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Genetic polymorphisms of xenobiotic metabolizing enzymes in human colorectal cancer

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It was proposed that both hereditary and environmental factors contribute to the development of colorectal cancer (CRC). Carcinogenic polycyclic aromatic hydrocarbons (PAHs) from food or tobacco smoke can form DNA adducts and thus initiate carcinogenesis after metabolic activation via cytochrome P4501A1 (CYP1A1). Intermediate metabolites are detoxified by conjugation with glutathione S-transferases. Our aim was to look for inherited metabolic susceptibility to CRC. We used PCR- based genotyping approach to determine the frequencies of polymorphic alleles of two cytochromes P450 (CYP2D6 and CYP1A1) and two glutathione S-transferases (GSTM1 and GSTT1) in DNA samples from 31 sporadic, 25 familial CRC cases and 73 healthy controls. The difference in frequencies of poor metabolisers due to CYP2D6 gene polymorphism was close to the limit of statistical significance between sporadic CRC and healthy control group ($\chi^2 = 5.52$, $m=2$, $p=0.06$) despite the small sample size. The frequencies of either CYP1A1 MspI, GST M1 or GST T1 genotypes were not significantly different in both CRC cases and in controls. Although our study suggests some difference in metabolic susceptibility between sporadic and familial CRC, further studies are needed to investigate the combined effect of polymorphic genes involved in carcinogen metabolism in a larger group of patients with defined exposure to dietary carcinogens and smoking.

Key words: colorectal neoplasms-genetics; polymorphism (genetics); cytochrome P-450CYP1A1; cytochrome P-450 CYP2D6; gluthatione transferases

Introduction

It is generally accepted that both hereditary and environmental factors contribute to the development of colorectal cancer (CRC). A genetic model for colorectal tumorigenesis suggested by Faeron and Vogelstein proposed that colorectal tumors arise due to mutations in oncogenes and tumor suppressor genes.[1] The sequences of adenoma to carcinoma transition are well established.[2] However, the earliest events in human colorectal tumor formation are not well defined. As far as environmental factors are concerned a large proportion of human cancers is known to be caused by chemicals from the environment.[3]

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A strong association has been observed between colorectal cancer and consumption of broiled and grilled meat. Exposure of food to pyrolysis can lead to the formation of compounds such as polycyclic aromatic hydrocarbons (PAHs), heterocyclic amines and others.[4] In addition to diet, tobacco smoking was also associated with colorectal cancer.[5] It is possible that PAHs in food exposed to pyrolysis and in tobacco smoke can play a role in some of the genetic alterations leading to colorectal tumor development.[5]

Most environmental chemicals have to be metabolically activated to their ultimate carcinogenic form and individual differences in metabolism of precarcinogenic substances exist due to polymorphisms in many enzymes involved in this process. So, after sufficient exposure to carcinogen individual differences in its metabolism may result in different genetic susceptibility to the development of cancer.[3]

Carcinogens as well as other xenobiotics are usually metabolized in two consecutive steps. Phase I is an activation step and phase II is the detoxification step. Enzymes from the cytochrome P450 families 1, 2 and 3 (CYP1, CYP2 and CYP3 respectively) are the main enzymes involved in the oxidative activation of chemical compounds into reactive electrophilic form.[3] Among others, CYP1A1 is involved in activation of variety of chemical carcinogens to their ultimate carcinogenic forms, particularly PAHs from tobacco smoke and PAHs and aromatic amines from food exposed to pyrolysis. CYP2D6 is involved in metabolism of many drugs and yet undefined carcinogens from tobacco smoke.[6] In the detoxification reaction the activated substance is conjugated with some organic acid or intracellular glutathione to form inactive water-soluble metabolites. This reactions are mainly catalyzed by N-acetyl transferases (NATs) and glutathione S-transferases (GSTs). There are four known mammalian classes of soluble GSTs: α, μ, π and τ, which allow for a broad overlapping substrate specificity. Among them, GST μ and τ are polymorphic and as such of interest in molecular epidemiological studies. GST μ is expressed in the liver, leukocytes, colon and other tissues, but it is absent in approximately half of the population due to a deletion (GSTM1*null allele). It catalyses conjugation of epoxides with glutathione and may thus protect individuals against chemical mutagens or carcinogens, among others also carcinogens from tobacco smoke. Although the predominant role of GST is in detoxification, certain glutathione conjugation reactions, particularly those involving halogenated alkanes and alkenes can result in the formation of reactive electrophiles that are toxic and carcinogenic.[6]

