

Genetic polymorphism and their contribution to cancer susceptibility

Polimorfismo genético e sua contribuição na susceptibilidade ao câncer

Nívea Conforti-Froes ¹
Randa El-Zein ²
William Au ²

¹ Instituto de Biociências,
Letras e Ciências Exatas,
Universidade Estadual
Paulista.

Cristóvão Colombo 2265,
São José do Rio Preto,
São Paulo, SP
15054-000, Brasil.

² Department of Preventive
Medicine, University
of Texas-Medical
Branch of Galveston.
Ewing Hall, 700 Harborside
Drive, 77555-1110
Galveston, TX, USA.

Abstract *Since the majority of chemical carcinogens are not capable of causing hazardous effects per se, the metabolism of these compounds is a crucial part of the initial host response to the environmental exposure. Disturbances in the balance between activation and detoxification may thus explain the individual variations in responses to exposures to carcinogens. The amount of the ultimate carcinogen produced depends on the action of competing activation and detoxification pathways involving cytochrome P450 and glutathione-S-transferases enzymes.*

Key words *Polymorphism (Genetics); Neoplasm; Molecular Epidemiology*

Resumo *Uma vez que a maioria dos carcinogênicos químicos não é capaz de causar efeitos danosos per se, o metabolismo desses compostos é a parte crucial da resposta inicial à exposição ambiental. Os distúrbios causados no balanço entre os processos de ativação e destoxificação podem, assim, explicar as variações individuais em resposta à exposição aos carcinogênicos. A quantidade de compostos carcinogênicos finais produzida depende da ação competitiva entre os passos de ativação e destoxificação, envolvendo as enzimas do citocromo P450 e das S-glutatioão transferases.*

Palavras-chave *Polimorfismo (Genética); Neoplasia; Epidemiologia Molecular*

Introduction

Genetic factors in individual responses to environmental exposure

According to the recent advances in molecular epidemiology, an emerging new field that combines highly sensitive and specific techniques for detecting early damage associated with cancer, it has been possible to identify risks and prevent adverse health effects related to environmental exposures.

There are approximately 500,000 cancer-related deaths annually in the United States; as many as 80% of those deaths could be prevented due to the fact that most malignancies are a result of external factors rather than inherent biological conditions (Eubanks, 1994). By combining knowledge about external factors related to lifestyle and environmental or occupational exposure to chemicals with how genetic differences cause variations in human response to environmental pollutants, it will be possible to answer questions like why certain groups of people have a higher incidence of cancer after exposure to a toxicant and others do not. Molecular epidemiology is an emerging new field that combines highly sensitive molecular techniques for detecting early damage associated with cancer.

For this purpose, it is necessary to use biological markers as "*indicators signaling events in biological systems or samples*" (National Research Council, 1987:3). Usually, three types of biological markers are identified: the first is a biological marker of exposure, or exposure biomarker, that may be an exogenous compound (or a metabolite) within the body, an interactive product between the compound (or metabolite) and an endogenous component, or another event related to the exposure. The second, a marker for the effect, which may be an endogenous component, or a measure of the functional capacity, or some other indicator of state or balance of the body or organ system, as affected by the exposure. Such effect markers are generally preclinical indicators of abnormalities. The third is a marker of susceptibility, whether inherited or induced, which serves as an indicator in that individual is particularly sensitive to the effect of a xenobiotic or to the effects of a group of such compounds (Grandjean et al., 1994).

Among all biomarkers, the markers of susceptibility are particularly relevant to determining inherited predisposition to risk of adverse health effects as a result of exposure to environmental chemicals. The underlying prin-

ciple of this marker is the interindividual differences that confer sensitivity or resistance to environmentally induced disease. There are three types of susceptibility markers: the first is based on the fact that most chemicals are altered by enzymes and these alterations may increase or decrease the ability of a chemical to interact with tissue macromolecules such as DNA, RNA, or proteins. The balance between enzymes that activate and detoxify chemicals differs among individuals and ethnic groups. A second type of susceptibility marker reflects genetic differences in the capacity of cells to repair DNA damage caused by environmental insult. People deficient in DNA repair genes may exhibit more DNA damage manifested as increased levels of DNA adducts; alterations in chromosome number, structural chromosome modification, activated oncogenes and their protein products and higher incidence of cancer. A third type of susceptibility marker is pre-existing inherited genetic defects that increase the risk of cancer. If a person has inherited one or more of the necessary genetic alterations, fewer steps are needed for a chemical to cause cancer, putting this person at a greater risk. We will concentrate on the first one related to enzyme metabolism.

