronk SF, Gores GJ. Increases of intracellular magnesium promote glycodeoxycholate-induced apoptosis in rat hepatocytes. *J Clin Invest* 1994; **94**: 2183-2192
65 **Kwo P**, Patel T, Bronk SF, Gores GJ. Nuclear serine protease activity contributes to bile acid-induced apoptosis in hepatocytes. *Am J Physiol* 1995; **268**: G613-G621
66 **Jones BA**, Rao YP, Stravitz RT, Gores GJ. Bile salt-induced apoptosis of hepatocytes involves activation of protein kinase C. *Am J Physiol* 1997; **272**: G1109-G1115
67 **Gumpricht E**, Dahl R, Devereaux MW, Sokol RJ. Beta-carotene prevents bile acid-induced cytotoxicity in the rat hepatocyte: Evidence for an antioxidant and anti-apoptotic role of beta-carotene in vitro. *Pediatr Res* 2004; **55**: 814-821
68 **Qiao L**, Studer E, Leach K, McKinstry R, Gupta S, Decker R, Kukreja R, Valerie K, Nagarkatti P, El Deiry W, Molkentin J, Schmidt-Ullrich R, Fisher PB, Grant S, Hylemon PB, Dent

P. Deoxycholic acid (DCA) causes ligand-independent activation of epidermal growth factor receptor (EGFR) and FAS receptor in primary hepatocytes: inhibition of EGFR/mitogen-activated protein kinase-signaling module enhances DCA-induced apoptosis. *Mol Biol Cell* 2001; **12**: 2629-2645

69 **Faubion WA**, Guicciardi ME, Miyoshi H, Bronk SF, Roberts PJ, Svingen PA, Kaufmann SH, Gores GJ. Toxic bile salts induce rodent hepatocyte apoptosis via direct activation of Fas. *J Clin Invest* 1999; **103**: 137-145

70 **Sodeman T**, Bronk SF, Roberts PJ, Miyoshi H, Gores GJ. Bile salts mediate hepatocyte apoptosis by increasing cell surface trafficking of Fas. *Am J Physiol Gastrointest Liver Physiol* 2000; **278**: G992-G999

71 **Higuchi H**, Bronk SF, Takikawa Y, Werneburg N, Takimoto R, El-Deiry W, Gores GJ. The bile acid glycochenodeoxycholate induces trail-receptor 2/DR5 expression and apoptosis. *J Biol Chem* 2001; **276**: 38610-38618

72 **Tan KP**, Yang M, Ito S. Activation of nuclear factor (erythroid-2 like) factor 2 by toxic bile acids provokes adaptive defense responses to enhance cell survival at the emergence of oxidative stress. *Mol Pharmacol* 2007; **72**: 1380-1390

73 **Strautnieks SS**, Byrne JA, Pawlikowska L, Cebecauerová D, Rayner A, Dutton L, Meier Y, Antoniou A, Stieger B, Arnell H, Ozçay F, Al-Hussaini HF, Bassas AF, Verkade HJ, Fischler B, Németh A, Kotalová R, Shneider BL, Cielecka-Kuszyk J, McClean P, Whitington PF, Sokal E, Jirsa M, Wali SH, Jankowska I, Pawłowska J, Mieli-Vergani G, Knisely AS, Bull LN, Thompson RJ. Severe bile salt export pump deficiency: 82 different ABCB11 mutations in 109 families. *Gastroenterology* 2008; **134**: 1203-1214

74 **Kim I**, Morimura K, Shah Y, Yang Q, Ward JM, Gonzalez FJ. Spontaneous hepatocarcinogenesis in farnesoid X receptor-null mice. *Carcinogenesis* 2007; **28**: 940-946

75 **Yang F**, Huang X, Yi T, Yen Y, Moore DD, Huang W. Spontaneous development of liver tumors in the absence of the bile acid receptor farnesoid X receptor. *Cancer Res* 2007; **67**: 863-867

76 **Goodman ZD**. Neoplasms of the liver. *Mod Pathol* 2007; **20** Suppl 1: S49-S60

77 **Perwaiz S**, Tuchweber B, Mignault D, Gilat T, Yousef IM. Determination of bile acids in biological fluids by liquid chromatography-electrospray tandem mass spectrometry. *J Lipid Res* 2001; **42**: 114-119

