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MINIREVIEWS

Genetic and environmental determinants of risk for cholangiocarcinoma in Thailand

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Abstract

Cholangiocarcinoma (CCA) is a difficult cancer to diagnose in the early stage and to treat by curative resection. The incidence of CCA in the northeast of Thailand is the highest in the world. To make progress in detecting a high risk group and in the prevention and detection of CCA, we have been analyzing the risk factors for CCA. Although liver fluke infection is known to be a risk factor, there are patients who are not infected with the liver fluke and not all people infected with the liver fluke will suffer from the disease. Therefore, it is of the utmost importance to analyze the risk factors and the mechanism to prevent the disease and also to detect the disease in its early stage to save patients' lives. Through collaboration among Thai and Japanese researchers, we analyzed the genetic and environmental determinants of risks for CCA. Also, we have been trying to develop methods to detect the disease in a non-invasive way. Without repeating findings reported in various reviews on CCA, we will first discuss the environmental and genetic determinants of the risks for CCA. Second, we will discuss the properties of CCA, including the etiological agents and the mechanism of cholangiocarcinogenesis, and finally, we will discuss future approaches to prevent and cure CCA from the standpoint of evidence-based medicine. We will discuss these points by including the data from our laboratories. We would like to emphasize the importance of the genetic data, especially whole genome approaches, to understand the properties of CCA, to find a high risk population for CCA and to develop effective preventative methods to stop the carcinogenic steps toward CCA in the near future. In addition, it is of the upmost importance to develop a non-invasive, specific and sensitive method to detect CCA in its early stage for the application of modern medical approaches to help patients with CCA.

Key words: Alcohol drinking; Cholangiocarcinoma; DNA polymorphism; Glutathione S transferase; 8-oxoguanine

glycosylase 1; Liver fluke; *Opisthorchis viverrini*; Thailand

Core tip: Cholangiocarcinoma (CCA) is an intractable cancer due to the difficulty of diagnosis in its early stage. The incidence of CCA in the northeast of Thailand is the highest in the world. It is of the utmost importance to analyze the risk factors and the mechanism to prevent the disease and to also detect the disease in its early stage to save patients' lives. We analyzed the genetic and environmental determinants of risks for CCA and discussed this with the findings already published by other researchers. It is of the utmost importance to develop a non-invasive, specific and sensitive method to detect CCA.

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INTRODUCTION

The age standardized rates (world ASR) of the incidence of liver and bile duct cancer in Thailand between 2001 and 2003 are 38.6 and 14.6 for men and women respectively. Most remarkably, world ASR of liver and bile duct cancer in Udon Thani, Khon Kaen, Nakorn Phanom, Ubon Ratchathani, Bangkok and Songkhla provinces for men are 115.0, 87.7, 78.4, 74.9, 21.5 and 10.9, respectively, and for women are 52.7, 36.3, 43.2, 34.7, 6.4 and 2.9, respectively. Cholangiocarcinoma (CCA) among the liver and bile duct cancer in the above provinces for men are 80.5%, 81.1%, 55.9%, 81.0%, 32.6% and 33.3%, respectively, and for women are 86.7%, 82.3%, 60.8%, 76.6%, 56.7% and 43.5%, respectively[1]. Thus, the incidence of CCA in the northeast of Thailand is extremely high in comparison to the rest of the world.

It was previously reported that the liver fluke, *Opisthorchis viverrini* (OV), and endogenous nitrosamines are the important risk factors for CCA in Thailand[2,3]. Multiple pathways on the tumorigenic OV infection to cause CCA from Thailand are nicely summarized in the recent review[4].

