Mammary Expression of Xenobiotic Metabolizing Enzymes and Their Potential Role in Breast Cancer

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Abstract
Breast cancer is the major cause of cancer death in women worldwide. High penetrance genes account for only 5% of cases, whereas polymorphic low penetrance genes acting in concert with lifestyle/environmental risk factors are likely to account for a much higher proportion. Genotoxic compounds implicated in human breast carcinogenesis include endogenous compounds, estrogens, and dietary or environmental xenobiotics—heterocyclic amines, aromatic amines, polycyclic aromatic hydrocarbons, and nitropolycyclic aromatic hydrocarbons. Here we review evidence for a role of mammary-expressed enzymes that metabolically activate and/or detoxify potential genotoxic breast carcinogens: cytochrome P-450s, catechol-O-methyltransferase, epoxide hydrolase, peroxidases, glutathione S-transferases, N-acetyltransferases, sulfotransferases, and other enzymes catalyzing conjugation reactions. This information is particularly relevant in the light of evidence for the presence of genotoxic agents that require metabolic activation in mammary lipid, in nipple aspirates and in breast milk, and for the presence of DNA adducts in human mammary epithelial cells (from which most breast carcinomas originate). The effect of polymorphisms in the genes encoding these enzymes on breast cancer risk are also considered. The evidence for the role of genotoxic carcinogens in the etiology of breast cancer is compelling, but mammary-specific enzyme polymorphism should be taken into account when considering the contribution of polymorphisms to risk.

Introduction
Each year breast cancer is diagnosed in 910,000 women worldwide, and 376,000 women die from the disease (1). Most of the cases are in industrialized countries (e.g., 180,000 in North America and 220,000 in Europe). The etiology of most breast cancer is obscure. Known risk factors including higher/exposed to estrogen are apparent in less than one-third of breast cancer cases, and the relative risks associated with them are numerically low (generally <3; Refs. 1–3). Although the mechanisms of estrogen-induced carcinogenesis are not entirely clear (4), it has been proposed that estrogens exert a genotoxic effect (reviewed in Ref. 5). Inherited mutations in high-penetrance cancer susceptibility genes, such as BRCA1 and BRCA2, are associated with many cases of familial breast cancer but account for only up to 5% of all breast cancer (2, 6). Thus, the majority of breast cancer cases occur in women who do not have an identifiable risk factor rendering them more susceptible to the disease.

There is a striking variation in breast cancer incidence in different countries. The highest reported rates of breast cancer incidence are for white or Hawaiian women in the United States, and significantly lower rates are observed for women in Africa and Asia (1). Female descendants (second and third generations) of migrants from low-risk countries approaching those of the host country (1), strongly implicating lifestyle factors as the major contributors to the development of the disease. Increased risk may be associated with exposure to genotoxic agents during breast development, because the undifferentiated ductal elements of the breast are more susceptible to the action of genotoxins early in life (7). Willett (3) has suggested that dietary and environmental causes may be responsible for up to 50% of breast cancer cases, although the precise nature of the “lifestyle” factors that are causative is unknown.

The ductal system of the adult human breast consists of 15–25 lactiferous ducts, opening at the nipple. The parenchyma of the ducts are composed of luminal epithelial cells lining the ducts and of myoepithelial cells in the outer layer (reviewed in Ref. 8). The luminal cells are the most common sites of origin of malignant transformation. Cell proliferation activity occurs in a fluctuating hormonal environment with a periodic variation in DNA synthesis in the terminal buds, and estrogen and progesterone have important roles in stimulating cell division in the breast (9). Chronic exposure to low levels of mutagens in the proliferating cells of the breast epithelium would result in an accumulation of gene mutations in target genes, leading to carcinoma development only when the combinations of targets release cell division from its tightly regulated control. In this way, functional mutations in “low penetrance” genes encoding carcinogen-metabolizing enzymes (i.e., mutations that resulted in an increase in the rate of an activating metabolic transformation of a carcinogen/mutagen, or in a decrease in the rate of an inactivating one) would lead to increased rates of breast cancer in older women.

Agents Implicated in Human Breast Carcinogenesis
The observation in the 17th century that nuns had a high incidence of breast cancer (10) can be regarded as one of the first examples of an occupational cancer. However, this is more likely to be related to the hormonal status of the women, resulting from nulliparity, than because of occupational exposure to a cancer-causing agent. Although there have been reports of occupational risk factors for breast cancer associated with employment in a variety of industries or jobs (11–13), the evidence is, for the most part, limited. The only exogenous agent for which there is sufficient evidence for breast cancer causation is ionizing radiation, both among atom bomb survivors (14) and in cohorts of women exposed to therapeutic doses of radiation (15). The human mammary gland is exposed to agents capable of inflicting DNA damage and thereby initiating tumor induction. The high lipid content of the mammary gland allows an accumulation of lipid-soluble compounds, including possible carcinogens (15), for the epithelial cell ducts to be exposed to (16). Mutagenic activity has been detected in nipple aspirates (17, 18) and breast cyst fluid (19). Recently, extracts of mammary lipid from women undergoing reduction mammoplasty have been shown to possess genotoxic activity (15, 20). Furthermore, extracts of human breast milk have been shown to be genotoxic (21–23), supporting the notion that functional elements of the mammary gland may be exposed to potential tumor initiators. The
components of these biological fluids that are responsible for the genotoxic activity require metabolic activation but remain to be identified.

The association between exposure to ionizing radiation and breast cancer (13, 14) indicates that the mammary gland is susceptible to tumor initiation by DNA-damaging agents. Many classes of carcinogens form chemically stable altered nucleotides in DNA (DNA adducts), and the detection and identification of the adducts in mammary tissue offers a means to identify the nature of human breast carcinogens. The presence of aromatic and/or hydrophobic DNA adducts in human mammary tissue has been detected in a number of studies (24–27). In some studies, the adducts detected have been shown to be partly characteristic of those formed by polycyclic aromatic hydrocarbons (24–26), whereas in another study, a contribution from aromatic amine-like species was implicated (27). However, these characterizations fall a long way short of definitive identification of the origins of reactive species responsible for DNA adduct formation. In one study (28), DNA adducts formed by malondialdehyde, a product of lipid peroxidation, were found to be at significantly higher levels in tumor-adjacent tissue from breast cancer patients than in tissue from disease-free controls, but the levels were still very low in both groups. The biological consequences of very low levels of DNA damage are far from clear. Levels of another marker of oxidative DNA damage, 8-hydroxydeoxyguanosine, were substantially lower in reduction mamoplasty tissues than in breast tumors (29). Two studies report no significant difference in 8-hydroxydeoxyguanosine levels between breast tumor and benign tumor-adjacent tissues (29, 30), but in another, levels were 50% lower in the tumor-adjacent tissue compared with the cancerous tissues (31). What is lacking, however, is a comparison of levels of oxidative damage in the normal, tumor-adjacent breast tissue of cancer patients, compared with the levels in disease-free women.