78 **Lazaridis KN**, Gores GJ. Primary sclerosing cholangitis and cholangiocarcinoma. *Semin Liver Dis* 2006; **26**: 42-51

79 **Scheimann AO**, Strautnieks SS, Knisely AS, Byrne JA, Thompson RJ, Finegold MJ. Mutations in bile salt export pump (ABCB11) in two children with progressive familial intrahepatic cholestasis and cholangiocarcinoma. *J Pediatr* 2007; **150**: 556-559

80 **Komichi D**, Tazuma S, Nishioka T, Hyogo H, Chayama K. Glycochenodeoxycholate plays a carcinogenic role in immortalized mouse cholangiocytes via oxidative DNA damage. *Free Radic Biol Med* 2005; **39**: 1418-1427

81 **Tucker ON**, Dannenberg AJ, Yang EK, Fahey TJ 3rd. Bile acids induce cyclooxygenase-2 expression in human pancreatic cancer cell lines. *Carcinogenesis* 2004; **25**: 419-423

82 **Adachi T**, Tajima Y, Kuroki T, Mishima T, Kitasato A, Fukuda K, Tsutsumi R, Kanematsu T. Bile-reflux into the pancreatic ducts is associated with the development of intraductal papillary carcinoma in hamsters. *J Surg Res* 2006; **136**: 106-111

83 **Ghadirian P**, Simard A, Baillargeon J, Maisonneuve P, Boyle P. Nutritional factors and pancreatic cancer in the francophone community in Montréal, Canada. *Int J Cancer* 1991; **47**: 1-6

84 **Binstock M**, Krakow D, Stamler J, Reiff J, Persky V, Liu K, Moss D. Coffee and pancreatic cancer: an analysis of international mortality data. *Am J Epidemiol* 1983; **118**: 630-640

85 **Wynder EL**, Mabuchi K, Maruchi N, Fortner JG. Epidemiology of cancer of the pancreas. *J Natl Cancer Inst* 1973; **50**: 645-667

86 **Larsson SC**, Wolk A. Meat consumption and risk of colorectal cancer: a meta-analysis of prospective studies. *Int J Cancer* 2006; **119**: 2657-2664

87 **Butler LM**, Wang R, Koh WP, Stern MC, Yuan JM, Yu MC. Marine n-3 and saturated fatty acids in relation to risk of colorectal cancer in Singapore Chinese: a prospective study. *Int J Cancer* 2009; **124**: 678-686

88 **Drasar BS**, Irving D. Environmental factors and cancer of the colon and breast. *Br J Cancer* 1973; **27**: 167-172

89 **Knox EG**. Foods and diseases. *Br J Prev Soc Med* 1977; **31**: 71-80

90 **Miller AB**, Howe GR, Jain M, Craib KJ, Harrison L. Food items and food groups as risk factors in a case-control study of diet and colo-rectal cancer. *Int J Cancer* 1983; **32**: 155-161

91 **McKeown-Eyssen GE**, Bright-See E. Dietary factors in colon cancer: international relationships. *Nutr Cancer* 1984; **6**: 160-170

92 **Willett W**. The search for the causes of breast and colon cancer. *Nature* 1989; **338**: 389-394

93 **Willett WC**, Stampfer MJ, Colditz GA, Rosner BA, Speizer FE. Relation of meat, fat, and fiber intake to the risk of colon cancer in a prospective study among women. *N Engl J Med* 1990; **323**: 1664-1672

94 **Hursting SD**, Thornquist M, Henderson MM. Types of dietary fat and the incidence of cancer at five sites. *Prev Med* 1990; **19**: 242-253

95 **Newmark HL**, Yang K, Lipkin M, Kopelovich L, Liu Y, Fan K, Shinozaki H. A Western-style diet induces benign and malignant neoplasms in the colon of normal C57Bl/6 mice. *Carcinogenesis* 2001; **22**: 1871-1875

96 **Newmark HL**, Yang K, Kurihara N, Fan K, Augenlicht LH, Lipkin M. Western-style diet-induced colonic tumors and their modulation by calcium and vitamin D in C57Bl/6 mice: a preclinical model for human sporadic colon cancer. *Carcinogenesis* 2009; **30**: 88-92