Susceptibility marker and enzyme metabolism

There is considerable variation among humans in the production of enzymes that either activate the formation of electrophilic metabolites that covalently bind to DNA or catalyze detoxification of chemical carcinogens (Harris, 1989; 1991).

Disturbances in the balance between activation and detoxification may thus explain the individual variations in responses to exposures to carcinogens (Hirvonen, 1995). In other words, the interindividual variation in response to xenobiotics and their potential carcinogenic effects is mediated by inherited predisposition (Shields, 1994). Certain individuals may be at significantly greater risk of chemically induced cancer than the average individual due to marked differences in the activation and detoxification processes.

Many of the enzymes involved in metabolic xenobiotic pathways have recently been shown to express genetic polymorphism in the population (Umeno et al., 1988; Idle, 1991; Hirvonen et al., 1993; Persson et al., 1993; Kato et al., 1994; Bois et al., 1995; Raunio et al., 1995; Kroemer & Eichelbaum, 1995).

The xenobiotic-metabolizing machinery contains two main types of enzymes: Phase I – mediating oxidative metabolism, and Phase II – conjugating enzymes. Many compounds are converted to reactive electrophilic metabolites by the oxidative Phase I enzymes, which are mainly cytochrome P-450 enzymes (CYPs). Phase II conjugating enzymes, such as glutathione-S-transferase (GST), UDP-glucuronosyltransferases and N-acetyltransferases (NAT), act usually as inactivating enzymes (Raunio et al., 1995).

The cytochrome P-450 gene superfamily carry out a myriad of diverse biotransformations including both anabolic and catabolic reactions (Wolf, 1986; Umeno et al., 1988). Due to the important role of cytochromes P450 in the metabolic activation of many procarcinogens, extensive research in the past has focused on the relationship between the distribution of polymorphic variants of different isozymes of P450 and cancer susceptibility. In this respect, three isozymes in particular have been studied: CYP1A1, CYP2D6, CYP2E1.

CYP genes and polymorphism

In recent years, increase attention has been focused on the possible relationship between interindividual differences in CYP gene structure and inducibility of CYP enzymes. This information has been used for the evaluation of increased susceptibility for cancer. As a matter of fact, many of the different isoforms of P450 activate precarcinogens and the interindividual differences in expression of P450 forms cause a pronounced variation in the level of the activated carcinogens in the cell.

It is not so long since it was believed that there were 2 cytochrome P450 enzymes, the methylcholanthrene-inducible P448 and the phenobarbitone-inducible P450. From cloned cDNA sequences, the deduced aminoacid sequences of some 57 P450 species have now been arrived at (Idle, 1991). This is also a likely explanation if polymorphically distributed variant forms of the isozyme, with different catalytic properties, occur within the population. P450 polymorphism in cancer susceptibility could also be lined to the fact that the P450 isozyme participates in the transformation of endogenous compounds, which are important during the process of differentiation of the initiated cell to the ultimate malignant stage. A genetic linkage between P450 and carcinogenesis might also be found at the level of inherited haplotypes, where an association could be related to linkage disequilibrium with

cancer-associated genes such as oncogenes and tumor suppressor genes, located in the same chromosomal region (Rannug et al., 1995).

• CYP1A

The CYP1A is the gene encoding P4501A1, a P450 isozyme that catalyzes the oxidation of polycyclic aromatic hydrocarbons (PAHs) to phenolic products and epoxides (Gonzales, 1989). The induction of CYP1A1 proceeds via binding of the inducer compound to the aryl hydrocarbon (Ah) receptor, and the liganded receptor activates transcription of several genes that encode proteins involved in xenobiotic metabolism.