What, then, are the putative agents involved in the initiation of breast cancer? Members of a number of classes of environmental chemicals have been found to be mammary carcinogens in rodents (32). These include polycyclic aromatic hydrocarbons (32), nitropoly-cyclic aromatic hydrocarbons (32), aromatic amines (33), and heterocyclic amines (34). Polycyclic aromatic hydrocarbons produced by incomplete combustion of carbon-containing compounds are present in the atmosphere, tobacco smoke, and in food (35), and nitropoly-cyclic aromatic hydrocarbons in particular are formed at high levels from diesel oil combustion (36). Aromatic amines are present in dyes and tobacco smoke (37). Heterocyclic amines are formed when proteinaceous foods are cooked to high temperatures, and a number of them are highly mutagenic (38). All of these agents appear to be carcinogenic by genotoxic mechanisms and must therefore be potentially carcinogenic to the human breast.

Tobacco smoking is well established as a risk factor for a number of cancers. To date, many but not all epidemiological studies have failed to show a relationship between smoking and breast cancer (39). It is, however, conceivable that women with certain alleles of polymorphic genes encoding metabolizing enzymes may generate greater levels of metabolically activated mammary carcinogens in this breast tissue. This could be attributable to increased levels of activating enzymes or decreased levels of detoxification enzymes. The increased risk for a susceptible subgroup may go unnoticed in studies where subjects are not stratified by genotype (39). The role of tobacco smoking as a cause of breast cancer is thus, at present, plausible but unproven.

A number of other environmental chemicals have been proposed to play a role in breast cancer induction, although not necessarily as initiators. These include the ubiquitous class of organochlorines–chlorinated pesticides, polychlorinated biphenyls, and polychlorinated dibenzo compounds. Because a small number of these mimic the properties of estrogen, it has been proposed that they may exert a hormonal influence on breast cancer (40). Because their estrogenicity is orders of magnitude weaker than that of natural estrogen, it is difficult to envisage the exposure to environmental estrogens having a significant influence on the growth of breast neoplasms via the estrogen receptor (40). However, they may persist in the body and so may have a long-term effect. Such compounds may be influential in the carcinogenic process by induction (or inhibition) of enzymes in the mammary gland that activate (or detoxify) genotoxic compounds. The epidemiological evidence for an association between organochlorine exposure and breast cancer is, however, contradictory (40–42).

Metabolites of natural estrogens may also be a source of DNA-damaging agents. When injected into the rat mammary gland, catechol estrogen-3,4-quinones have been shown to cause unstable DNA adducts; this form of DNA damage results in depurination (43). Whether such events can or do occur in vivo from natural concentrations of these metabolites is not known. In other circumstances, there are doubts about the biological significance of unstable DNA adducts that result in depurination, relative to the consequences of stable DNA adduct formation (44). In female CD rats fed a high fat diet, the polycyclic aromatic hydrocarbon-derived electrophile, 3,4-dihydroxy-1,2-epoxy-1,2,3,4-tetrahydrobenzo[c]phenanthrene, was shown to be a strong mammary carcinogen after injection under the nipples, whereas injection of estrone-3,4-quinone under the nipples did not cause mammary tumorigenesis (45).

Cholesterol metabolites may also be a source of the mutagens in nipple aspirates (46), but the significance is unknown. Human milk is known to contain a large number of compounds of exogenous origin (47), but whether they are present at biologically significant levels is open to question. Recently, some monocyclic aromatic amines have been detected in human milk at parts-per-billion levels, including one, o-toluidine (48), that is a mammary carcinogen in rats (49). An earlier study reported similarly low levels of polycyclic aromatic hydrocarbons in human lipid (50).

In summary, several classes of environmental chemicals have been implicated in the development of breast cancer (51, 52). Where their mechanisms of carcinogenicity might be thought to be primarily genotoxic, most of these chemicals require metabolic activation to exert a biological effect.

Evidence has also been found for the genotoxic activity of breast fluids or extracts, when tested in the presence of metabolizing systems. Human mammary epithelial cells are capable of metabolically activating compounds that are mammary carcinogens in rodents. These include polycyclic aromatic hydrocarbons (53, 54), nitropoly-cyclic aromatic hydrocarbons (55), heterocyclic amines (56–58), and aromatic amines (22). In considering the roles of potential initiators of breast cancer, it is appropriate to consider the metabolic capabilities of the human mammary gland.

Metabolizing Enzymes Expressed in the Breast

Most of the potential human mammary carcinogens require multiple enzyme-catalyzed steps to effect biotransformation to DNA-reactive metabolites. This may proceed through a primary metabolic step carried out by hepatic metabolism, followed by a secondary conjugation with a suitable leaving group, or through complete metabolic activation in the breast in situ, or through a combination of both. Determining the relative contributions of hepatic and mammary carcinogen activation is an important task not yet undertaken and would depend on the potential mammary carcinogen of interest and on the metabolic stability of activated intermediates of the compounds in human plasma (59).

