The Human Glutathione S-Transferase Supergene Family, Its Polymorphism, and Its Effects on Susceptibility to Lung Cancer

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Introduction

Considerable effort has been put into studies intended to determine the mechanisms whereby tobacco smoking brings about carcinogenesis in the lung, but much remains to be learned. The activity of enzymes that toxify and detoxify carcinogens known to be present in tobacco smoke has been an important area of study; recently, genetic polymorphisms in both the cytochrome P450 CYP2D6 (debrisoquine hydroxylase) (1-3) and one of the glutathione S-transferase isoenzymes (EC 2.5.1.18) (GSTM1 [GSTM1] (4-6)], have been implicated in susceptibility to the disease. This paper focuses on the GSTs and examines what is known of their possible roles in pulmonary and extrapulmonary tissues with respect to anticarcinogenesis in human lungs exposed to cigarette smoke.

Glutathione S-Transferases and Anticarcinogenesis

GSTs are multifunctional proteins that catalyze many reactions between glutathione (GSH) and lipophilic compounds with electrophilic centers, including those which are cytotoxic and genotoxic. When the reaction of GSH with an electrophile forms a stable covalent bond, the resultant adduct, referred to as a GSH conjugate, is usually no longer toxic and is excreted. All compounds that possess an electrophilic carbon, or acquire one by metabolism, form adducts by spontaneous reaction, at rates that
vary considerably depending on the nature of the electrophilic center (e.g., the second-order rate constant for the reaction of GSH at pH 7 with the cytoxton N-acetyl-l-p-benzoquinoneimine is 3 x 10^4 M^-1 s^-1, but that for the carcinogen metabolite 1-nitropyrene-4,5-oxide is only 0.17 M^-1 s^-1), GSTs catalyze many, but not all, of these reactions. These enzymes also catalyze GSH-dependent reactions that do not form stable adducts, such as hydroperoxide reduction and certain isomerizations, which might also be involved in tobacco smoke carcinogenesis during tumor promotion and tumor progression. These activities are not discussed in the present paper (7).

Electrophiles such as benzo[a]pyrene (B1 -7,8-diol-9,10-oxide (BPDE), which react with DNA to produce a carcinogenic lesion, have a slow spontaneous rate of reaction with GSH; however, if this rate is sufficiently enhanced by GST catalysis, the resulting reaction competes effectively with DNA adduct formation and GSTs are powerful anticarcinogens (7). The importance of GSH conjugation in anticarcinogenesis is illustrated by the case of aflatoxin B1 (AFB1) carcinogenesis in rat liver. AFB1 is metabolized to the electrophile AFB1-8,9-oxide, which reacts readily with DNA but is poorly conjugated at normal GST levels in the rat liver; with the result that AFB1 is a powerful rat hepatocarcinogen. Inducers of GST expression reduce AFB1 binding to very low levels, and AFB1-induced carcinogenesis is all but abolished (8). So far, evidence as direct as this is not available for other carcinogens: The effect of GSTs on carcinogenesis is usually inferred from the known enzymatic activities of purified enzymes and knowledge of their cellular concentrations.

Glutathione S-Transferase Supergene Family

In rats and humans, numerous soluble GSTs have been identified and shown to be dimers, the subunits of which are members of a supergene family composed of four multigene families, referred to as α, β, π, and θ. The most extensively studied GSTs are those of the rat, in which five α, five β, one π, and two θ class GST subunits have been identified by the combined efforts of a number of laboratories (7). As work progresses, the existence of a similar multiplicity of forms is becoming apparent among the human enzymes. The nomenclature is at present very confusing, but a rational system based on published guidelines for human gene nomenclature (9), similar to that used for the cytochrome P450 supergene family, was devised recently and agreed upon by many of the principal workers in the field. This system is used in the present paper (Table 1).

Many of the biologically significant substrates for GSTs are difficult to synthesize, are labile, and are not ideal for enzymatic assay, with the result that the supply of data is limited. Table 2 shows the values that have been obtained for BP-4,5-oxide (BPO), the GSH conjugate of which is a major excretory product of BP in the rat (10) and perhaps also in man; (+)-anti-PDE, which is the ultimate carcinogenic electrophile derived from metabolism of BP; and trans-stilbene oxide, which is used in studies of GSTM1-1 polymorphisms (7,24).