97 **Yang K**, Kurihara N, Fan K, Newmark H, Rigas B, Bancroft L, Corner G, Livote E, Lesser M, Edelmann W, Velcich A, Lipkin M, Augenlicht L. Dietary induction of colonic tumors in a mouse model of sporadic colon cancer. *Cancer Res* 2008; **68**: 7803-7810

98 **Reddy BS**, Hanson D, Mangat S, Mathews L, Sbaschnig M, Sharma C, Simi B. Effect of high-fat, high-beef diet and of mode of cooking of beef in the diet on fecal bacterial enzymes and fecal bile acids and neutral sterols. *J Nutr* 1980; **110**: 1880-1887

99 **Hill MJ**. Bile flow and colon cancer. *Mutat Res* 1990; **238**: 313-320

100 **Cheah PY**. Hypotheses for the etiology of colorectal cancer--an overview. *Nutr Cancer* 1990; **14**: 5-13

101 **Hill MJ**, Drasar BS, Hawksworth G, Aries V, Crowther JS, Williams RE. Bacteria and aetiology of cancer of large bowel. *Lancet* 1971; **1**: 95-100

102 **Hill MJ**, Taylor AJ, Thompson MH, Wait R. Fecal steroids and urinary volatile phenols in four Scandinavian populations. *Nutr Cancer* 1982; **4**: 67-73

103 **Crowther JS**, Drasar BS, Hill MJ, Maclennan R, Magnin D, Peach S, Teoh-chan CH. Faecal steroids and bacteria and large bowel cancer in Hong Kong by socio-economic groups. *Br J Cancer* 1976; **34**: 191-198

104 **Reddy BS**, Wynder EL. Large-bowel carcinogenesis: fecal constituents of populations with diverse incidence rates of colon cancer. *J Natl Cancer Inst* 1973; **50**: 1437-1442

105 **Reddy BS**, Hedges AR, Laakso K, Wynder EL. Metabolic epidemiology of large bowel cancer: fecal bulk and constituents of high-risk North American and low-risk Finnish population. *Cancer* 1978; **42**: 2832-2838

106 **Jensen OM**, MacLennan R, Wahrendorf J. Diet, bowel function, fecal characteristics, and large bowel cancer in Denmark and Finland. *Nutr Cancer* 1982; **4**: 5-19

107 **Lee DH**, Anderson KE, Harnack LJ, Folsom AR, Jacobs DR Jr. Heme iron, zinc, alcohol consumption, and colon cancer: Iowa Women's Health Study. *J Natl Cancer Inst* 2004; **96**: 403-407

108 **Giovannucci E**, Martínez ME. Tobacco, colorectal cancer, and adenomas: a review of the evidence. *J Natl Cancer Inst* 1996; **88**: 1717-1730

109 **Crowley-Weber CL**, Dvorakova K, Crowley C, Bernstein H, Bernstein C, Garewal H, Payne CM. Nicotine increases oxidative stress, activates NF-kappaB and GRP78, induces apoptosis and sensitizes cells to genotoxic/xenobiotic stresses by a multiple stress inducer, deoxycholate: relevance to colon carcinogenesis. *Chem Biol Interact* 2003; **145**: 53-66

110 **Casellas F**, Mourelle M, Papo M, Guarner F, Antolin M, Armengol JR, Malagelada JR. Bile acid induced colonic irritation stimulates intracolonic nitric oxide release in humans. *Gut* 1996; **38**: 719-723

111 **Venturi M**, Hambly RJ, Glinghammar B, Rafter JJ, Rowland IR. Genotoxic activity in human faecal water and the role of bile acids: a study using the alkaline comet assay. *Carcinogenesis* 1997; **18**: 2353-2359

112 **Booth LA**, Gilmore IT, Bilton RF. Secondary bile acid induced DNA damage in HT29 cells: are free radicals involved? *Free Radic Res* 1997; **26**: 135-144

113 **Washo-Stultz D**, Crowley-Weber CL, Dvorakova K, Bernstein C, Bernstein H, Kunke K, Waltmire CN, Garewal H, Payne CM. Role of mitochondrial complexes I and II, reactive oxygen species and arachidonic acid metabolism in deoxycholate-induced apoptosis. *Cancer Lett* 2002; **177**: 129-144