The liver plays a major role in the control of systemic levels of...
xenobiotics, and for some compounds such as nitrosamines (60) or polycyclic aromatic hydrocarbons (61, 62), there is evidence that hepatic metabolic activation produces short-lived electrophiles that could damage DNA in extrahepatic organs. From early work on heterocyclic and aromatic amine activation in rats, there was speculation on the central role of the liver in producing proximate mutagens that could be transported to the breast for final metabolic activation to form the ultimate DNA-reactive metabolites, e.g., the heterocyclic metabolism of the heterocyclic amine PhIP to produce systematically circulating N-hydroxylated metabolites (63, 64). More recent research has suggested that the breast is the principal site of heterocyclic amine metabolic activation. N-Hydroxy-PhIP, a proximate metabolite of PhIP formed mainly by CYP1A2-mediated metabolism in the liver (65), was not detectable in human blood plasma after an oral dose of PhIP (59). Hepatic amine reductases have been discovered that reduce N-hydroxylated carcinogenic amines produced by human CYP enzymes back to their respective parent compounds (66). Also, after incubation of another heterocyclic amine, MeIQx, in human hepatocytes, only the N-hydroxy-MeIQx glucuronide was formed and not the free N-hydroxy metabolite (67).

CYP1A2 and xenobiotic metabolizing enzymes expressed in the breast may be “activating” (metabolizing substrates to more reactive species) or “detoxifying” (reducing the DNA reactivity of genotoxic species and increasing their excretion). Some enzymes may possess activating and detoxifying roles, depending on the substrate. For example, NAT enzymes can catalyze N- or C-acetylation of aromatic amines, which are considered to be detoxification steps, or they may be activating, via O-acetylation of N-hydroxy derivatives. The roles of locally expressed enzymes in breast cancer risk are described in the following sections, with particular focus on those with the potential to activate endogenous and xenobiotic compounds.

Studies of metabolizing enzymes have been carried out using homogenized breast tissue obtained from reduction mammoplasties (68, 69) and mastectomies (70) and cultures of human mammary epithelial cells (56–58, 71) or mammary fibroblasts (72, 73). The use of cultured cells has the advantage of allowing regulation of the metabolism of enzymes and their respective genes (54, 58, 72). Table 1 summarizes those enzymes that have been shown to be expressed in mammary tissue or in cultures of breast-derived cells and their potentially carcinogenic substrates. Table 2 details the evidence for breast-specific mechanisms of enzyme regulation, including induction, which is the up-regulation of active enzyme via increased gene transcription, mRNA levels, or protein stability. Reference to endogenous functions of mammary expressed enzymes is given in the following sections where this has been established.

### CYPs

CYP enzymes are necessary for normal development in mammals (74), and their wide substrate specificity and inducibility indicates multiple roles in endogenous compound synthesis and metabolism (75, 76). Many of the CYPs identified to date that catalyze the metabolism of potential breast carcinogens are activating, via C- or N-hydroxylation (Table 1). Mutations in the genes that increase catalytic efficiency or expressed levels of the active enzyme can therefore be expected to increase levels of metabolic activation. Until the advent of reverse transcription-PCR, there was little information on the expression of CYPs in breast tissue. CYP expression levels are up to 500 times lower in the breast than in the liver (68). Some attempts (77, 78) to measure CYP protein by Western blot analysis were not able to detect expression of all of the proteins that have been identified subsequently by this method (68, 70).

#### CYP1A1, CYP1A2, and CYP1B1

Human CYP1A2 is the most abundantly expressed CYP enzyme in the liver (79). Substrates for metabolic activation by the CYP1 family of enzymes include polycyclic aromatic hydrocarbons (54, 80) and heterocyclic amines (58, 81). CYP1A1 catalyzes the hydroxylation of estrogen at the C-2

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**Table 1** Substrates of human carcinogen-metabolizing enzymes expressed in the human mammary gland

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Detected as</th>
<th>Substrate(s) implicated in breast carcinogenesis*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A1 (54)</td>
<td>mRNA levels, immunoreactive protein and enzyme activity</td>
<td>DMBA (54) and other PAHs (80) activated by dihydrodiol epoxide formation</td>
</tr>
<tr>
<td>CYP1A1 (58)</td>
<td>mRNA levels</td>
<td>IQ (58) activated via N-hydroxylation</td>
</tr>
<tr>
<td>CYP1B1 (54)</td>
<td>mRNA levels, immunoreactive protein and enzyme activity</td>
<td>DMBA (54) activated by dihydrodiol epoxide formation</td>
</tr>
<tr>
<td>CYP1B1 (58)</td>
<td>mRNA levels</td>
<td>IQ (58) activated via N-hydroxylation</td>
</tr>
<tr>
<td>CYP1B1</td>
<td></td>
<td>17β-estradiol (83) activated by 4-hydroxylation (also see review of estrogen and cancer, ref. 5) PAHs activated by dihydroepioid formation (80)</td>
</tr>
<tr>
<td>CYP2C (78)</td>
<td>Immunoreactive protein</td>
<td>17β-estradiol (101) activated by C2-hydroxylation</td>
</tr>
<tr>
<td>CYP2D6 (68, 70)</td>
<td>mRNA levels and immunoreactive protein</td>
<td>N-acyclidialkylamines activated (183)</td>
</tr>
<tr>
<td>CYP2E1 (68)</td>
<td>Immunoreactive protein</td>
<td>MeIQ activated by N-hydroxylation (109), aflatoxin B1 activated (110)</td>
</tr>
<tr>
<td>CYP3A4/5 (70)</td>
<td>mRNA levels</td>
<td>Catechol estrogens (120) detoxified by O-methylation</td>
</tr>
<tr>
<td>CYP3A4/5</td>
<td></td>
<td>PAHs (184) activated by epoxide hydrolysis</td>
</tr>
<tr>
<td>Catechol-O-methyltransferase (115)</td>
<td>Enzyme activity</td>
<td>N-OH-IQ and N-OPH-PhIP (56) activated by O-acetylation</td>
</tr>
<tr>
<td>Epoxide hydrolase (77)</td>
<td>Immunoreactive protein</td>
<td>N-OH-IQ (69) activated by O-acetylation</td>
</tr>
<tr>
<td>N-Acetyltransferase (NAT1) (56)</td>
<td>mRNA levels and enzyme activity</td>
<td>AsA detoxified by N-acetylation (reviewed in Ref. 125)</td>
</tr>
<tr>
<td>N-Acetyltransferase (NAT1) (69)</td>
<td>mRNA levels, immunoreactive protein, and enzyme activity levels</td>
<td>N-OH-IQ (71) activated by O-sulfation</td>
</tr>
<tr>
<td>(Estragon) sulfotransferase (142)</td>
<td>Immunoreactive protein and enzyme activity</td>
<td>HAx detoxified by N-sulfation (185)</td>
</tr>
<tr>
<td>Sulfatase (77)</td>
<td>Enzyme activity</td>
<td>NA</td>
</tr>
<tr>
<td>UDP-glucuronosyltransferase (77)</td>
<td>Enzyme activity</td>
<td>HAx (185) detoxified by N-glucuronidation</td>
</tr>
<tr>
<td>Glucurondiase (77)</td>
<td>Enzyme activity</td>
<td>NA</td>
</tr>
<tr>
<td>Glutathione-S-transferase (78)</td>
<td>Immunoreactive protein and enzyme activity</td>
<td>N-OH-PhIP (154) detoxified by glutathione conjugation</td>
</tr>
<tr>
<td>Lactoperoxidase (160)</td>
<td>mRNA levels</td>
<td>NA</td>
</tr>
<tr>
<td>12-Lipoxygenase (178)</td>
<td>mRNA levels</td>
<td>Aflatoxin B1 activated by epoxidation (186)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4-Aminobiphenyl activated by oxidation (187)</td>
</tr>
</tbody>
</table>