Distribution of Glutathione S-Transferase Isoenzymes in Human Lung and Liver

Tissues vary considerably in overall GST concentration and also have very specific distributions of GST subunits. Such quantitative and qualitative differences between tissues are presumably important in determining the susceptibility of a tissue to a carcinogen. Tables 3 and 4 address the situation seen in man, in whom the individual differences can be very great (7). Table 3 shows quantitative values obtained by high-performance liquid chromatography (HPLC), using the method of Ostlund Farrants et al. (25), for expression of GST subunits A1, A2, and M1 in a number of human liver samples (26). x

The absence of GST subunit M1 in a number of cases is noteworthy. It will be seen below that the GSTM1 null phenotype occurs in about 50% of the population. Table 4 shows the results of quantitative western blotting for method, see Towbin et al. (27) of 11 resected samples of human lung. Preliminary HPLC analyses indicated that A1 and A2 are detected in the α class GST subunits M1 and M3 in the μ class GST subunits, and P1 in the π class GST subunit.

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Table 1. Nomenclature for human glutathione S-transferases (GSTs).

| Previous designation for GST | New designation | References | Locus design | Chromosome | References |
|-----------------------------|-----------------|------------|--------------|-------------|------------|
| GST 1, 2 | GSTA1-1 | (11-13) | GSTA1 | 6 | (12) |
| GST2-1 | GSTA2 | (13,14) | GSTA2 | 6 | (12) |
| GST3-1 | GSTM1-1a | (15) | GSTM1-1 | 1 | (15) |
| GST4-1 | GSTM1-1b | (16) | GSTM1-1 | 1 | (15) |
| GST5 | GSTM2-1 | (17) | GSTM2 | 11 | (20) |
| GST6 | GSTM3-1 | (18,19) | GSTM3 | 11 | (20) |
| GST7 | GSTP1-1 | (21) | GSTP1 | 12 | (23) |

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*Available data on amino acid sequence demonstrate unequivocally the existence of a distinct class named θ, but until complete primary structure is known it will not be given a designation in the new system (21).
Lung Tissue and Lung Cancer

The two lobes of the lung begin at the end of the trachea, which divides into two bronchi; each of these extends and further divides into bronchioles, which undergo increasing ramification, ending in the terminal bronchioles, which open into the mass of alveoli. Proximally, the pseudostratified epithelium of the bronchi is lined with flattened ciliated cells, together with mucus-secreting goblet cells. Proceeding along the bronchiolar tree, the larger bronchioles have a simple columnar epithelium, which is also ciliated, while the smaller bronchioles have a cuboidal epithelium lacking cilia. Finally, in the alveoli, the principal cells are types I and II pneumocytes, the former providing...
most of the alveolar surface, as they are remarkably thin in transverse section, and the principal site of gaseous exchange with the pulmonary capillaries. The vascularization of the lung in this region is more extensive than that in any other part of the body. Type I cells are capable of the active pinocytosis of particles that reach their surface, resulting in their transfer to the interstitium. Both the interstitium and the lumen of the alveolus contain macrophages which engulf microorganisms and other particulates, such as those derived from tobacco smoke. Those that remain in the interstitium contribute to the dark lung color seen in smokers, while those that enter the alveolar lumen (millions per day) pass up and out of the lung by mucociliary action of the bronchi and upper bronchioles (28,29).

These are the major cellular components of the lung, but minor components should not be overlooked, such as the neuroepithelial APUD (amine uptake and decarboxylation) cells which accumulate and secrete biogenic amines and are believed to be the cells of origin of small-cell lung carcinoma. Cells of origin for squamous-cell carcinoma and adenocarcinoma, which are the most frequently occurring lung cancers, and of the rarer large-cell carcinomas have not been identified. It is important to note that all these cancers usually occur in the more proximal parts of the lung—hence the term bronchiogenic carcinoma. Both squamous-cell and goblet-cell metaplasia have been observed in the bronchi of smokers, but whether or not these are preneoplastic lesions has not been ascertained (30).