ENVIRONMENTAL DETERMINANTS

From the epidemiological study, it was previously known that the infection of the liver fluke, OV, is an important risk factor of CCA[2] (Table 1). In addition to OV infection, some of the chemical carcinogens like nitrosamine are also suggested to be factors in the risk for CCA[3]. We performed a population-based case-control study in which sex, age and place of residence were matched individually. We confirmed that the presence of the antibody against OV significantly increased the risk for CCA; odds ratio (OR) = 27.09 [95% confidence interval (CI): 6.30-116.57]. The results confirmed the previously reported data by Parkin *et al*[2]. In addition, we found that alcohol drinking is another risk factor for CCA. Ex-regular and regular alcohol drinkers showed OR = 6.23 (95%CI: 1.23-31.57) and OR = 4.31 (95%CI: 1.12-16.57), respectively (Table 1)[5]. We examined the possibility that alcohol consumption affects the risk for CCA due to OV infection, as well as smoking and dietary habits during the past 10 years, and found only the risks due to smoking and eating fermented fish (*pla-ra* and/or *pla-chao*) were altered with alcohol consumption (P for interaction < 0.01 and 0.07, respectively). The interactions between alcohol drinking and selected variables are shown in Table 2. The odds ratios are slightly different from those appearing in our previous paper[5] due to a typing error although the conclusion is materially the same. The increased risk for CCA due to ever-smoking was more prominent among ever-drinkers than among never-drinkers and a similar observation was made for the risk by eating *pla-ra* and/or *pla-chao*. Conversely, vitamin C was suggested to reduce the risk[3]. Recently, Songserm *et al*[6] confirmed that alcohol drinking increased the risk for CCA and they reported that the consumption of fruits and vegetables decreased the risk for CCA (Table 1). Manwong *et al*[7] also reported that a family history of cancer was a significant risk factor (Table 1).

INTERACTION BETWEEN GENETIC AND ENVIRONMENTAL DETERMINANTS

Since not all patients with CCA are infected with OV and not all individuals infected by OV develop CCA, it is possible that some other environmental and genetic determinants are involved in the pathogenesis of CCA. We examined the genetic polymorphism on the risk for CCA. We first examined the effect of carcinogen detoxification enzyme gene polymorphisms, namely *GSTM1* and *GSTT1*, which are well-known. DNA polymorphism of *GSTM1* or *GSTT1* alone was not associated with the risk of CCA. However, the null genotype of *GSTM1* enhanced OR of the risk for CCA in anti-OV antibody positive subjects was 18.00 (95%CI: 3.33-97.40) compared to that of *GSTM1* wild in anti-OV antibody positive subjects of 10.34 (95%CI: 1.31-81.63) and the null genotype of *GSTT1* enhanced OR in ex-regular alcohol drinkers was OR = 27.93 (95%CI: 1.84-424.60) compared to that of *GSTT1* wild in ex-regular drinkers of OR = 1.28 (95%CI: 0.12-14.08)[5].

Songserm *et al*[6] analyzed methylenetetrahydrofolate reductase gene polymorphism (*MTHFR*) at 677 and at 1298 for interaction with beef sausage consumption (Table 3). They found that *MTHFR*677 TT variants and *MTHFR*1298 CC variants showed increased risks when the individuals ate beef sausage daily. The data attained by the above researchers which showed an interaction are listed in Table 3.