*Only published information on human enzymes is included; NA, information not available. Substrate abbreviations: DMBA, 7,12-dimethylbenz[a]anthracene (a polycyclic aromatic hydrocarbon); HAs, heterocyclic amines; IQ, 2-amino-3-methylimidazo[4,5-f]quinoline (heterocyclic amine); MeIQ, 2-amino-3,4-dimethylimidazo[4,5-f]quinoline (heterocyclic amine); MeIQx, 2-amino-3,4-dimethylimidazo[4,5-f]quinoline (heterocyclic amine); NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (tobacco nitrosamine); PAHs, polycyclic aromatic hydrocarbons. |
cells from seven individuals detected expression of 17β-estradiol to a promutagenic metabolite (83). Huang et al. (70) reported CYP1A1 mRNA expression in 5 of 11 reduction mammaryplasty tissues and CYP1B1 mRNA in 100% of tissues investigated. Hellmold et al. (68) reported CYP1A1 mRNA expression in 8 of 15 reduction mammaryplasty samples and positive Western blot signals in five of eight positive samples, and CYP1B1 mRNA expression was also detected in 100% of reduction mammaryplasty samples investigated (68).

Reverse transcription-PCR analysis of gene expression in primary human mammary epithelial cell cultures (2–3 days of age) from four individuals detected CYP1B1 mRNA transcripts at 1.5-fold higher levels than CYP1A1 transcripts (relative to β-actin expression), but CYP1A2 mRNA was undetectable (58). Interestingly, CYP1A2 expression has been detected in the pancreas (84, 85) and prostate (86). Another study of 6-day-old cultures of human mammary epithelial cells from seven individuals detected CYP1A1 mRNA and protein expression at levels 7–70-fold lower than that of CYP1B1 (54). Both studies found that aryl hydrocarbon receptor ligand-mediated induction of expression by tetrachlorodibenzodioxin (54) or benz[a]anthracene (58) was greater for CYP1A1 than for CYP1B1. Interindividual variation in CYP1A1 expression is greater than in CYP1B1 expression in human mammary epithelial cells; a 21-fold variation in tetrachlorodibenzodioxin-induced CYP1A1 levels was observed in cultured human mammary epithelial cells from seven individuals, whereas basal and induced levels of CYP1B1 expression showed only a 2.5-fold difference (54). Human mammary fibroblasts, located in breast stroma adjacent to human mammary epithelial cells, also express tetrachlorodibenzodioxin-inducible CYP1A1 mRNAs and protein but not CYP1A1 (72). This provides further evidence for tissue- and cell-specific regulation of CYP1 genes in the breast. Evidence for posttranscriptional regulation of CYP1A1 expression has been seen in studies in MCF-7 cells and showed a decrease in CYP1A1 mRNA stability in the presence of dehydroepiandrosterone (87), a finding that requires replicating in human mammary epithelial cells.

Induction of CYP1 expression in human mammary epithelial cells increases the metabolic activation of potential breast carcinogens via increased translation of CYP1 mRNA. Tetrachlorodibenzodioxin treatment increased formation of the precursor dihydrodiols of reactive diol-epoxides of the polycyclic aromatic hydrocarbon 7,12-dimethylbenz[a]anthracene (54). Similarly, benz[a]anthracene-mediated induction of CYP1A1 expression is paralleled by increases in the metabolic activation of IQ to form IQ-DNA adducts (58).

The expression of active CYP1B1 enzyme in human mammary epithelial cells may affect estrogen-initiated carcinogenesis as well as the metabolic activation of polycyclic aromatic hydrocarbons, aromatic amines, and heterocyclic amines. CYP1B1 hydroxylates estradiol to form 4-hydroxyestradiol (83), which has been implicated in the production of depurinating genotoxic semi-quinone and quinone estrogen byproducts (5, 43). These products may also be carcinogenic (Ref. 88; see also COMTs).

Four polymorphisms of the CYP1A1 gene have been reported (89), and two (exon 7 and MspI) may modulate breast cancer risk via metabolic activation of estrogen or exogenous genotoxins. Possession of the exon 7 (isoleucine→valine) allele was associated with increased CYP1A1 gene inducibility in one study (90) but not in another (91). Increased catalytic efficiency of the CYP1A1 enzyme encoded by the exon 7 allele in lymphocytes was reported in both studies (90, 91), but direct analysis of enzyme activity using in vitro expression systems has shown no difference between the enzymes encoded by the variant and wild-type alleles (92, 93). Women who have the exon 7 allele have an increased risk (but not statistically significant) of postmenopausal breast cancer [216 cases, 282 controls; OR, 1.61; 95% CI, 0.94–2.75 (94)], especially in combination with heavy smoking (OR, 5.22; CI, 1.16–23.56). A cytosine→thymine transition in the 3′ non-coding region of the CYP1A1 gene, generating an MspI restriction site, increases CYP1A1 mRNA inducibility (95, 96). One study of African-American women showed that the OR of breast cancer with the MspI homozygous variant was 9.7 (95% CI, 2.0–47.9), possibly because of effects on estrogen metabolism (97), but the confidence limits were large, reflecting the small size of the study. A nested case-control study of breast cancer risk (466 cases and 466 controls), however, found no association between possession of at least one of these polymorphic alleles compared with homozygous wild-type individuals, although an increased risk (MspI relative risk, 5.65; 95% CI, 1.50–21.3; exon 7 relative risk, 3.61; 95% CI, 1.11–11.7) was observed for those women who had commenced smoking before the age 18 (98). A multicentric study in Taiwanese women examining polymorphisms in genes encoding enzymes that are involved in estrogen biosynthesis (CYP17, aromatase), hydroxylation (CYP1A1, MspI polymorphism), and conjugation (COMT) showed that possession of the CYP1A1 MspI polymorphism contributed independently (OR, 2.11; 95% CI, 1.08–4.20) and in conjunction with other “high risk” phenotypes (OR not reported) to overall risk (99). A case-control study (Ref. 100: 219 breast cancer cases and 219 controls) has failed to demonstrate an association of CYP1B1 polymorphisms in exon 3 with breast cancer risk.