114 **Washo-Stultz D**, Hoglen N, Bernstein H, Bernstein C, Payne CM. Role of nitric oxide and peroxynitrite in bile salt-induced apoptosis: relevance to colon carcinogenesis. *Nutr Cancer* 1999; **35**: 180-188

115 **Payne CM**, Weber C, Crowley-Skillicorn C, Dvorak K, Bernstein H, Bernstein C, Holubec H, Dvorakova B, Garewal H. Deoxycholate induces mitochondrial oxidative stress and activates NF-kappaB through multiple mechanisms in HCT-116 colon epithelial cells. *Carcinogenesis* 2007; **28**: 215-222

116 **Longpre JM**, Loo G. Protection of human colon epithelial cells against deoxycholate by rottlerin. *Apoptosis* 2008; **13**: 1162-1171

117 **Dall'Agnol M**, Bernstein C, Bernstein H, Garewal H, Payne CM. Identification of S-nitrosylated proteins after chronic exposure of colon epithelial cells to deoxycholate. *Proteomics* 2006; **6**: 1654-1662

118 **Craven PA**, Pfanstiel J, DeRubertis FR. Role of reactive oxygen in bile salt stimulation of colonic epithelial proliferation. *J Clin Invest* 1986; **77**: 850-859

119 **Bernstein H**, Holubec H, Bernstein C, Ignatenko N, Gerner E, Dvorak K, Besselsen D, Ramsey L, Dall'Agnol M, Blohm-Mangone KA, Padilla-Torres J, Cui H, Garewal H, Payne CM. Unique dietary-related mouse model of colitis. *Inflamm Bowel Dis* 2006; **12**: 278-293

120 **Wang L**, Baudhuin LM, Boardman LA, Steenblock KJ, Petersen GM, Halling KC, French AJ, Johnson RA, Burgart LJ, Rabe K, Lindor NM, Thibodeau SN. MYH mutations in patients with attenuated and classic polyposis and with young-onset colorectal cancer without polyps. *Gastroenterology* 2004; **127**: 9-16

121 **Jenkins MA**, Croitoru ME, Monga N, Cleary SP, Cotterchio M, Hopper JL, Gallinger S. Risk of colorectal cancer in monoallelic and biallelic carriers of MYH mutations: a population-based case-family study. *Cancer Epidemiol Biomarkers Prev* 2006; **15**: 312-314

122 **Kulkarni MS**, Yielding KL. DNA damage and repair in epithelial (mucous) cells and crypt cells from isolated colon. *Chem Biol Interact* 1985; **52**: 311-318

123 **Pool-Zobel BL**, Leucht U. Induction of DNA damage by risk factors of colon cancer in human colon cells derived from biopsies. *Mutat Res* 1997; **375**: 105-115

124 **Payne CM**, Crowley C, Washo-Stultz D, Briehl M, Bernstein H, Bernstein C, Beard S, Holubec H, Warneke J. The stress-response proteins poly(ADP-ribose) polymerase and NF-kappaB protect against bile salt-induced apoptosis. *Cell Death Differ* 1998; **5**: 623-636

125 **Rosignoli P**, Fabiani R, De Bartolomeo A, Fuccelli R, Pelli MA, Morozzi G. Genotoxic effect of bile acids on human normal and tumour colon cells and protection by dietary antioxidants and butyrate. *Eur J Nutr* 2008; **47**: 301-309

126 **Booth LA**, Bilton RF. Genotoxic potential of the secondary bile acids: a role for reactive oxygen species. In: Arouma OI, Halliwell B, eds. DNA and free radicals: Techniques, mechanisms and applications. London: OICA International, 1998: 161-177

127 **Powolny A**, Xu J, Loo G. Deoxycholate induces DNA damage and apoptosis in human colon epithelial cells expressing either mutant or wild-type p53. *Int J Biochem Cell Biol* 2001; **33**: 193-203

128 **Glinghammar B**, Inoue H, Rafter JJ. Deoxycholic acid causes DNA damage in colonic cells with subsequent induction of caspases, COX-2 promoter activity and the transcription factors NF-kB and AP-1. *Carcinogenesis* 2002; **23**: 839-845