The bioactivation of several PAHs has been shown to begin with stimulation of the aromatic hydrocarbon (Ah) receptor, which then activates CYP1A1, epoxide hydrolase, and other enzymes (Hirvonen, 1995). Beside PAHs, CYP1A1 is also inducible by xenobiotics found in cruciferous vegetables such as flavones, and by indole derivatives such as acid condensation products of indole-3-carbinol and photooxidized derivatives of tryptophan (McDonnell et al., 1992). Additionally, studies in mice and rats have indicated that UV light induces CYP1A1 in the skin and in the liver (Rannug et al., 1995).

The catalytic activities and the mode of induction of CYP1A1 appear to be very well conserved in higher animals, indicating that it has some important physiological function. The activity and inducibility pattern in humans of this enzyme, which forms DNA-reactive metabolites from PAH, is therefore of great importance when assessing the cancer risk to humans. CYP1A1 is considered to be primarily an extrahepatic enzyme in humans. It is induced in lung, in lymphocytes, and in placenta after exposure to PAHs and tobacco smoke (Antilla et al., 1991).

• CYP2D6

The genetic polymorphism related to the anti-hypertensive drug debrisoquine was the first one described associating the metabolism of drugs with expression of a P450 enzyme. The gene CYP2D6 encodes P450IID6 or debrisoquine 4-hydroxylase, sometimes referred to as P450dbl (Gonzales, 1995).

The CYP2D6 polymorphism is one of the most thoroughly studied human enzyme deficiencies. A genetic polymorphism in the CYP2D6 gene locus affects 5-10% of the Caucasian population and is responsible for impaired de-

brisoquine hydroxylase activity (Hirvonen, 1995). This polymorphism has conventionally been determined by the administration of a test drug (usually debrisoquine) and subsequent analysis of the urinary metabolic ratio. According to that, individuals have been classified as poor metabolizers (PMs) or extensive metabolizers (EMs) of debrisoquine (Idle, 1991). Using polymerase chain reaction (PCR)-based analysis together with the (restriction fragment length polymorphism (RFLP) analysis, the three most common defective alleles of CYP2D6 in Caucasians are CYP2D6A, a deletion of A2637 in exon 5 causing a frameshift mutation; CYP2D6B, a transition involving G1934 to A causing a splicing defect and CYP2D6D, where a deletion of the entire CYP2D6 functional gene has occurred (Rannug et al., 1995).

There is a pronounced interethnic difference in the distribution of these defect alleles, where the CYP2D6B and CYP2D6A alleles are almost exclusively present in the Caucasian population. By contrast, the CYP2D6D allele is more evenly distributed between black, Asian, and Caucasian populations.

Several studies have shown that the extensive metabolizer (EM) phenotype is associated with increased risk of various cancers, especially tobacco-smoke-induced lung cancer (Raunio et al., 1995). Recent genotyping studies have either confirmed (Hirvonen et al., 1993) or refuted (Rannug et al., 1995) such an association.

According to Raunio et al. (1995), the overrepresentation of CYP2D6 EMs among lung cancer patients can be explained in two ways: first, the CYP2D6 may mediate the activation of procarcinogenic agents present in tobacco smoke. The demonstration that CYP2D6 is capable of activating the tobacco-specific procarcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), directly supports this hypothesis. Second, the CYP2D6 gene may be in a linkage disequilibrium with the causative gene.

- CYP2E1

The cytochrome P4502E1 (CYP2E1) is a major ethanol-inducible P450 isozyme that metabolizes a wide variety of chemicals with different structures, particularly small and hydrophilic compounds (Persson et al., 1993). Today, more than 100 specific substrates for CYP2E1 have been identified, such as benzene, dimethylnitrosoamine, and vinyl chloride all of them potentially important carcinogens (Rannug et al., 1995). CYP2E1 is localized in several different tissues, among them the brain and lung, but

the highest expression is in the liver. The regional distribution of CYP2E1 correlates well to the areas where the hepatotoxic chemicals, known to be activated by CYP2E1, exert their toxic action (Persson et al., 1993).