CYP2C, CYP2D, CYP2E, CYP3A, and CYP4A. Human CYP2C enzymes have been shown to 2-hydroxylate estradiol (101) to form catechol estrogens (5). They may be subject to aberrant mammary expression (Table 2), as the mammary- and ovarian-specific expression of a protein with immunoreactive properties similar to CYP2C6 has been reported (78). The mammary and ovarian expressed protein was of a slightly different molecular weight to CYP2C6 and other CYP2C enzymes that are expressed in the liver. The contribution of this mammary enzyme to carcinogen activation is, however, unknown. mRNA transcripts of the CYP2D6 gene are alternatively spliced in the human breast, where full-length mRNA is a minor transcript, and shorter variant splice versions of the transcribed gene predominate (102). Alternative splicing of the CYP2D6 gene may therefore represent a mechanism for mammary-specific regulation (102).

CYP2E1 metabolizes small molecules (103), including the rat mammary carcinogen benzene (104) and nitrosamines that are present in tobacco smoke (see Table 1). In mammary tissues from 15 of 15 individuals, CYP2E1 mRNAs and immunoreactive protein were de-

### Table 2 Mammary-specific regulation of enzymes expressed in the breast

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Difference in expression compared with the liver</th>
<th>Method of detection</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C</td>
<td>Distinct molecular weight of protein</td>
<td>Western blotting</td>
<td>(78)</td>
</tr>
<tr>
<td>CYP2D</td>
<td>Alternate mRNA splicing</td>
<td>RT-PCR, reverse transcription-PCR</td>
<td>(103)</td>
</tr>
<tr>
<td>GST µ</td>
<td>Two immunoreactive proteins (compared with one in the liver)</td>
<td>Western blotting</td>
<td>(102)</td>
</tr>
<tr>
<td>NAT2</td>
<td>Expression of mRNA but not of enzyme activity in the breast, whereas the liver expresses both</td>
<td>Semiquantitative RT-PCR</td>
<td>(69)</td>
</tr>
</tbody>
</table>

* RT-PCR, reverse transcription-PCR; GST, glutathione S-transferase.

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tectable (68). Hepatic CYP2E1 is induced by alcohol (105), but the regulation of mammary expressed CYP2E1 has not been investigated. Ethanol ingestion has been shown to enhance rat mammary carcinogenesis induced by 7,12-dimethylbenz[a]anthracene (106) and N-methyl-N-nitrosourea (107). Moderate consumption of alcohol is associated with slightly increased breast cancer risk in women (108), but it is unclear whether this effect is attributable to increases in endogenous estrogen levels or carcinogen activation.

Enzymes from the CYP3A family are the most important in terms of drug metabolism because of their hepatic abundance and wide substrate specificity (103). CYP3A enzymes are able to activate aflatoxins (109), heterocyclic amines (110), and nitrosamines (for references, see Table 1). CYP3A4 also catalyzes the 16α-hydroxylation of estrogen, a metabolite implicated in breast carcinogenesis (5). Expression of CYP3A4 and CYP3A5 mRNAs has been detected in most mammary tissues investigated (70). CYP4A hydroxylates fatty acids (see Table 1), and both CYP4A mRNAs and protein are expressed in breast tissues (68).

CYP19 (Aromatase). The CYP19 gene encodes a steroid aromatase that mediates the rate-limiting step in the metabolism of C19 androgen steroids to estrogens. CYP19 mRNA protein and have been detected in reduction mammoplasty tissues (68). Both immunocytochemistry and in situ hybridization assays identified aromatase enzyme and mRNA expression in the epithelial cells of the terminal ductal lobular units and surrounding stromal cells of the normal human breast (111). Increased activities of this enzyme may affect breast cancer risk (112), possibly by providing more estrogen for conversion to genotoxic metabolites, and/or by stimulating breast epithelial cell division. Possession of the rare A1 allele of this gene [incorporating 12 tetranucleotide (TTTA) repeats in intron 5] was here found to be 2.42 times more common among breast cancer patients than in controls (367 patients and 252 controls; 95% CI, 1.03–5.80; Ref. 112). Interestingly, nipple aspirate fluid has very high levels of dehydroepiandrosterone compared with plasma (113), indicating that androgen precursors are available for estrogen synthesis. It has been hypothesized that inhibitors of aromatase, such as aminoglutethimide, may act as chemopreventive agents by lowering levels of potentially carcinogenic estrogens (114).

COMT

COMT conjugates and inactivates catechol (i.e., 2-hydroxy) estrogens and is expressed at high levels in the liver and kidney but also in the breast, red blood cells, and the endometrium (115). The enzyme exists in both soluble and membrane-bound forms (116). Immunohistochemical staining has shown that COMT in normal mammary tissue is confined to ductal epithelial cells (116). Both 2-hydroxy and 4-hydroxy estrogens have been implicated in breast carcinogenesis via a genotoxic mechanism (Ref. 5; see also CYP enzymes, above).