Table 1 Effects of environmental determinants on risks for cholangiocarcinoma

Environmental determinants		Cases	Controls	OR	95%CI		*P* value	Ref.	Ethnic group
					LL	UL			
Anti-OV Ab	ref: < 1/40	101 matched case-control pairs		5.00	2.30	11.00	< 0.001	Parkin *et al*[2] 1991	Thai
				Adjusted OR					
Anti-OV Ab (ELISA)	< 0.200	61	119	1.00	Reference			Honjo *et al*[5] 2005	Thai
	⩾ 0.200	65	8	27.09	6.30	116.57	< 0.01		
Alcohol drinking	Never	30	46	1.00	Reference		-		
	Occasional	41	54	2.20	0.65	7.45	0.21		
	Ex-regular	15	7	6.23	1.23	31.57	0.03		
	Regular	41	21	4.31	1.12	16.57	0.03		
	Missing	2	-	-	-	-	-		
Raw fish	0	30	57	1.00	Reference				
	< 2/mo	54	41	2.70	1.28	5.68	< 0.01		
	⩾ 2/mo	45	31	2.94	1.24	6.96	0.01		
Fermented fish or pork	0	28	41	1.00	Reference				
	< 2/mo	58	63	2.95	0.98	8.90	0.06		
	⩾ 2/mo	43	25	4.50	1.30	15.54	0.02		
				Adjusted OR					
Alcohol drinking	Non-drinker	57	254	1.00	Reference			Songserm *et al*[6] 2012	Thai
(Units of alcohol per month)	< 14	79	92	5.60	2.85	10.95	< 0.001		
	⩾ 14	83	92	9.50	4.55	19.79	< 0.001		
Total vegetables (average times/ month)	< 52	136	214	1.00	Reference				
	⩾ 52	83	224	0.40	0.23	0.76	0.004		
Total fruits (average times/ month)	< 35	131	217	1.00	Reference				
	⩾ 35	88	221	0.60	0.33	0.98	0.04		
Family history of cancer	No	85	107	1.00	Reference			Manwong *et al*[7] 2013	Thai
	Yes	38	16	4.34	1.80	10.43	0.001		

OR: Odds ratio; CI: Confidence interval; LL: Lower limit; UL: Upper limit.

EFFECTS OF GENETIC DETERMINANTS AND DNA POLYMORPHISM ON RISK FOR CCA

There are several reports of the effects of DNA polymorphisms on the risk of CCA. Among various enzymes involved in carcinogen metabolism, CYP1A2, one of the phase I enzymes in the activation of such a carcinogen in cigarette smoke, has a DNA polymorphism. *CYP1A2* polymorphism, found in intron 1, might be involved in the risk of CCA. Prawan *et al*[8] found that *CYP1A2*1A/*1A* polymorphism had a protective effect on the risk of CCA in men but not in women (Table 4). Since men smoke more than women in Thailand, it is considered that in the individuals with *CYP1A2*1A* polymorphism, the CYP1A2 enzyme might be less inducible compared to that with *CYP1A2*1F*, although the effect of these mutations on the induction of the enzyme is not clear.

Arylamine *N*-acetyltransferase (NAT) catalyzes *N*- and *O*-acetylation of various arylamines and heterocyclic amines, thereby regulating the metabolic activation and detoxification of xenobiotics and carcinogens. Individuals with three *NAT2* alleles, *NAT2*13*, **6B* and **7A*, are associated with a decreased risk for CCA, while those with *NAT2*4, *5, *6A* and **7B* were not, suggesting that the *NAT2* polymorphism may modify the risk of CCA (Table 4)[8].

Glutathione *S*-transferases (GSTs), a family of Phase II detoxifying enzymes, can conjugate reduced glutathione to various compounds. Concerning polymorphism of *GSTO1* and *GSTO2*, Marahatta *et al*[9] found that individuals with *GSTO1*D140* had a significantly increased risk for CCA, hepatocellular carcinoma and breast cancer (Table 4). A study with a larger sample size will better clarify the function of GSTO1.

Natural killer cell receptor G2D (NKG2D) haplotypes were found to be associated with the natural cytotoxic activity of individuals. NKG2D triggers cell-mediated cytotoxicity in natural killer cells. Various NKG2D haplotype alleles showed a significant difference between cases and controls[10]. Primary sclerosing cholangitis (PSC) is an inflammatory bowel disease suggested to be a predisposing disease to hepatobiliary malignancy.

