COMMENTARY

Single nucleotide polymorphisms, metabolic activation and environmental carcinogenesis: why molecular epidemiologists should think about enzyme expression

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This commentary was written to stimulate thoughts on, and consideration of, enzyme expression data in target organs when investigating possible associations between polymorphisms in carcinogen activation enzymes, lifestyle/dietary factors and cancer risk. The lung and breast are taken as examples. There is overwhelming evidence for a genotoxic mechanism in lung cancer development, and compelling evidence for the contribution of genotoxins to breast cancer aetiology. A consistent association has been shown where lung cancer risk is decreased by a G→A polymorphism in the myeloperoxidase (MPO) gene, which is expressed in neutrophils recruited to the lung after chemical or immunological insults. In the breast, a consistent lack of association has been observed for women who are fast N-acetyltransferase type 2 (NAT2) acetylators consuming cooked meat. This could be explained by the lack of detectable NAT2-associated sulfamethazine acetylation activity in cytosols prepared from mammary tissue, suggesting a minor contribution to carcinogen activation. The recent identification in mammary cytosols of detectable sulfotransferase isofoms (SULT1A1 and SULT1A3), which have high catalytic efficiency for activating N-hydroxylated heterocyclic amines (HCAs, mutagens in cooked meat), offers a more important role for these enzymes in the metabolic activation of genotoxins in the breast. The possible contribution of MPO and lactoperoxidase enzymes to carcinogen activation in mammary tissue is also considered. Sulfotransferases and peroxidases have wide substrate specificity in terms of carcinogen activation (HCAs, aromatic amines and polycyclic aromatic hydrocarbons—all present in cooked meat and tobacco smoke) compared with NATs (HCAs and aromatic amines only). For gene–environment interactions, investigations into functional polymorphisms in SULT and peroxidase genes may, therefore, offer new evidence for the involvement of genotoxins in the initiation of carcinogenesis. Identification of the isoforms (if any) of carcinogen activation enzymes that are expressed in the organs of interest will help to determine which genes to investigate in these studies.

Introduction

Epidemiological studies in combination with mechanistic studies can determine the causes of cancer at various organ sites (1). The mutational spectra of the TP53 gene in lung and breast tumours suggests that exogenous agents may play a greater part in carcinogenesis here than in other tissues, such as the colon (2). Knowledge of the aetiological agents responsible for and the mechanisms leading to tumour formation is essential for achieving cancer prevention (3–6). It is argued in this article that assessment of enzyme expression studies in the target organ should be incorporated into molecular epidemiology studies when investigating the potential effects of polymorphisms in carcinogen metabolizing enzymes on cancer risk. This is particularly appropriate for organs such as the lung and breast (7) where evidence exists for the involvement of a genotoxic mechanism in the carcinogenic process (2,8–11) and where evidence of metabolic activation of suspected carcinogens exists in the form of DNA adducts (3,5,12). First, the effect of polymorphisms in the myeloperoxidase (MPO) gene on lung cancer risk will be briefly reviewed. Secondly, reasons will be suggested for the apparent lack of association between polymorphisms in the N-acetyltransferase 2 (NAT2) gene, levels of meat consumption and breast cancer risk. Further, suggestions will be made as to why the recent characterization of sulfotransferase (SULT) isofoms in cytosols prepared from mammary tissues should direct molecular epidemiologists to investigate SULT1A polymorphisms in gene–environment interaction studies. Full characterization of the carcinogen-activating enzymes expressed in prostatic tissues could establish whether exposures to environmental agents are risk factors for cancer at these sites. Finally, it will be suggested that when associating genotype and lifestyle or environmental factors with cancer risk, investigation of polymorphisms in genes encoding enzymes with wide substrate specificity with regard to carcinogen activation (e.g. peroxidases and SULTs) will be more fruitful than studies of those polymorphic genes encoding enzymes with a narrow substrate specificity (e.g. NATs).