A genetic polymorphism (guanine→adenine), creating a valine→methionine [COMT (Val)→COMT (Met)] substitution at codon 158, is associated with decreased activity (117). This has led to the suggestion that carriers, ~25% of Caucasians, may be less able to conjugate catechol estrogens and therefore may be at increased risk of breast cancer. One epidemiological study (654 cases and 642 controls) that has evaluated this has found no increase in breast cancer risk for individuals homozygous or heterozygous for the variant allele (118). Another study (281 cases and 289 community controls) associated increased risk of premenopausal breast cancer with COMT (Met/Met) genotypes (OR, 2.1; 95% CI, 1.4–4.3), compared with women with the homozygous COMT (Val/Val) genotype (119). Overweight women with the COMT Met/Met genotype were found to be especially at risk (OR, 5.7. 95% CI, 1.1–30.1). Possession of COMT Met/Met homozygosity was associated with decreased relative risk for postmenopausal women (OR, 0.4; 95% CI, 0.2–0.7) in one study (120) but with increased risk in another (Ref. 120; 112 cases and 112 controls; OR, 2.18; 95% CI, 0.93–5.11). Those women homozygous for the Met polymorphism with a high body mass index were at particularly high risk (OR, 3.58; 95% CI, 1.07–11.98; Ref. 120). A multicentric study focusing on estrogen metabolism in Taiwanese women (150 cases and 150 controls) found that of the three genes considered (CYP17, CYP1A1, and COMT), possession of the “low activity” COMT genotype remained the most significant risk factor (4-fold relative risk; 95% CI, 1.12–19.08; Ref. 99).

Epoxide Hydrolase

Polycyclic aromatic hydrocarbons are activated by a pathway that involves both CYP enzymes and epoxide hydrolase. A number of studies have shown the ability of human mammary epithelial cells to form DNA-binding products from polycyclic aromatic hydrocarbons (53, 55). An investigation of immunoreactive epoxide hydrolase protein gave positive signals in all samples of peritumoral breast tissues (11 of 11 individuals; Ref. 77). Polymorphisms in the epoxide hydrolase gene have been identified that may alter enzyme activity (121), and polymorphisms that affect mRNA levels have also been identified in 5’ flanking regions (122). Polymorphisms in the epoxide hydrolase gene may influence risk of liver, ovarian (123), and lung cancer (121), but no studies have evaluated the effect of epoxide hydrolase polymorphisms on breast cancer risk.

NATs (NAT1 and NAT2)

The NAT genes are intronless genes situated at two loci on chromosome 8p22 (124). Hepatically expressed NAT enzymes and mammary expressed NAT enzymes may have opposing effects on breast carcinogenesis, e.g., hepatic detoxication and increased excretability of potential breast carcinogens (48) and metabolic activation of N-hydroxylated HAS and AAs by O-acetylation in the mammary epithelium. N-hydroxylated NAT1 activates N-hydroxylated aminobiphenyls by O-acetylation, whereas both NAT1 and NAT2 (O-acetylation) activate N-hydroxy metabolites of heterocyclic amines (125).

There are at least 15 NAT1 alleles.4 NAT1*4 is the wild-type, whereas NAT1*14 and NAT1*15 are defective alleles with low catalytic activity (126, 127). Possession of NAT1*10 has been associated with elevated NAT1 activity in the bladder and colon (128) but not in the placenta or breast (69, 129). Possession of the NAT1*10 allele may increase the risk of breast cancer among former or recent smokers, although to date only two studies have been reported (130, 131). Among postmenopausal women (498 cases and 473 controls), recent smokers with the NAT1*10 allele were reported to be at greater risk of developing breast cancer (OR, 9.0; 95% CI, 1.9–41.8) than those without the NAT1*10 allele (OR, 2.5; 95% CI, 0.9–7.2) compared with nonsmokers (130). In another study (154 cases and 330 controls), Zheng et al. (131) reported a higher risk of postmenopausal breast cancer among smokers who possessed the NAT1*10 allele (OR, 3.3; 95% CI, 1.2–9.5) or the NAT1*11 allele (OR, 13.2; 95% CI, 1.5–116.0) compared with nonsmokers.

Fifteen NAT2 alleles have been characterized. NAT2*4 (125) and NAT2*14A (132) are associated with fast acetylation status. In Caucasian populations, 50% of individuals are “slow” NAT2 acetylators (two copies of low activity variant alleles). Both NAT1 and NAT2 mRNAs are detectable in human breast tissue (56, 69, 133). NAT1 mRNA transcript levels are 2–3-fold more abundant than NAT2, and

4 Internet address: http://www.louisville.edu/medschool/pharmacology/NAT.html.
NAT1 and NAT2 proteins are detectable in the breast epithelium using immunohistochemical assay (69). Immunohistochemical staining has shown an absence of NAT expression in the stromal cells (69). NAT1, but not NAT2, enzyme activity is detectable in cytosols prepared from human mammary tissue (56, 69) and human mammary epithelial cell lysates (56). The presence of NAT2 mRNA and the lack of measurable NAT2 enzyme activity is consistent with NAT2 enzyme expression in human mammary epithelial cells being low.

Two studies (131, 134) have reported an association between NAT2 status, cigarette smoking, and increased postmenopausal breast cancer, but another found no association (135). One study, based on 304 cases and 327 controls, showed a dose-dependent risk effect of previous smoking 2 and 20 years previously, with relative risks of 4.4 (95% CI, 1.3–14.8) and 3.9 (95% CI, 1.4–10.8), respectively (134). This association could possibly be explained by a slower hepatic detoxication of carcinogenic aromatic amines. An interesting finding was that smoking intensity, and not duration, seemed to be more important in elevating risk among postmenopausal slow NAT2 acetylators (134). This suggests that increased risk results from saturation of detoxication or of DNA repair pathways. In contrast, Millikan et al. (130) reported (based on 498 cases and 473 controls) greater risks for NAT2-fast acetylators (OR, 7.4; 95% CI, 1.6–32.6) than NAT2-slow acetylators (OR, 2.8; 95% CI, 0.4–8.0) for those smoking within the last 3 years. Another report (466 cases and 466 controls) showed no influence of NAT2 genotype on postmenopausal breast cancer risk among cigarette smokers (135).