Initiation of tumorigenesis by a chemical compound *in vivo* requires at least three successive reactions: First, metabolic conversion of chemically inert compounds to a reactive electrophilic form has to occur. This is an obligatory initiation step in chemical carcinogenesis. Next, adducts have to form between these reactive metabolites and DNA, resulting in base changes or DNA rearrangements. Finally, these DNA alterations must be fixed and must lead to oncogene activation. Enhanced activity of phase I enzymes or decreased activity of phase II enzymes result in higher amount of activated carcinogen and increased formation of DNA adducts.[3] This mechanism implies that the presence and activity of relevant xenobiotic metabolizing enzymes contribute to the risk of cancer development.[3] However, genetic susceptibility factor only becomes relevant if sufficient exposure to carcinogen occurs. Risk factors for tumor development include the extent of environmental exposure to a procarcinogen as well as the host susceptibility factor due to the polymorphism of drug metabolizing enzymes involved in the formation of activated carcinogen.

In our study we wished to find out if

genetic polymorphism of xenobiotic metabolism represents a risk factor for CRC. The frequency of polymorphic alleles of two cytochrome P450 dependent monooxygenases (CYP1A1 and CYP2D6) and two glutathione S-transferases (GST M1 and GST T1), supposed to be associated with higher cancer risk, were determined in DNA samples from Slovenian sporadic and familial CRC patients and healthy controls.

Materials and methods

DNA samples from 31 sporadic and 25 familial CRC patients were analyzed. Seventy-three DNA samples from 107 healthy controls previously analyzed for CYP2D6[7] were included in the study. No clinical data regarding patient characteristics, the localization or stage of tumor or epidemiological data considering exposure were obtained at this stage of the study.

PCR- based genotyping approach was used to determine the frequencies of polymorphic alleles of two cytochrome P450-dependent monooxygenases (CYP2D6 and CYP1A1) and two glutathione S-transferases (GSTM1 and GSTT1).

PCR followed by restriction with BstNI enzyme was used to distinguish between the wild type allele (CYP2D6wt) and deficiency allele CYP2D6B.[8] Allele specific PCR with two pairs of primers was used to distinguish between the wild type allele and deficiency allele CYP2D6A.[9]

The polymorphism in 3' flanking region of CYP1A1 gene was analyzed by PCR and restriction with MspI.[10]

Triplex PCR was used to analyze for GST M1 and T1 null alleles. Conserved beta globin primers were used as internal control.[11]

Results and discussion

The frequencies of CYP2D6 wt, A and B alleles were 90.9 %, 7.6 % and 1.5 % in sporadic CRC cases and 80.8 %, 19.2 % and 0 % in familial CRC cases. The frequencies of CYP2D6 alleles in the healthy control population studied previously were as follows: CYP2D6A 1 %, CYP2D6B 21 %, and CYP2D6wt 78 % of all alleles.[7] CYP2D6 phenotype frequencies in CRC patients and in controls are presented in Table 1. The phenotype was predicted according to the genotype.[12] Individuals homozygous for two deficiency alleles were considered poor (slow) metabolizers (PM), heterozygotes for one deficiency allele were considered heterozygous extensive metabolizers (HEM) and homozygotes for two wild type alleles were considered extensive metabolizers (EM). As presented in Table 1, no PM of debrisoquine was identified in sporadic CRC cases, while 8 % of familial CRC and 7 % of healthy controls were identified as PM. The difference in PM frequencies between sporadic CRC and healthy control group is close to the limit of statistical significance (χ^2 = 5.52, m=2, p=0.06) despite the small sample size. There