Activation of Carcinogens in the Lung

Although cancer is mostly associated with the bronchial region, it cannot be assumed that most carcinogens are removed and activated in the upper regions of the lungs and either do not reach the alveoli in sufficient quantity to be dangerous or are very effectively detoxified there. Levels of activating enzymes, such as aryl hydrocarbon hydroxylase and epoxide hydrolase, are present in similar amounts in bronchial and alveolar tissue in smokers (31), and 32P-postlabeling studies on bronchial and alveolar tissues from lungs of smokers show that the two regions of the lung have similar levels of DNA adducts (32).

Bronchoalveolar macrophages are also active in xenobiotic metabolism and contain aryl hydrocarbon hydroxylase, epoxide hydrolase, and GST. The macrophages engulf smoke particulates, which carry adsorbed carcinogens such as polycyclic aromatic hydrocarbons (PAH) and are capable of metabolizing them (33,34). The principal function of these macrophages is to produce bacteriocidal (and cytotoxic) oxidizing agents in response to ingested bacteria; however, other particulates, such as those found in tobacco smoke, also activate the cytotoxic activity of macrophages. Tobacco smoke is also toxic to the ciliated epithelium, greatly reducing the effectiveness of mucociliary clearance of the lung. It is possible that, as a result, the rate of passage of macrophages through the bronchi is reduced and that their cytotoxins, together with some of the carcinogenic metabolites they produce, have the opportunity to affect the bronchial epithelium (35).

Glutathione S-Transferases of Normal and Smokers’ Lungs

Table 4 shows the GST composition of samples of lung tissue from a number of adults. In agreement with the work of Di Lilio et al. (36) and Fryer et al. (37), who separated native enzymes, GSTP1-1 is the major component. Ontogenetic studies have shown that GSTP1-1 is very dominant at the earliest stages, when the primitive lung is an invagination of the endoderm and undergoing the branching associated with the growing bronchial tree. As development proceeds, the level falls steeply and then remains stable during the rest of fetal life, the postnatal period, and adult life (the lung is vascularized early in fetal life). The α class enzymes occur at low levels throughout all stages of development, and GSTM1-1 occurs at even lower levels in those individuals in whom it is expressed. The studies in which these findings were made were done before GSTM3-3 and other members of the M family (apart from GSTM1-1) had been isolated and identified.

Preparations of bronchial and peripheral tissue from adults have similar BPO-GST activities (31). Smoking causes a small but significant depression in BPO-GST activity in lung tissue overall (38), but bronchial macrophages undergo a considerable reduction (31).

Relationship between Level of DNA Adducts and Glutathione S-Transferase Content of the Lungs of Smokers

Although the properties and tissue distribution of GSTs have been described, it is unclear how they might affect detoxication of the many components of tobacco smoke that have been shown to be carcinogenic and that may be involved in the etiology of smoking-induced lung cancer. A list of some of the carcinogens known to be present in cigarette smoke includes PAH, nitrosamines, and aromatic amines (35). So far, only PAH metabolites have been shown to be substrates for GSTs. Thus, the assays for BPO-GST activity described above are relevant for humans (and are believed to be important in rats) (10), to the extent that BPO-GSH conjugation is an important pathway of BP detoxication. BPO is known to be a good substrate for GSTM1-1, less so for GSTP1-1, and even less so for GSTs containing A1 and A2. (+) anti-BPDE, however, which is known to be the ultimate carcinogen in some tissues (e.g., skin), is a good substrate for GSTM1-1, and an even better one for GSTP1-1, which not only results in higher rates than other GSTs but is more selective for the metabolically produced (+) anti-enantiomer (7). Unfortunately, no data are available on GSTM3-3 and other μ-class enzymes. Since the GSTs M1-1 and P1-1 are known to catalyze the detoxication of PAH in vitro, one might expect to see some effect of GSTs on PAH—DNA adduct levels in smokers. Figure 2 shows a comparison between the levels of nuclease P1-enhanced 32P-postlabeled DNA adducts [see the method described in Talaska et al. (39)] and of GST subunits M1 + M3 in smokers and nonsmokers. These are preliminary results; nevertheless, there appears to be an
The level of GST in the lungs is inversely correlated. No correlation was seen with GST subunits A1 + A2 or P1.