129 **Romagnolo DF**, Chirnomas RB, Ku J, Jeffy BD, Payne CM, Holubec H, Ramsey L, Bernstein H, Bernstein C, Kunke K, Bhattacharyya A, Warneke J, Garewal H. Deoxycholate, an endogenous tumor promoter and DNA damaging agent, modulates BRCA-1 expression in apoptosis-sensitive epithelial cells: loss of BRCA-1 expression in colonic adenocarcinomas. *Nutr Cancer* 2003; **46**: 82-92

130 **Qiao D**, Im E, Qi W, Martinez JD. Activator protein-1 and CCAAT/enhancer-binding protein mediated GADD153 expression is involved in deoxycholic acid-induced apoptosis. *Biochim Biophys Acta* 2002; **1583**: 108-116

131 **Scott DW**, Mutamba S, Hopkins RG, Loo G. Increased GADD gene expression in human colon epithelial cells exposed to deoxycholate. *J Cell Physiol* 2005; **202**: 295-303

132 **Narahara H**, Tatsuta M, Iishi H, Baba M, Uedo N, Sakai N, Yano H, Ishiguro S. K-ras point mutation is associated with enhancement by deoxycholic acid of colon carcinogenesis induced by azoxymethane, but not with its attenuation by all-trans-retinoic acid. *Int J Cancer* 2000; **88**: 157-161

133 **Payne CM**, Bernstein C, Dvorak K, Bernstein H. Hydrophobic bile acids, genomic instability, Darwinian selection, and colon carcinogenesis. *Clinical and Experimental Gastroenterology* 2008; **1**: 19-47

134 **Bernstein C**, Bernstein H, Payne CM, Garewal H. DNA repair/pro-apoptotic dual-role proteins in five major DNA repair pathways: fail-safe protection against carcinogenesis. *Mutat Res* 2002; **511**: 145-178

135 **Payne CM**, Bernstein H, Bernstein C, Garewal H. Role of apoptosis in biology and pathology: resistance to apoptosis in colon carcinogenesis. *Ultrastruct Pathol* 1995; **19**: 221-248

136 **Samaha HS**, Bernstein C, Payne CM, Garewal HS, Sampliner RE, Bernstein H. Bile salt induction of apoptosis in goblet cells of the normal human colonic mucosa: relevance to colon cancer. *Acta Microsc* 1995; **4**: 43-58

137 **Garewal H**, Bernstein H, Bernstein C, Sampliner R, Payne C. Reduced bile acid-induced apoptosis in "normal" colorectal mucosa: a potential biological marker for cancer risk. *Cancer Res* 1996; **56**: 1480-1483

138 **Bernstein C**, Bernstein H, Garewal H, Dinning P, Jabi R, Sampliner RE, McCuskey MK, Panda M, Roe DJ, L'Heureux L, Payne C. A bile acid-induced apoptosis assay for colon cancer risk and associated quality control studies. *Cancer Res* 1999; **59**: 2353-2357

139 **Bernstein H**, Holubec H, Warneke JA, Garewal H, Earnest DL, Payne CM, Roe DJ, Cui H, Jacobson EL, Bernstein C. Patchy field defects of apoptosis resistance and dedifferentiation in flat mucosa of colon resections from colon cancer patients. *Ann Surg Oncol* 2002; **9**: 505-517

140 **Hague A**, Elder DJ, Hicks DJ, Paraskeva C. Apoptosis in colorectal tumour cells: induction by the short chain fatty acids butyrate, propionate and acetate and by the bile salt