Table 2 Effect of modification of alcohol drinking on relationships between smoking, eating fermented fish and risk for cholangiocarcinoma

Variable	Category	Alcohol drinking							
		Never drinkers				Ever[1] drinkers			
		Adjusted[2] OR	95%CI		*P* value	Adjusted[2] OR	95% CI		*P* value
			LL	UL			LL	UL	
Smoking	Never	1	Reference			4.25	1.02	17.63	0.05
	Occasional	4.36	0.4	47.49	0.23	1.07	0.06	20.66	0.96
	Ex-regular					9.09	1.27	65.18	0.03
	Regular	3.64	0.19	71.41	0.39	7.99	1.56	40.94	0.01
Pla-ra,	< 3/d	1	Reference			14.07	1.46	135.36	0.02
Pla-chao	≥ 3/d	12.34	1.22	124.75	0.03	20.88	2.27	192.06	< 0.01

[1]Including occasional, ex- and currently regular drinkers; [2]Adjusted for anti-OV Ab when calculating the OR of smoking, and adjusted for anti-OV Ab and smoking when calculating the OR of eating of fermented fish (pla-ra and/or pla-chao). Nakorn Phanom (Thailand): based on the conditional logistic regression model. CI: Confidence interval; LL: Lower limit; UL: Upper limit; OR: Odds ratio. Adapted from Honjo *et al*[5] 2005. Allowing for absence of control subject in the category for occasional smoking and absence of case subject in the category for ex-regular smoking among never drinkers, we combined these two categories and confirmed the conclusion in the table is the materially unchanged from that in the table in our previous paper (Honjo *et al*[5] 2005).

Table 3 Interaction between genetic and environmental determinants on risks for cholangiocarcinoma

Genetic determinants		Environmental determinants		OR	95%CI		*P* value	Ref.	Ethnic group
					LL	UL			
				Adjusted OR					
GSTMI	Wild	Anti-OV antibody	Negative	1.00	Reference			Honjo *et al*[5] 2005	Thai
	Wild		Positive	10.34	1.31	81.63	0.03		
	Null		Negative	0.48	0.21	1.11	0.09		
	Null		Positive	18.00	3.33	97.40	< 0.01		
GSTMI	Wild	Toilet	Inside the house	1.00	Reference				
	Wild		Outside or none	0.20	0.04	1.02	0.05		
	Null		Inside the house	0.22	0.06	0.88	0.03		
	Null		Outside or none	0.25	0.07	0.91	0.04		
GSTTI	Wild	Alcohol drinking	Never	1.00	Reference				
	Wild		Occasional	3.58	0.71	17.95	0.12		
	Wild		Ex-regular	1.28	0.12	14.08	0.84		
	Wild		Regular	4.69	0.93	23.51	0.06		
	Null		Never	0.75	0.23	2.43	0.63		
	Null		Occasional	1.12	0.22	5.80	0.89		
	Null		Ex-regular	27.93	1.84	424.60	0.02		
	Null		Regular	3.28	0.35	30.91	0.30		
				Crude OR					
MTHFR 677	CC	Beef sausage	< 1/mo	1.0	Reference			Songserm *et al*[6] 2012	Thai
	CT		< 1/mo	1.1	0.51	2.37	0.82		
	TT		< 1/mo	0.6	0.25	1.53	0.32		
	CC		Weekly	0.9	0.45	1.83	0.80		
	CT		Weekly	1.2	0.57	2.43	0.65		
	TT		Weekly	1.6	0.80	3.31	0.18		
	CC		Daily	3.3	1.51	7.07	0.003		
	CT		Daily	3.2	1.33	7.62	0.01		
	TT		Daily	8.3	2.23	30.82	0.002		
MTHFR 1298	AA	Beef sausage	< 1/mo	1.0	Reference				
	AC		< 1/mo	1.3	0.63	2.55	0.51		
	CC		< 1/mo	0.8	0.28	2.15	0.63		
	AA		Weekly	1.3	0.71	2.45	0.39		
	AC		Weekly	1.0	0.49	1.79	0.84		
	CC		Weekly	3.8	1.48	9.89	0.01		
	AA		Daily	3.8	1.71	8.62	0.001		
	AC		Daily	3.5	1.56	7.85	0.002		
	CC		Daily	18.3	3.68	90.80	< 0.001		

Thirteen percent of patients with primary sclerosing cholangitis developed CCA[11]. When NKG2D single nucleotide polymorphisms (SNPs) were compared between the PSC patients with CCA and the PSC patients