A proportion of the smoke that enters the lungs is taken up by the epithelium during its passage down the bronchial tree, but the remainder gains ready access to the bloodstream in the alveoli. Figure 1 shows that the detoxification capacity of the lung is very much lower than that of the liver and perhaps of other extrapulmonary tissues. The same is true of the activating enzymes (30). The consequence is that tobacco smoke produces DNA adducts not only in lung tissue but also in a number of extrahepatic tissues, ranging from those most immediately accessible, such as the heart, to those more distant, such as the liver and kidney (40). Table 2 shows that little effect of GSTs containing subunits A1 and/or A2 is to be expected; even the major component GSTP1-1 occurs at low levels compared to the levels of GSTs found in the liver. Products derived from tobacco that reach the blood may recirculate and be further metabolized by the lung, but their amount will be considerably reduced and they will be changed qualitatively after passage through the liver. It may be that the adducts that are found in the lung depend not only on the metabolism of inhaled aerosol entering tissue from the lumen but also on the activation of progoitoxins (e.g., BP-7,8-diol, 9-OH-BP) entering lung tissue from the bloodstream. Levels of extrapulmonary PAH and PAH derivatives may be affected profoundly by whether or not GSTM1-1 is expressed in the liver, since cytochrome P450s are inhibited by the products of their action. GST and epoxide hydrolase have important functions in removing these inhibitors (10). The results shown in Table 3 might then be the result of the combined effects of primary and secondary pulmonary metabolism, the latter being most important in the peripheral lung where the blood supply is so rich. It should be noted, however, that, regardless of their origin, the levels of DNA adducts are similar in the bronchial and peripheral regions of the lung although lung cancer is much more common in the bronchi.

**Table 5. Association between risk for lung cancer among smokers and the GSTM1 null phenotype.**

| Subjects                | GSTM1-1/GSTM1 null | p-Value |
|-------------------------|--------------------|---------|
| Smokers                 |                    |         |
| Small-cell carcinoma    | 3/12               | 0.02    |
| Large-cell carcinoma    | 4/10               | 0.25    |
| Squamous-cell carcinoma | 27/62              | 0.04    |
| Adenocarcinoma          | 28/38              | 0.0001  |
| Other                   | 8/14               | 0.58    |
| Total                   | 76/191             | 0.001   |
| Controls                | 112/192            |         |

*Data from Seidegard et al. (5).*

**Relationship between Lung Cancer and Glutathione S-Transferase Genotype**

Evidence has been given that DNA adduct formation is inversely correlated with the GST μ-class phenotype and there may be a similar correlation with susceptibility to lung cancer. Thus, Seidegard and his colleagues (4,5,24) have demonstrated a significant correlation between the occurrence of carcinoma of the lung and low levels of trans-stilbene oxide-GSH transferase activity in blood leukocytes, this compound having been shown to be a particularly good substrate for GSTM1-1 (Table 2). They compared smokers with and without lung cancer and found that the GSTM1 null phenotype was associated with susceptibility to lung cancer and that this association was highly significant in patients with adenocarcinoma (Table 5). Using Southern blots of DNA obtained from blood cells, probed with subunit GSTM1b cDNA, they compared phenotype with genotype in a handful of cases and showed that the GSTM1 null phenotype was associated with a deletion; thus, in the GSTM1*O/GSTM1*O genotype, the GSTM1 gene is partly or totally absent (5).

In this particular population sample, studied by phenotyping, there was a preponderance of adenocarcinomas, which is regarded as not typical. Squamous-cell carcinomas are the commonest among smokers in western society, although adenocarcinomas appear to be on the increase (30). In a recent study by Zhong et al. (6), involving genotyping rather than phenotyping, in which probes were derived from highly conserved regions of μ family genomic DNA (41), the correlation between GSTM1*O/GSTM1*O and adenocarcinoma was, if anything, negative. There was, however, a small but positive correlation of GSTM1*O/GSTM1*O with squamous-cell carcinoma. This population had the more usual preponderance of squamous cell carcinomas (Table 6) (30). The discrepancy between the results of these two studies is not yet understood.