deoxycholate. *Int J Cancer* 1995; **60**: 400-406
141 **Marchetti MC**, Migliorati G, Moraca R, Riccardi C, Nicoletti I, Fabiani R, Mastrandrea V, Morozzi G. Possible mechanisms involved in apoptosis of colon tumor cell lines induced by deoxycholic acid, short-chain fatty acids, and their mixtures. *Nutr Cancer* 1997; **28**: 74-80
142 **Milovic V**, Teller IC, Faust D, Caspary WF, Stein J. Effects of deoxycholate on human colon cancer cells: apoptosis or proliferation. *Eur J Clin Invest* 2002; **32**: 29-34
143 **Martinez JD**, Stratagoules ED, LaRue JM, Powell AA, Gause PR, Craven MT, Payne CM, Powell MB, Gerner EW, Earnest DL. Different bile acids exhibit distinct biological effects: the tumor promoter deoxycholic acid induces apoptosis and the chemopreventive agent ursodeoxycholic acid inhibits cell proliferation. *Nutr Cancer* 1998; **31**: 111-118
144 **Powell AA**, LaRue JM, Batta AK, Martinez JD. Bile acid hydrophobicity is correlated with induction of apoptosis and/or growth arrest in HCT116 cells. *Biochem J* 2001; **356**: 481-486
145 **Yui S**, Saeki T, Kanamoto R, Iwami K. Characteristics of apoptosis in HCT116 colon cancer cells induced by deoxycholic acid. *J Biochem* 2005; **138**: 151-157
146 **Yui S**, Kanamoto R, Saeki T. Deoxycholic acid can induce apoptosis in the human colon cancer cell line HCT116 in the absence of Bax. *Nutr Cancer* 2008; **60**: 91-96
147 **Crowley CL**, Payne CM, Bernstein H, Bernstein C, Roe D. The NAD+ precursors, nicotinic acid and nicotinamide protect cells against apoptosis induced by a multiple stress inducer, deoxycholate. *Cell Death Differ* 2000; **7**: 314-326
148 **Im E**, Martinez JD. Ursodeoxycholic acid (UDCA) can inhibit deoxycholic acid (DCA)-induced apoptosis via modulation of EGFR/Raf-1/ERK signaling in human colon cancer cells. *J Nutr* 2004; **134**: 483-486
149 **Zhou J**, Liu M, Zhai Y, Xie W. The antiapoptotic role of pregnane X receptor in human colon cancer cells. *Mol Endocrinol* 2008; **22**: 868-880
150 **Washo-Stultz D**, Crowley C, Payne CM, Bernstein C, Marek S, Gerner EW, Bernstein H. Increased susceptibility of cells to inducible apoptosis during growth from early to late log phase: an important caveat for in vitro apoptosis research. *Toxicol Lett* 2000; **116**: 199-207
151 **Katona BW**, Anant S, Covey DF, Stenson WF. Characterization of enantiomeric bile acid-induced apoptosis in colon cancer cell lines. *J Biol Chem* 2009; **284**: 3354-3364
152 **Haza AI**, Glinghammar B, Grandien A, Rafter J. Effect of colonic luminal components on induction of apoptosis in human colonic cell lines. *Nutr Cancer* 2000; **36**: 79-89
153 **Schlottman K**, Wachs FP, Krieg RC, Kullmann F, Schölmerich J, Rogler G. Characterization of bile salt-induced apoptosis in colon cancer cell lines. *Cancer Res* 2000; **60**: 4270-4276
154 **Wachs FP**, Krieg RC, Rodrigues CM, Messmann H, Kullmann F, Knüchel-Clarke R, Schölmerich J, Rogler G, Schlottmann K. Bile salt-induced apoptosis in human colon cancer cell lines involves the mitochondrial transmembrane potential but not the CD95 (Fas/Apo-1) receptor. *Int J Colorectal Dis* 2005; **20**: 103-113
155 **Payne CM**, Waltmire CN, Crowley C, Crowley-Weber CL, Dvorakova K, Bernstein H, Bernstein C, Holubec H, Garewal H. Caspase-6 mediated cleavage of guanylate cyclase alpha 1 during deoxycholate-induced apoptosis: protective role of the nitric oxide signaling module. *Cell Biol Toxicol* 2003; **19**: 373-392
156 **Holubec H**, Payne CM, Bernstein H, Dvorakova K, Bernstein C, Waltmire CN, Warneke JA, Garewal H. Assessment of apoptosis by immunohistochemical markers compared to cellular morphology in ex vivo-stressed colonic mucosa. *J Histochem Cytochem* 2005; **53**: 229-235