Table 4 Effects of genetic determinants on risks for cholangiocarcinoma

Genotype		No. CCA (%)	No. control (%)	OR	95%CI		*P* value	Ref.	Ethnic group
					LL	UL			
				Adjusted OR					
CYP1A2, Male	*1F/*1F	85 (57.4)	88 (51.2)	1.0	Reference			Prawan *et al*[8] 2005	Thai
	*1A/*1F	59 (39.9)	69 (40.1)	0.9	0.55	1.47	0.677		
	*1A/*1A	4 (2.7)	15 (8.7)	0.28	0.08	0.94	0.039		
NAT2	All, except *6B, *7A and *13	193 (89.4)	162 (69.5)	1.0	Reference				
	One or two alleles (All, except *6B, *7A and *13)	23 (10.6)	71 (30.5)	0.26	0.15	0.44	< 0.001		
				Crude OR					
GST01	A140/A140	13 (43.33)	26 (86.67)	1.0	Reference			Marahatta *et al*[9] 2006	Thai
	A140/D140 + D140/D140	17 (56.67)	4 (13.33)	0.86	2.07	37.85			
		Minor allele frequency							
	Alleles	PSC[2] with CCA (n = 49)	PSC without CCA (n = 316)	OR			Corrected P		
NKG2D[1]	rs11053781 (Intron 5) G *vs* A	0.66	0.49	2.08	1.31	3.29	0.011	Melum *et al*[12] 2008	Norwegian
	rs2617167 (Intron 1) A *vs* G	0.39	0.22	2.32	1.47	3.66	0.002		
		PSC with CCA (n = 49)	Healthy controls (n = 368)						
	rs11053781 (Intron 5) G *vs* A	0.66	0.5	1.95	1.23	3.07	0.021		
	rs2617167 (Intron 1) A *vs* G	0.39	0.23	2.2	1.40	3.44	0.003		
		Counts (frequencies) of alleles/genotypes							
		2n = 120	2n = 146	Crude OR				Hoeblinger *et al*[13] 2009	Caucasian
MRP2/ ABCC2[3]	*ABCC2 c.3972* C (exon 28, synonymous SNP)	73 (0.61)	108 (0.74)						
	ABCC2 c.3972 T	47 (0.39)	38 (0.26)	1.83	1.087	3.08	0.022		
				OR					
MYH rs3219476	T/T	25 (42.4)	26 (26.0)	1.0	Reference			You *et al*[14] 2013	Han Chinese
	T/G	20 (33.9)	58 (58.0)	0.359	0.17	0.758	0.006		
	G/G	14 (23.7)	16 (16.0)	0.91	0.369	2.246	0.838		
	T/G + G/G	34 (57.6)	74 (74.0)	0.478	0.241	0.946	0.033		
MYH rs3219472	G/G	28 (47.5)	46 (46.0)	1.0	Reference				
	G/A	19 (32.2)	47 (47.0)	0.664	0.326	1.351	0.258		
	A/A	12 (20.3)	7 (7.0)	2.816	0.992	7.999	0.047		
	G/A + A/A	31 (52.5)	54 (54.0)	0.943	0.495	1.797	0.859		

[1]Natural killer cell receptor G2D; [2]Primary sclerosing cholangitis; [3]Multidrug resistance-associated protein 2 gene. OR: Odds ratio.

without CCA in a Norwegian population, there was significantly increased allele frequencies in two SNPs, namely rs11053781 and rs11053781, both of which are non-coding. The odds ratio for G *vs* A in the rs11053781 was 2.08 (95%CI: 1.31-3.29) and that for A *vs* G in rs2617167 was 2.32 (95%CI: 1.47-3.66). When they were compared between PSC patients with CCA and healthy controls, there was also a significant increase of allele frequencies in the above two SNPs. The odds ratio for G *vs* A in the rs11053781 was 1.95 (95%CI: 1.23-3.07) and that for A *vs* G in rs2617167 was 2.20 (95%CI: 1.40-3.44) (Table 4)[12]. The functional role of the changes of these SNPs on the susceptibility to CCA remains to be elucidated.