The major known potential procarcinogenic substrates present in cooked meat are heterocyclic amines (135, 136) and polycyclic aromatic hydrocarbons (35). One study (273 cases and 657 controls) reported an association between “well done” cooked meat and increased breast cancer risk, especially for those who habitually consumed well-done bacon, beef, or hamburgers compared with those who consumed rare or medium meat (OR, 4.62; 95% CI, 1.36–15.70; Ref. 137). However, two investigations (Ambrosone et al. (138), based on 740 cases and 793 controls; and Gertig et al. (139), based on 466 cases and 466 controls) showed no increased risk of breast cancer with NAT2 acetylator status and meat consumption. Experiments with mammary cytosols (56, 69) show that NAT1 and SULT enzymes are the major enzymes contributing to the metabolic activation of N-hydroxy-heterocyclic amines (via O-acetylation), which may help to explain why fast NAT2 acetylator status does not influence breast cancer risk.

SULTs

SULTs can be broadly classified into two classes—the phenol SULTs (including estrogen SULT), encoded by SULT1 genes, and the hydroxysteroid SULTs, encoded by SULT2 genes. Conjugation with sulfate results in the inactivation of estrogens, because the addition of the charged sulfate group prevents the binding of the steroid to its receptor, thereby ameliorating its mitogenic effects. Sulfation also plays an important role in the activation and detoxification of carcinogens (Table 1). SULTs have been shown to activate some metabolites of polycyclic aromatic hydrocarbons (140) and the N-hydroxylated derivatives of aromatic amines (33) and heterocyclic amines (71, 141). SULT1A1 phenotype also seems to correlate with 4′-PhIP-SO₄ levels in the urine of individuals given [¹⁴C]PhIP. When SULT and immunoreactive protein have been investigated in human mammary epithelial cells, the presence of estrogen SULT, and not phenol or hydroxysteroid SULT (142), was detected, although the cells investigated had been passaged; hence, SULT expression may have been influenced by the culture conditions (142). This clearly requires further investigation, because another study of mammary cytosols derived from reduction mammmoplasty tissues revealed expression of the phenol SULTs SULT1A1 and SULT1A3, but estrogen SULT (SULT1E1) was undetectable (69). In passaged cultures of human mammary epithelial cells, estrogen SULT appears to contribute to the metabolic activation of N-hydroxylated heterocyclic amines by O-sulfation (Table 1; Ref. 71). Among the SULTs, SULT1A1 seems to be the most catalytically efficient in activating N-hydroxylated heterocyclic amines (143).

SULT-catalyzed sulfate transfer to N-hydroxylated aromatic and heterocyclic amines produces chemically unstable sulfoxo esters that readily generate DNA-reactive nitrenium ions. C-sulfation of these compounds can, however, be regarded as a detoxification step, except in circumstances where the sulfate is subsequently released by the action of sulfatasas, (e.g., 4′-PhIP-SO₂→PhIP), thus regenerating the parent compounds. A study of peritumoral tissues from 12 women (using 4-methylumbelliferone as substrate) reported sulfatase activities in the same order of magnitude as SULT activity (using umbelliferone sulfate as substrate; Ref. 77), suggesting similar levels of conjugation/deconjugation of SULT substrates (compare with glucuronidation, below). Although the chemical consequences of sulfation are well defined (e.g., for estrogen or N-hydroxylated heterocyclic amines), the consequences of desulfation are not fully understood but may have biological relevance. Polymorphisms in the SULT1A genes encoding phenol SULTs have been identified (144), but the effects of these polymorphisms on cancer risk have not been evaluated (145).

UDP-Glucuronyltransferases

Glucuronidation catalyzed by UDP-glucuronyltransferases is a major pathway of metabolism of estrogens (146) and androgens (147) in the breast. Glucuronidation is also a major pathway of detoxification metabolism for polycyclic aromatic hydrocarbons (148), heterocyclic amines (59), and aromatic amines (149). The relative pH stability of O-glucuronides of aromatic amines is consistent with a role in detoxification and excretion, whereas the acid lability of aromatic amine N-glucuronides is consistent with delivery of these amines to the bladder epithelium for activation. Like sulfation, glucuronidation produces polar conjugates that are readily excreted. A study of glucuronidation (of umbelliferone) in human breast peritumoral tissues (12 individuals) found levels of activity two orders of magnitude lower than levels of glucuronidase activity (77). This indicates a very low level of deglucuronidation of progencotoxic compounds such as N-hydroxy-PhIP glucuronide, which may be deglucuronidated to N-hydroxy-PhIP to provide a substrate for metabolic activation by SULT or NAT. In the rat, dietary supplementation with 3-glucaro-1,4-lactone, a β-glucuronidase inhibitor, inhibits proliferation of mammary glandular cells in vivo (150). It has been hypothesized that potent nontoxic inhibitors of β-glucuronidase may be useful in the prevention of estrogen-related cancers (146). This may also be relevant to degraduronidation of putative breast carcinogens, e.g., N-OH-PhIP glucuronides, which are the major metabolites in plasma after PhIP ingestion (59). Polymorphisms resulting in altered glcyuronidation activity have been identified (151, 152), but no studies on the effect of these polymorphisms on cancer risk have been reported.

Glutathione S-Transferases

Glutathione S-transferases play an important role in drug, carcinogen, and reactive oxygen species detoxication and act both as peroxi-dases and as catalysts of glutathione transfer to hydrophobic electro- philes. There are six major gene families (α, -M, -P, -S, -T, and -Z), respectively encoding six families of enzymes (α, μ, γ, π, θ, and ξ) with overlapping substrate specificities (153). Western blot analysis of
glutathione S-transferase proteins in breast tissues from 14 individuals showed expression of π and μ as the major isoforms, although glutathione S-transferase α was also detectable at lower levels (78). Variation in the mammary expression of glutathione S-transferase isoforms may be relevant to protecting against the genotoxic effects of HAs in the breast; glutathione S-transferase α (A1-1) detoxifies N-acetoxy-PhIP (thereby preventing DNA binding) in the presence of glutathione (154).

Anti-glutathione S-transferase μ antibody identified two immunoreactive proteins in the breast (see Table 2), but only one protein is detectable in the liver (78); glutathione S-transferase π was expressed in the mammary epithelial cells. Total glutathione S-transferase enzyme activity in mammary tissue determined by using 1-chloro-2,4-dinitrobenzene as substrate varied 19-fold (78). Although two enzymes have glutathione peroxidase activity, one selenium-dependent and one selenium-independent, the former was found to make up the majority of glutathione peroxidase activity in the breast (78). The selenium-dependent enzyme can reduce both hydrogen peroxide and organic hydroperoxides. The selenium-independent form will only metabolize organic hydroperoxides, although other xenobiotic substrates have not yet been investigated.