Table 1. CYP2D6 phenotype frequencies in CRC cases and controls

	Cases (number)	CYP2D6 EM	CYP2D6 HEM	CYP2D6 PM
CRC total	56	0.75	0.21	0.04
- sporadic	31	0.84	0.16	0.00
- familial	25	0.64	0.28	0.08
Controls	107	0.63	0.31	0.07

Table 2. CYP1A1 MspI genotype frequencies in CRC cases and controls

	Cases (number)	CYP1A1 A	CYP1A1 B	CYP1A1 C
CRC total	56	0.86	0.12	0.02
- sporadic	31	0.84	0.13	0.03
- familial	25	0.88	0.12	0.00
Controls	73	0.81	0.18	0.01

was no statistically significant difference in CYP2D6 phenotype distribution between familial CRC cases and healthy controls.

CYP1A1 MspI genotype frequencies in CRC patients and in controls are presented in Table 2. The predominant genotype in both CRC cases as well as healthy controls is genotype A which indicates homozygotes for two wild type alleles in which the MspI restriction site is absent. Genotype B indicates heterozygotes for one wild type allele and one allele with mutation creating MspI restriction site. Genotype C which indicates homozygotes for two alleles with MspI restriction site present is very rare in both CRC cases and in controls. It was shown that CYP1A1 inducibility and enzymatic activity is increased in individuals with genotype C.[10] Genotype C frequency seemed to be slightly higher in sporadic CRC cases than in familial CRC cases or controls, however, the difference in frequencies of CYP1A1 MspI genotypes was not statistically significant (χ^2 = 1.67, m=4, p=0.80).

GST M1 and GST T1 genotype frequencies in CRC patients and in controls are presented in Table 3. GST M1 and GST T1 positive genotype indicates individuals homozygous or heterozygous for the presence of GST M1 or GST T1 gene respectively, while the null genotype indicates individuals homozygous for the deletion of GST M1 or GST T1 gene respectively. The frequencies of GST M1 as well as GST T1 genotypes were not significantly different in both CRC cases and controls.

On the basis of our results we can conclude that inherited metabolic susceptibility to carcinogens from the environment seems to be higher in sporadic than in familial CRC. Polymorphic CYP2D6 gene probably has some role in colorectal carcinogenesis in our population. Enhanced metabolic activation of PAHs was observed in CYP1A1 MspI C genotype[10] but its frequency in Slovenian population is probably to low to contribute significantly to colorectal tumor formation. It is interesting that this polymorphism was related to an increased risk for in situ CRC in the Japanese and Hawaiians, but not in the Caucasians.[13] The discrepancy between the above and our study may be due to a low frequency of CYP1A1 susceptibility allele in our as well as in other Caucasian populations, which limits the statistical power of analysis in small groups of patients. Another possible explanation is that PAHs may influence the earliest genetic alterations leading to colorectal tumorigenesis and their role may be masked in advanced disease. Our results also indicate that the polymorphic GST genes probably do not represent a significant risk factor for colorectal carcinogenesis.

However, we must keep in mind that cancer is a polygenic disease and the penetrance of any single gene is not sufficient to produce an observable effect. Colorectal cancer results from multiple mutations and it may be difficult to demonstrate a direct involvement of P450s and GSTs in colorectal cancer, especially in advanced disease. However, genetic differences in metabolism of carcino-

Table 3. GST M1 and GST T1 genotype frequencies in CRC cases and controls

	Cases (number)	GST M1 positive	GSTM1 null	GSTT1 positive	GSTT1 null
CRC total	56	0.50	0.50	0.79	0.21
- sporadic	31	0.52	0.48	0.81	0.19
- familial	25	0.48	0.52	0.76	0.24
Controls	73	0.48	0.52	0.78	0.22

gens may influence the earliest genetic alterations leading to colorectal tumorigenesis.

Although our study suggests a difference in metabolic susceptibility in sporadic and familial CRC, further studies are needed to investigate the combined effect of polymorphic genes involved in carcinogen metabolism in a larger group of patients with defined exposure to dietary carcinogens and smoking.

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