Multidrug resistance-associated protein 2 (MRP2/ABCC2), one of the ATP-binding cassette transporter proteins, is suggested to be involved in the excretion of the conjugates of carcinogens into bile, a metabolic step classified as so called "Phase Ⅲ metabolism". Thus, it might play an important role in cellular defense against toxic substances. The frequency of the *c.3972C > T ABCC2* gene variant (synonymous SNP) was compared between patients with CCA and healthy individuals.

Table 5 Interaction among genetic determinants on risks for cholangiocarcinoma

Genetic determinant		Genetic determinant		OR	95%CI		*P* value	Ref.	Ethnic group
					LL	UL			
MTHFR C677T[1]	CC	*TSER*[2]	*2R* (-)	1	Reference			Ko *et al*[17] 2006	South Korean
	CC		*2R* (+)[3]	5.38	1.23	23.56	0.026		
	CT		*2R* (-)	1.08	0.68	1.07			
	CT		*2R* (+)[3]	1.19	0.71	2.01			
	TT		*2R* (-)	1.02	0.7	1.5			
	TT		*2R* (+)[3]	1.24	0.9	1.71			
hOGG1(*Codon326*)	Ser/Ser	*GSTM1*	wild	1	Reference			Zeng *et al*[18] 2013	Thai
	Ser/Ser + Cys/Cys		wild	0.06	0.01	0.54	0.01		
	Ser/Ser		null	0.06	0.01	0.53	0.01		
	Ser/Ser + Cys/Cys		null	0.14	0.02	1.08	0.06		

[1]5,10-Methylenetetrahydrofolate reductase; [2]Thymidylate synthase enhancer region; [3]Including 2R2R and 2R3R.

There was a significant association between the SNP and the risk in a Caucasian population (Table 4)[13].

The DNA repair mechanism is protecting DNA damage caused by various kinds of carcinogenic factors. Among them, base excision repair (BER) plays an important role in the oxidative DNA damage caused by reactive oxygen species. MutY homolog, MYH, is involved in BER and functions as a DNA glycosylase which removes adenine paired with 8-hydroxy-2'-deoxyguanine residue. Individuals with T/G genotype in MYH rs3219476 had a reduced risk (OR = 0.478, 95%CI: 0.17-0.758, $P = 0.006$). Individuals with A/A genotype in MYHrs3219472 had an increased risk (OR = 2.816, 95%CI: 0.992-7.999, $P = 0.047$) (Table 4)[14].

Concerning other variants or mutations related to the risk for CCA, a mutation in bile salt export pump (ABCB11) was found in two children with progressive familial intrahepatic cholestasis and cholangiocarcinoma[15]. Biliary papillomatosis is considered to be a premalignant lesion with a high probability to develop to CCA, although the genetic changes have not been clarified[16].

INTERACTION AMONG GENETIC DETERMINANTS

Susceptibility to cancer might be regulated not only by one gene or one environmental determinant. Thus, interaction of genetic determinants could easily be imagined in regulating various cellular processes. However, there are few reports on the interaction among genetic determinants. Ko *et al*[17] reported the interaction of polymorphisms of 5,10-methylenetetrahydrofolate reductase (MTHFR *C677T*) and thymidylate synthase enhancer region (TSER) and the risk for CCA in a South Korean population (Ko *et al*[17] 2006). MTHFR is involved in the pathway of folate metabolism and DNA methylation. Thymidylate synthase (TS) catalyzes the formation of dTMP from dUMP, an important step for production of dTTP for use in DNA synthesis. Both TS and MTHFR use the common substrate 5,10-methylanetetrahydrofolate and might affect DNA synthesis and repair. Therefore, the interaction between *MTHFR C677T* and *TSER* polymorphisms were analyzed. Ko *et al*[17] found that the individuals with *MTHFR 677CC* with *TSER 2R(+)* genotypes (2R2R, 2R3R, 2R5R) showed an increased risk for CCA compared to *677CC* with *TSER 2R(-)* genotypes (3R3R, 3R4R, 3R5R) ($P = 0.0257$) (Table 5)[17]. There was no association between *MTHFR C677T* polymorphism or *TSER* polymorphism alone and the risk for CCA.