Polymorphism of CYP2E1 gene has recently been shown to be one of the potential indicators of a cytochrome P450-associated susceptibility to cancer in man (Hirvonen et al., 1993). Genetic polymorphism, identified by RFLP using the restriction enzyme *Dra I*, *Pst I*, (*Rsa I*) and *Taq I* of CYP2E1 gene, have been shown to be associated with human cancer. In Japanese, the distribution of two *Dra I* genotypes among lung cancer cases is significantly different from that among controls and an association was found to exist between the amount of lifelong smoking exposure and the distribution of the *Dra I* genotypes among lung cancer patients. Subsequent studies have revealed profound ethnic differences in the frequencies of the polymorphic alleles. In a study involving Finnish lung cancer patients, homozygosity for the rare *Dra I* allele (minor, allele C) was found to be less frequent than in the Japanese population, and the distribution was similar among lung cancer patients and their controls (Hirvonen et al., 1993) which is in agreement with the results obtained by Persson et al. (1993).

Another CYP2E1 polymorphism is identified by RFLP, using *Pst I* (*Rsa I*) is also identified. This restriction site is located in the transcription region of the gene and might have a biological effect (Kato et al., 1994).

GSTs genes and polymorphism

The glutathione S-transferases (GSTs) are a family of multifunctional dimeric proteins which can conjugate electrophilic molecules with glutathione to render them less toxic and have the additional property of acting as binding proteins or ligandins in the liver (Idle, 1991). Carcinogens detoxified by GSTs include polycyclic aromatic hydrocarbons present in the diet and tobacco smoke.

Human GSTs have been grouped on the basis of their isoelectric points into alpha (basic) mu (near-neutral) and theta (acidic) forms. Members of the same class share 75-95% amino acid similarity, but between classes the identity drops to 25-30%. It appears that while all human livers express several alpha forms of GST, only about 50% of them express a mu isozyme (Mannervik et al., 1992).

Within the human mu class there is a specific isozyme GSTM1 that is frequently lacking in about 40-45% among Caucasians, i.e., they

inherit two deficient alleles and are homozygous for the null allele (Raunio et al., 1995). The GSTM1 activity is lacking in about 38-65% of different ethnic populations (Board et al., 1990).

As DNA adducts formation is inversely correlated with GSTM1 expression, individuals missing GSTM1 may have reduced capacity to detoxify reactive metabolites (Seidegard et al., 1990; Nakajima et al., 1995). Hirvonen et al. (1993) reported that the GSTM1 null (GSTM1 0/0) genotype is associated with increased risk of squamous cell carcinoma of the lung. Others found an association between adenocarcinoma and lack of GSTM1 expression (Seidegard et al., 1990; Bell et al., 1993). However, Brockmoller et al. (1993) did not find any significant correlation between GSTM1 genotypes and various types of lung cancer.

Individuals lacking GSTM1 could be at a greater risk for developing cancer due to deficient detoxification processes. There are, however, several putative confounding factors that are known to affect the phenotype, such as environmental exposures, nutrition and differences in smoking habits. Studies conducted by Hirvonen et al. (1993) with Finnish lung cancer patients showed that 62 % of individuals with squamous cell carcinoma of the lung were of the GSTM1 null genotype compared with 44% in the control population. Lack of the GSTM1 gene is also associated with susceptibility to bladder, skin and colon cancers (Raunio et al., 1995).

Nakajima et al. (1995) recently reported that patients lacking the GSTM1 gene have a less efficient pulmonary detoxification system than GSTM1 positive genotypes due to an overall low GST activity. Such individuals with the GSTM1 null genotype were also found to express significantly lower levels of the μ -class GSTM3-3 in the lung than individuals who possessed the GSTM1 gene. Antilla et al. (1991) reported that all GST enzymes are most abundant in the ciliated epithelium of the bronchi, and least in the distal airways. Further analysis of the lung tissues by high performance liquid chromatography demonstrated that GST subunits A1, A2, M1, M3 & P1 were present in varying amounts with the different individuals studied. The lack of GSTs (especially among GST null individuals) in the bronchial wall could favor the development of SCC which usually originates from the bronchial epithelium (Antilla et al., 1991). However, in view of the relatively high content of GSTP1 and GSTM3 and low content of GSTM1 in the lung, the influence of GSTM1 on susceptibility to lung