Some μ-class genes are clustered. The remarkable conservation in genomic structure in both exons and regions of introns which has been shown in this family shows that gene conversion has occurred and should continue to do so (41). A situation can be envisaged in which a gene conversion gives a positive genotype but a null phenotype, because a conversion event has occurred, and results in a negative regulatory effect on the gene. Such a
hybrid would not have the GSTM1*0-associated deletion or ever give rise to a GSTM1*0/GSTM1*0 genotype; however, such gene conversions would not be of sufficient frequency to explain the discrepancy between the results of Seidegard et al. (5) and those of Zhong et al. (6), although they could explain occasional inconsistencies between genotype and phenotype. One should also be aware that genotyping by polymerase chain reaction of members of the GST μ multigene family, which has such a high level of sequence identity in introns and exons arising from gene conversion, may result in an artefactually positive attribution of genotype.

Discussion

The lung has the capacity to activate and detoxify BP, and presumably other PAHs, in both the bronchiolar and alveolar epithelia (31), but the levels of many activating and detoxifying enzymes in this organ are low overall, and a significant proportion of the PAH load that a tobacco smoker encounters may pass into the systemic blood to be toxified and detoxified in extrapulmonary tissues (40). Among the members of the GST supergene family for which the specificity toward some substrates is known, GSTM1-1 is effective with both a presumed major epoxide metabolite of BP, namely BPO, and the ultimate carcinogen BPDE (7) and is therefore important for their detoxication and excretion. (The activities of the GSTs M2-2 and M3-3 with these substrates have yet to be determined, and the activities toward other electrophilic substrates such as BP-7,8-oxide are not known.) Even so, the importance of the μ family for the detoxication of PAH is borne out by our preliminary experiments in which the level of immunochemically determined μ family GSTs in the lungs of smokers appears to be inversely correlated with its nuclease-P1 32P-postlabeled DNA adducts. The GSTM1 locus is polymorphic and has a null allele that occurs at such frequency that GSTM1 null phenotypes are present in approximately 50% of the population (4-6,40). Individuals with null phenotypes are expected to have reduced abilities to detoxify BPO and BPDE. Thus, the expression of GSTM1-1 could affect the development of PAH-dependent cancer; this should be apparent when either the GSTM1-1 phenotype or the GSTM1 genotype is determined.

It should be noted that, in the two studies performed so far, the analysis of phenotype has shown much more significant correlations than studies of the genotype (4-6). This might be due to the different epidemiological designs of the two studies, to the different origins of the two populations studied (the phenotyping having been done on a U.S. group and the genotyping on a British group), or to other undetermined factors. It should be borne in mind that at least one other genetic polymorphism is associated with lung cancer, namely that involving the debrisoquine hydroxylase (CYP2D6) locus (1-3). There is no linkage with the GSTM1 polymorphism, with the result that the effect of one may either obscure or enhance the effects of the other or be neutral, according to inheritance. Simultaneous genotyping of the same individuals for the GSTM1 deletion and CYP2D6 mutants might reveal populations with very significant differences in susceptibility to lung cancer. A glance at Table 3 shows individuals in which GSTM1 and debrisoquine hydroxylase expression might work with each other (HL15, FH80) or against each other (FH55) to promote susceptibility to lung cancer.

Since the levels of GSTs, including GST subunit M1, in the lung are low (Fig. 1), a significant proportion of the detoxication of PAH from tobacco smoke may occur in the extrapulmonary tissue and some DNA damage in the lung may be secondary, due to bloodborne progenotoxic PAH metabolites released from other organs.

Data currently available do not explain why lung tumors are mostly associated with the bronchi rather than with the alveoli, to which PAH are similarly accessible. Unknown but critical promotion events may occur in the bronchi which do not occur more distally. In this respect, bronchoalveolar macrophages may play a role: not only are their levels of BPO-GST sharply reduced by smoking (34), but they may also spend more time in the bronchial area of smokers, since muccosal action is inhibited by tobacco smoke (35). Wherever they are, they produce not only PAH metabolites but also cytotoxic active oxygen, which is a promotor in the skin and perhaps also in the lung [see also Kadlubar et al. (42)].

This manuscript was presented at the Conference on Biomonitoring and Susceptibility Markers in Human Cancer: Applications in Molecular Epidemiology and Risk Assessment that was held in Kailua-Kona, Hawaii, 28 October–1 November 1991.

The authors thank L. Christodoulides for her help in preparing this manuscript.

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