Human 8-oxoguanine glycosylase 1 (hOGG1) is involved in the repair of 8-hydroxy-2'deoxyguanine residue in oxidatively damaged DNA, one of the most mutagenic lesions among base modification produced by reactive oxygen species. While polymorphisms of DNA repair enzymes, including hOGG1 (codon 326), XRCC1 (codon 194, 280 and 399) and PARP1 (codon 762), alone had no association with the risk for CCA[18], there is a significant interaction between hOGG1 and *GSTM1* polymorphisms for the risk for CCA. When *GSTM1* polymorphism was considered, the hOGG1 codon 326 polymorphism was related to the decreased risk: OR = 1.00 (reference), OR = 0.06 (95%CI: 0.01-0.53), OR = 0.06 (95%CI: 0.01-0.54) and OR = 0.14 (95%CI: 0.02-1.08) for subjects with hOGG1 Ser/Ser and *GSTM1* wild, ones with Ser/Ser and *GSTM1* null, ones with Ser/Cys or Cys/Cys and *GSTM1* wild, and ones with Ser/Cys or Cys/Cys and *GSTM1* null, respectively (P for interaction < 0.01) (Table 5). Although the effect of hOGG1 polymorphism is not clear when amino acid Ser 326 is changed to Cys, the DNA repair capacity might decrease. However, the above data showed the decreased risk of CCA. It could be considered that if DNA repair capacity is inhibited when relatively abundant DNA damage is present in the presence or absence of *GSTM1* enzyme, the cells would die before malignant transformation[18]. Kim *et al*[19] reported that hOGG1 326 Cys/Cys genotypes were associated with lowered risk of bladder cancer occurrence and recurrence in South Korean subjects, while hOGG1 326 Ser/Cys genotype was a risk factor. The protective effect of *GSTM1* null variant could be

due to the slow metabolism caused by *GSTM1* deficiency of some dietary materials, such as isothiocyanates contained in cruciferous vegetables, known to be a chemopreventive compound. The protective effects of *GSTM1* null variant were reported in breast carcinoma[20] and hepatocellular carcinoma[21]. The concerted action of a DNA-repair enzyme and *GSTM1* on the risk for CCA should give a new insight in understanding the mechanism of the carcinogenesis of CCA.

ETIOLOGICAL AND ENHANCING AGENTS FOR CHOLANGIOCARCINOGENESIS AND THEIR PATHOGENICITY

Concerning the etiological agents for CCA, epidemiological studies implicated various chemicals and occupational risks. One of the examples is thorium dioxide (thorotrast) used for radiological examination[22]. Animal experiments showed that *N*-nitrosodimethylamine could induce CCA in the Syrian Golden hamster[23]. Although OV infection alone did not induce CCA, the OV infection enhanced CCA production by *N*-nitrosodimethylamine in the hamsters[24,25]. Actually, a small amount of nitrosamine was detected in the food[4]. Quite recently, 1, 2-dichloropropane and/or dichloromethane used in the color proof-printing factory were considered to be the etiological agents from a precise epidemiological study in Japan[26]. Other than the liver fluke, viral infections like hepatitis B and C virus infections are also related to the increased risk for CCA[27].

There have been many findings on the abnormalities of gene expression caused by the reorganization of the genome through endogenous and environmental factors in many types of cancers[28]. It is also true for CCA that many genetic changes are found in CCA. One of the examples from our laboratory is the mutation of the tumor suppressor protein genes, *p16Ink4/CDKN2* and *p15Ink4B/MTS2*[29]. However, the precise mechanisms of cholangiocarcinogenesis are not well clarified. We have been using a hamster model of cholangiocarcinoma and found that a molecule, protein kinase A regulatory subunit 1 α (Prkar1a), is overexpressed in the cholangiocarcinoma tissues[30,31]. *PRKAR1A* gene overexpression is also found in humans and this is associated with production of extracellular protein kinase A (ECPKA), especially its catalytic subunit (PRKACA)[31], as found in prostate cancer. Although the function of the extracellular protein kinase A is not clear, it might contribute to the development of cancer cells[32].