cancer probably originates in extrapulmonary compartments such as the liver where GSTM1 is the only active GST detected (Randerath et al., 1989). A considerable amount of PAHs from tobacco smoke passes into systemic blood to be activated or detoxified in extrapulmonary tissues, e.g. liver, and causes cancer in non-pulmonary tissues (Randerath et al., 1989). It is therefore reasonable to assume that more carcinogenic electrophiles enter the circulation from the liver in GSTM1 null individuals compared to GSTM1 +/+ individuals. This assumption is also consistent with the association of the GSTM1 null genotype with increased risk of other cancers such as the stomach, colon, urinary bladder and larynx (Lafuente et al., 1993; Anwar et al., 1996).

Glutathione S-transferase theta (GSTT1) is another polymorphic gene which is deleted in 38% of a European population (Pemble et al., 1994) and 20% in North Americans (Nelson et al., 1995). The deficiency is highly correlated with the inability to conjugate glutathione with small molecular weight toxicants. However, its association with the development of cancer has not been adequately investigated.

Since metabolism of chemicals requires the interactions with multiple genes, investigators have begun to document the inheritance of multiple polymorphic genes in the development of cancer (Raunio et al., 1995).

The frequency of the GSTT1 null genotype has not yet been studied extensively. Three studies have reported that the null allele frequency ranged between 20.4% to 38% depending on the size and ethnicity of the studied population (Pemble et al., 1994; El-Zein et al., 1997; Abdel-Rahman et al., 1996).

The association between the GSTT1 deletion and increased incidence of lung cancer can be explained in different ways. GSTT1 deficiency was shown to be responsible for increased induction of chromosomal damage by epoxide substrates such as ethylene oxide *in vitro* (Hallier et al., 1993).

Genetic predisposition and chromosome aberrations may be mechanistically related to the initiation of lung carcinogenesis. We proved that lung cancer patients missing one or the other GST (GSTM1 or GSTT1) genes were found to have significantly higher chromosome aberrations compared to lung cancer patients with both genes present (Conforti-Froes et al., 1997). According of that, individuals lacking one or both genes are expected to have increased body burden of these toxic metabolites. Another possible mechanism is that cigarette smoke is known to contain multiple chemicals

that are substrates for both GSTM1 and GSTT1 gene products. Therefore, the combined polymorphic deletion of the two genes is expected to interact positively to modify cancer risk.

Molecular epidemiology in Brazil

The molecular epidemiology in Brazil is a new field of expertise. Despite, a great interest has been emerged in scientific community. In our laboratory we are currently searching the link between some types of cancer and environmental exposures. To better understand that relationship, we must know the Brazilian distribution concerning the xenobiotic metabolizing genes, such as the CYPs and GSTs family. According of that, this is our first priority ongoing project we are concentrated in order to have those data soon. Along with, we are conducting two different studies regarding genetic susceptibility and environmental-cancer related.

One of them concerns the link between hepatocellular carcinoma (HCC) and the polymorphic activating and detoxifying genes. The HCC is the most frequent malignant tumor of the liver and the commonest cancer occurring in males in the world. The annual incidence of

the disease worldwide is estimated to be one million cases (Lau & Lai, 1990). There is a strong and specific association between chronic hepatitis B and C virus infection and HCC. Hepatic cirrhosis due to alcohol is another etiologic factor incriminated. However, genetic predisposition may also act to promote hepatocarcinogenesis, once heavy drinkers do not always develop alcoholic liver disease (Tsutsumi et al., 1994) and polymorphism of CYP2E1 may be related to liver disease incidence in Japanese population (Kato et al., 1995).

The other ongoing project concerns the study of carcinogen-metabolizing enzymes and skin cancer. The genetic factors that mediate the pathogenesis of skin tumors is unknown (Lear et al., 1996). Skin cancers are the most common type of human neoplasia. Excessive ultraviolet irradiation, with consequent reactive oxygen species attack on target molecules such as DNA, appears to be the major cause, although the effects of UV are complex and other factors, including soot, cigarette smoke, and arsenic are relevant (Heagerty et al., 1994). Consequently, polymorphism associated with impaired activation and detoxification may determine susceptibility to multiple skin cancers.

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