Pathways of activation of three classes of suspected human chemical carcinogens catalyzed by human xenobiotic-metabolizing enzymes are shown in Figure 1. The enzymes catalyzing metabolic activation of these compounds include cytochrome P450 (CYP) enzymes, NATs, SULTs and peroxidase enzymes.

Myeloperoxidase expression in the lung

Neutrophil recruitment into lung tissue occurs after exposure to a variety of insults known to increase lung cancer risk, including tobacco smoke particulates, infection, asbestos and ozone (13–15). Following immunological and/or chemical insults, neutrophils release MPO (13) and undergo a ‘respiratory burst’, which is characterized by a massive increase in oxygen consumption and a consequent NADPH-dependent production of superoxide and other free radicals. MPO is present in the primary granules of neutrophils and catalyzes the production of the potent bacteriotoxic oxidizing agent hypochlorous acid (a one- and two-electron oxidant that can

Abbreviations: CYP, cytochrome P450; HCAs, heterocyclic amines; MPO, myeloperoxidase; NAT, N-acetyltransferase; PAHs, polycyclic aromatic hydrocarbons; SULT, sulfotransferase.
Fig. 1. Pathways of metabolic activation of suspected human carcinogens. References are given in parentheses. *Refers to rat enzyme.

Possible pathways of activation of suspected human carcinogens

- **Heterocyclic amines**
  - e.g. IQ, PhIP

- **Aromatic amines**
  - e.g. 4-ABP

- **PAHs**
  - e.g. B[a]P, DMBA

\[ \text{DNA-reactive products of metabolic activation} \]

\[ \text{NH}^+ \]

\[ \text{C}^+ \]

\[ \text{Dihydrodiol epoxide} \]

\[ \text{Electrophile} \]

- **B[a]P**

- **7,12 DMBA**

- **4-ABP**

- **IQ**

- **PhIP**

- **CYP (22), LPO (37)**

- **PHS (74)**

- **SULT (39)**

- **NAT1 (38, 39)**

- **SULT (57, 59)**

- **CYP, MPO (89)**

- **SULT (56)**

- **Electrophile**

**Expression of N-acetyltransferases and sulfotransferases in the breast**

The majority of lung cancer cases (87%) can be attributed to a single agent, tobacco smoking (29), but the aetiology of...
The limit of detection (38,39). Although reverse transcription–metabolites (39) are the major adducts formed (36). Parent HCAs can also be potential risk factors e.g. consumption of well-done meat.

Cooked meat contains a variety of mutagens (30), including HCAs formed from the cooking of proteinaceous foodstuffs (particularly meat and fish) at high temperatures. These compounds are carcinogens in the rat mammary (31) and prostate (32) glands following high-dose oral administration, and are suspected of being mammary carcinogens in women (33) and prostate carcinogens in men (32,34). HCAs require metabolic activation to form DNA-damaging metabolites (Figure 1). This can occur via a two-step pathway involving first a CYP catalyzed N-hydroxylation (22,35) followed by O-esterification catalyzed by NAT and/or SULT enzymes (Figure 1). The acetoxy or sulfoxy esters formed are unstable and generate a nitrenium ion as the ultimate DNA-reactive intermediate (Figure 1), and quantitatively, N-(deoxyxynanosin-8-yl)–HCAs are the major adducts formed (36). Parent HCAs can also be activated by peroxidases to generate nitrenium ions (22,37).

Determination of NAT and SULT (38,39) enzyme expression in the breast has revealed the major isozymes to be NAT1, SULT1A1 and SULT1A3, all of which can metabolically activate promutagenic derivatives of HCAs to DNA-reactive metabolites (39–41) (Figure 1). In mammary cytosols, NAT1-specific para-aminobenzic acid acetylation activity was measurable (with a 6-fold interindividual variation), whereas NAT2-specific sulfamethazine acetylation activity was below the limit of detection (38,39). Although reverse transcription–polymerase chain reaction and immunohistochemical analyses have shown that the NAT2 gene is expressed in the ductal epithelial cells of the breast (39), the absence of enzyme activity is consistent with low mammary expression of NAT2. These advances in the determination of enzyme expression in the breast, and others detailed below, may explain why no association has been found between polymorphisms in the NAT2 gene, cooked meat consumption and breast cancer risk; for example, in studies by Ambrosone et al. (42) (740 cases, 810 controls), Gertig et al. (43) (466 cases, 466 controls) and Delfino et al. (44) (114 cases, 280 controls).