The precise mechanism of liver fluke infection causing CCA (cholangiocarcinogenesis) is also not known. OV produces mechanical injury to the biliary epithelia by attachment with suckers, inflammation caused by OV and mitogenic factors secreted by OV to help the biliary epithelial cells transform to CCA[33]. In particular, TGF-β and EGF signal transduction pathways are indicated as the possible pathways of OV-induced cell proliferation of fibroblasts[34]. It could be speculated that CCA-associated fibroblasts induce tumor progression of the initiated epithelium, as found in human prostate epithelium[35]. This would be a novel target for chemoprevention and treatment of fibrosis in CCA which might delay the formation of CCA. Gene expression profile of OV infection-related CCA and non-OV associated CCA was reported by Jinawath *et al*[36]. Enhanced expression of RAD51 associating protein-1 was also involved in the growth of CCA cells[37]. These genes upregulated in CCA would be expected to serve as diagnostic and therapeutic targets for CCA. The up-to-date findings of the mechanism of tumorigenesis by OV infection and the prevention of OV infection, including the education and trial for vaccine development against OV, is reviewed by Sripa *et al*[4].

FUTURE PROSPECTS FOR PREVENTION AND EARLY DETECTION OF CCA

The present work is intended to analyze the effects of environmental and genetic determinants on the risk of CCA and to know the mechanisms of CCA to prevent the disease. At the same time, it is also important to detect the disease during its early phase so that medical intervention could possibly prevent the death of patients with cholangiocarcinoma. Therefore, the method to detect the high risk population and patients with cholangiocarcinoma using a non-invasive procedure is quite important. To find the possible tumor marker of CCA, we use the sera and label the compounds with fluorescent chemicals to try and find a certain compound found in the serum of patients with cholangiocarcinoma. One of our preliminary results showed that a new peak (named peak B) was found in 50% of CCA patients but in 6.3% in normal individuals[38]. In addition, Loilome *et al*[31] found that in liver fluke-associated CCA, PRKR1A overexpression is associated with an increased extracellular PKA autoantibody. The antibody titers in the sera from patients with CCA (0.154 ± 0.077), adenocarcinoma (0.150 ± 0.061) and OV infected individuals with fibrosis (0.157 ± 0.045) were significantly higher than that in healthy control subjects (0.129 ± 0.028), while there was no significant difference between the sera from OV infected individuals without fibrosis (0.139 ± 0.053) and that of healthy control subjects[31]. Recently, Matsuda *et al*[39] found the *Wisteria floribunda* agglutinin-positive mucin 1 and the L1 cell adhesion molecule[40] were sensitive biliary biomarkers for CCA. Silsirivanit *et al*[41] reported a novel Lewis a associated carbohydrate epitope, CA-S27, as a diagnostic and prognostic biomarker for CCA.

Although the prognosis of CCA is not good, there are several reports on the relationship of genetic changes and the prognosis of patients with CCA. One example would be with the classical comparative genomic hybridization studies. It was suggested that amplification of the D22S283 region of the chromosome was a favorable prognostic marker[42].

With recent rapid advancement of DNA sequencing technology, it becomes possible to analyze the whole genome sequence relatively less expensively. Therefore, it should be possible to search the responsible chromosomal region involved in the genetic determinants of the risk for CCA and the progression or inhibition of the growth of CCA in more detail. With the technology of genomics, proteomics and glycobiology, one can expect to find the high risk population for CCA more easily, to help the population better adjust their lifestyles for prevention of CCA and to detect the patients with CCA in its early phase.

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