157 **Badvie S**, Hanna-Morris A, Andreyev HJ, Cohen P, Saini S, Allen-Mersh TG. A "field change" of inhibited apoptosis occurs in colorectal mucosa adjacent to colorectal adenocarcinoma. *J Clin Pathol* 2006; **59**: 942-946
158 **Fang C**, Dean J, Smith JW. A novel variant of ileal bile acid binding protein is up-regulated through nuclear factor-kappaB activation in colorectal adenocarcinoma. *Cancer Res* 2007; **67**: 9039-9046
159 **Crowley-Weber CL**, Payne CM, Gleason-Guzman M, Watts GS, Futscher B, Waltmire CN, Crowley C, Dvorakova K, Bernstein C, Craven M, Garewal H, Bernstein H. Development and molecular characterization of HCT-116 cell lines resistant to the tumor promoter and multiple stress-inducer, deoxycholate. *Carcinogenesis* 2002; **23**: 2063-2080
160 **Bree RT**, Neary C, Samali A, Lowndes NF. The switch from survival responses to apoptosis after chromosomal breaks. *DNA Repair* (Amst) 2004; **3**: 989-995
161 **Roos WP**, Kaina B. DNA damage-induced cell death by apoptosis. *Trends Mol Med* 2006; **12**: 440-450
162 **Plesca D**, Mazumder S, Almasan A. DNA damage response and apoptosis. *Methods Enzymol* 2008; **446**: 107-122
163 **Matés JM**, Segura JA, Alonso FJ, Márquez J. Intracellular redox status and oxidative stress: implications for cell proliferation, apoptosis, and carcinogenesis. *Arch Toxicol* 2008; **82**: 273-299
164 **Payne CM**, Crowley-Skillicorn C, Holubec H, Dvorak K, Bernstein C, Moyer MP, Garewal H, Bernstein H. Deoxycholate, an endogenous cytotoxin/genotoxin, induces the autophagic stress-survival pathway: Implications for colon carcinogenesis. *J Toxicol* 2009; Epub ahead of print
165 **Schreck R**, Albermann K, Baeuerle PA. Nuclear factor kappa B: an oxidative stress-responsive transcription factor of eukaryotic cells (a review). *Free Radic Res Commun* 1992; **17**: 221-237
166 **Azad MB**, Chen Y, Gibson SB. Regulation of autophagy by reactive oxygen species (ROS): implications for cancer progression and treatment. *Antioxid Redox Signal* 2009; **11**: 777-790
167 **Wang Q**, Gao F, May WS, Zhang Y, Flagg T, Deng X. Bcl2 negatively regulates DNA double-strand-break repair through a nonhomologous end-joining pathway. *Mol Cell* 2008; **29**: 488-498
168 **Makishima M**, Okamoto AY, Repa JJ, Tu H, Learned RM, Luk A, Hull MV, Lustig KD, Mangelsdorf DJ, Shan B. Identification of a nuclear receptor for bile acids. *Science* 1999; **284**: 1362-1365
169 **Parks DJ**, Blanchard SG, Bledsoe RK, Chandra G, Consler TG, Kliewer SA, Stimmel JB, Willson TM, Zavacki AM, Moore DD, Lehmann JM. Bile acids: natural ligands for an orphan nuclear receptor. *Science* 1999; **284**: 1365-1368
170 **Wang H**, Chen J, Hollister K, Sowers LC, Forman BM. Endogenous bile acids are ligands for the nuclear receptor FXR/BAR. *Mol Cell* 1999; **3**: 543-553
171 **Modica S**, Murzilli S, Salvatore L, Schmidt DR, Moschetta A. Nuclear bile acid receptor FXR protects against intestinal tumorigenesis. *Cancer Res* 2008; **68**: 9589-9594
172 **Makishima M**, Lu TT, Xie W, Whitfield GK, Domoto H, Evans RM, Haussler MR, Mangelsdorf DJ. Vitamin D receptor as an intestinal bile acid sensor. *Science* 2002; **296**: 1313-1316
173 **Xie W**, Radominska-Pandya A, Shi Y, Simon CM, Nelson MC, Ong ES, Waxman DJ, Evans RM. An essential role for nuclear receptors SXR/PXR in detoxification of cholestatic bile acids. *Proc Natl Acad Sci USA* 2001; **98**: 3375-3380
174 **Staudinger JL**, Goodwin B, Jones SA, Hawkins-Brown D, MacKenzie KI, LaTour A, Liu Y, Klaassen CD, Brown KK, Reinhard J, Willson TM, Koller BH, Kliewer SA. The nuclear receptor PXR is a lithocholic acid sensor that protects against liver toxicity. *Proc Natl Acad Sci USA* 2001; **98**: 3369-3374

S- Editor Tian L **L- Editor** O'Neill M **E- Editor** Lin YP