The liver plays a major role in the control of systemic levels of xenobiotics, and for some compounds, such as nitrosamines (45) or PAHs (46,47), there is evidence that hepatic metabolic activation produces short-lived electrophiles that could damage DNA in extra-hepatic organs (for review on carcinogen metabolism see ref. 48). For heterocyclic and aromatic amines, however, local metabolic activation at the target site (i.e. the breast) may be more important than hepatic activation (7,49–51).

There are 26 reported alleles of the NAT2 gene (for the NAT allele website see http://www.louisville.edu/medschool/pharmacology/NAT.html) but NAT2 activities in the human population are bimodally distributed (50–60% of Caucasians have low acetylation activity for NAT2 substrates, and are designated ‘slow acetylators’ ) (52). The influence of the NAT2 genotype on NAT2 enzyme activity has been well characterized (40). An important opportunity was therefore presented to study gene–environment interactions, since the slow NAT2 phenotype is present in a significant proportion of the Caucasian population, and the enzyme has potential human carcinogens as substrates (53) (Figure 1). These include carcinogenic aromatic amines that are good substrates for hepatic clearance by N-acetylation. However, where genotoxic carcinogens are metabolically activated by NAT2 (via NAT-catalyzed O-acetylation of N-hydroxylated aromatic or HCAs), expression at the target site, e.g. the breast, is likely to be more important since the short-lived acetoxy esters would not be expected to survive transport to the breast.

The identification of the SULT isoforms expressed in mammary tissue (39) may be more promising in providing information on breast cancer aetiology. The SULTs are a supergene family (54), and one of the two isoforms detected in mammary cytosols, SULT1A1 (the other is SULT1A3) (39) has the highest O-esterification activity for N-hydroxylated heterocyclic amines (41). SULT enzymes metabolically activate a wider range of promutagenic substrates (39,55–57) and rodent mammary carcinogens than NATs (Figure 1). It might therefore be expected that functional polymorphisms in the SULT1A1 and SULT1A3 genes would have more influence on breast cancer risk than NAT polymorphisms, especially as humans are most often exposed to mixtures of genotoxins through a particular potential risk factor e.g. consumption of well-done meat resulting in exposure to mixtures of PAHs and HCAs (30,58). In model systems, methylated PAHs such as 7,12-dimethylbenz[a]anthracene can be activated via sulfation of the 7-hydroxylated derivative (57,59). However the major pathway of metabolic activation of PAHs via sulfation is thought to occur via conjugation of a tetrolic derivative (56). The relative importance of these SULT-requiring pathways in human tissues is unknown. A functional polymorphism has been described for the SULT1A1 gene (60), a G→A transition in codon 213 (Arg213→His213). The frequency of the IA1*Arg allele in Caucasian population samples has been reported to be 0.63 in Germany, (60), 0.69 in the USA (61) and 0.68 in the UK (62). In a Nigerian population the frequency of the IA1*Arg allele was reported to be 0.63 (62). The His→Arg213 polymorphism has been reported to decrease mutagen activation by 10–300 fold (63). The high frequency of the low activity sulfation allele in the study population, coupled with wide substrate specificity, could offer a practical method of identifying populations at risk of developing cancer in the mammary gland and in other organs where SULT1A1 is expressed.

Expression of cytochrome P450 enzymes in prostate and pancreatic tissues

The mutagenic activity of PAHs and HCAs present in cooked meat provides a plausible mechanism for carcinogenesis in human tissues. There is an association between levels of cooked meat consumption and risk of prostate (64) and pancreatic cancer (65), but a case-control study based in Auckland, New Zealand (317 patients and 480 age-matched controls), found no clear association between intake of known HCAs and prostate cancer risk (65). In this New Zealand-based study, however, an increased risk for prostate cancer was observed for high intake-levels of well-done beefsteak. CYP1A2 is expressed in the prostate (34) and pancreas (66,67) and in experiments using recombinantly-expressed CYP1 enzymes, the catalytic efficiency for N-hydroxylation of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine was far greater for CYP1A1 and CYP1A2 than for CYP1B1 (68). An association between CYP1A2 expression, HCA activation and cancer risk could be investigated if functional polymorphisms in the CYP1A2 gene could be fully understood (69–71).
Peroxidases in the breast

Two interesting epidemiological studies have shown that women with allergies/atopic diseases (i.e. those associated with inflammation and elevated peroxidase activity), compared with women without atopic diseases, have an elevated risk of developing cancer of the breast [relative risk 2.5; 95% confidence interval 1.01–5.16 (72) and relative risk, 1.25; 95% confidence interval 0.76–2.07 (90)]. A mechanistic role for peroxidase-activation of mutagens has been suggested (22,37,73,74) (Figure 1). Levels of MPO-containing neutrophils are elevated in inflamed mammmary tissues (75) compared with non-inflamed tissues, and are present in breast milk and breast secretions. The gene encoding the human form of another peroxidase enzyme, lactoperoxidase (76), is arranged tail-to-tail with the MPO gene. The enzyme encoded by this gene also has bacteriocidal functions, and is secreted into the milk ducts. The catalytic activity of the human form of this enzyme has not been well studied, but the bovine form of this enzyme can activate 17β-estradiol (77), aromatic amines (78) and HCAs (37) to mutagenic metabolites. A possible causal link between peroxidase-catalyzed carcinogen activation and/or free radical production and breast carcinogenesis could be investigated by assessing the effect of the reported MPO polymorphism (25–27) and/or functional single nucleotide polymorphisms in the lactoperoxidase gene on breast cancer risk.

Summary and future perspectives

It would be particularly informative to make a quantitative assessment of the contributions of metabolizing enzymes to carcinogen activation in the organs of interest. This could be done by measuring mRNA levels and enzyme activity levels in the target tissues, inter-individual variations in carcinogen metabolism and/or enzyme activity, as well as catalytic efficiency of the enzymes for metabolically activating carcinogenic substrates. Allocation of an endogenous role for carcinogen metabolizing enzymes will help our understanding of enzyme expression in target tissues; e.g. a role for folate metabolism has recently been shown for NAT1 (79).

The functional effects of single nucleotide polymorphisms on enzyme expression can be assessed by measuring gene transcription (25), mRNA stability, immunoreactive protein or enzyme activity (60,80; see ref. 7 for a review). Caution should be taken when using just one method of measurement, as enzyme activity levels do not always correspond to mRNA transcript levels (39,81). Furthermore, for metabolizing enzymes, increases in protein are not always mirrored by increases in active enzyme (82). If possible, a combination of methods should be employed to increase confidence in the perceived functional effect of a single nucleotide polymorphism on the expression level or activity of the enzyme (7).

In summary, knowledge of tissue-specific enzyme expression can provide useful information for molecular epidemiologists when investigating gene–environment interactions. This can include profiling of the isoforms expressed (39,83,84), localization of enzyme expression within the tissue (38,39,67,83,84) and quantitative determination of enzyme activity within subcellular tissue fractions (38,39,83,84). Information on all the competing pathways of metabolic activation and detoxification of suspected human carcinogens should also be taken into account. Peroxidases and SULT enzymes have wider substrate specificities than NAT enzymes. Thus, while NAT enzymes are limited to activation of N-hydroxylated heterocyclic and aromatic amines, peroxidases can metabolically activate both parent and hydroxylated heterocyclic and aromatic amines and hydroxylated PAHs, and SULTs can activate the hydroxylated promutagenic derivatives of all three classes of mutagens. Such mixtures are more representative of the mixtures present in cooked meat and tobacco smoke. Investigation of polymorphisms in enzymes with wide substrate specificity, with regard to carcinogen activation therefore looks promising for the detection of links between environmental or lifestyle factors and cancer risk.

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References


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