Polymorphisms exist in many of the glutathione S-transferase genes, in some cases resulting in an absence of the gene in some individuals, e.g., GSTM1 null genotype. Epidemiological evidence regarding the role of glutathione S-transferases in influencing breast cancer risk is equivocal. In a study of 110 cases and 113 controls, Helzlsouer et al. (155) reported an increased breast cancer risk in those with the GSTM1 null genotype (OR, 2.10; 95% CI, 1.22–3.64). In a French Caucasian population (361 cases and 437 controls), there was an association between deletion of the GSTM1 allele and breast cancer in the oldest group of patients (above the age of 55) but not in the younger age groups (156). However, a larger United States study (740 cases and 810 controls) found no association between possession of the null genotype and increased susceptibility (157).

**Peroxidases**

Peroxidase enzymes such as lactoperoxidase and myeloperoxidase have been implicated in mutagen and carcinogen activation in the human mammary gland (158, 159). The major endogenous function of mammary lactoperoxidase is thought to be to provide free radicals to combat bacterial and fungal infections. Lactoperoxidase mRNA is expressed in mammalian tissue (160), and lactoperoxidase immunoreactive protein is up-regulated in women during lactation (161). Peroxidase enzymes have wide substrate specificity (e.g., human, mammalian, and plant peroxidases perform N-oxidation reactions; Ref. 162). The human gene has been little studied, but bovine lactoperoxidase (in the presence of hydrogen peroxide) metabolically activates 17β-estradiol (163), 4-hydroxy catechol estrogens (43), IQ (73), benzidine (164), and other aromatic amines (162) and causes free radical-induced DNA damage via oxidation of polychlorinated biphenyls (165).

Human myeloperoxidase is expressed in neutrophils and is present in breast milk and blood. Sodium azide-inhibitable myeloperoxidase is responsible for the metabolic activation of IQ to DNA-binding products in phorbol myristate acetate-stimulated neutrophils (58), which can also metabolically activate MeIQx (166), aromatic amines (167), and polycyclic aromatic hydrocarbons (168). A study by London et al. (Ref. 169; 182 cases and 459 controls) found that individuals with two inherited copies of myeloperoxidase alleles that favored lower transcription of the gene were at reduced risk (OR, 0.30; 95% CI, 0.10–0.93) of developing lung cancer compared with heterozygotes and those with two wild-type alleles. Peroxidases may therefore be significant in the activation of carcinogens in extrahepatic tissues (including the breast). Myeloperoxidase also catalyzes the production of cholesterol epoxides (113). These compounds are mutagenic, and possibly carcinogenic in breast tissue (170).

The inducible form of COX, also known as prostaglandin synthetase, is the rate-limiting enzyme in prostaglandin biosynthesis. Xenobiotics have been suggested to act as reducing factors in the peroxidative reduction of prostaglandins (171), and it has been shown in vitro that a number of chemicals including the heterocyclic amine IQ (172) and the polycyclic aromatic hydrocarbon benz[a]pyrene (173), can be cooxidized by this pathway into reactive intermediates by ram vesicle COX. COX may, therefore, play an important part in carcinogen activation in extrahepatic tissues, especially where CYP enzyme activity is low. Immunohistochemical and Western blot analysis of COX-1 showed expression in 14 of 14 breast tissue samples (174). In human mammary epithelial cells, however, the COX inhibitor indomethacin did not significantly affect metabolic activation of IQ (58), although the enzyme has been reported to bioactivate N-hydroxy-PhIP in a proportion of human mammary epithelial cell microsomes (141).

**Lipoxygenase**

Eicosanoids derived from lipoxygenase- and cyclooxygenase-derived metabolism have been implicated in tumor promotion, progression, and metastatic disease (175). Lipoxygenase has also been implicated in carcinogen activation. Rat lung lipoxygenase metabolically activates rat carcinogens such as benzidine (176), ortho-dianisidine (176), and benzo[a]pyrene (177). This activation can be inhibited by lipoxygenase inhibitors (176). In one study, human 12-lipoxygenase mRNA was barely detectable in normal breast tissue (178), and the lipoxygenase inhibitor eicosatetraenoic acid did not significantly affect metabolic activation of the heterocyclic amine IQ in human mammary epithelial cells (58). Therefore, it is not yet clear whether a lipoxygenase-mediated activation pathway is significant in human mammary tissue.

**Concluding Remarks and Future Perspectives**

Greater understanding of the endogenous role and regulation of mammary expressed enzymes should be useful in determining the contributions of polymorphic enzymes to carcinogenesis. Laboratory-based studies can lead or complement epidemiology studies focusing on gene-environment interactions. Although hepatic enzymes are likely to play a role in the metabolism of potential breast carcinogens, it is probable that enzymes locally expressed in the target site also have an important influence in modulating levels of DNA-reactive species.

Breast cancer is a multifactorial disease. An energy-rich diet may put women at risk by a mechanism affecting estrogen levels, but the number of cases attributable to this is difficult to estimate (179). Hormonal factors that reflect reproductive habits and inherited high penetrance genes are not identifiable factors in two thirds of cases. Alternatively, induction of breast cancer by ionizing radiation indicates that mammary epithelial cells are vulnerable to DNA-damaging agents. It would therefore be surprising if exposure of the breast to environmental or exogenous genotoxins did not have consequences for tumor initiation. What is currently lacking, however, is knowledge of how significant this risk is and what the genotoxin(s) is. Nevertheless, cumulative analysis of p53 gene mutations in breast tumors has revealed a mixture of mutations associated with apparently endogenous events (e.g., transitions at CpG dinucleotides attributable to spontaneous deamination of 5-methylcytosine residues at CpG sequences) and mutations (typically transversions at non-CpG sites)
characteristic of those induced by exogenous, DNA-binding agents (180).

Numerous studies have looked for associations between genetic polymorphisms and risk of breast cancer (90, 181). However, little attention has been paid to the genes that are actually expressed in the mammary gland. Future studies should incorporate qualitative assessment of isoforms expressed in human mammary epithelial cells or mammary cytosols. It will also be important to make quantitative assessment of enzyme activities using isoform-specific substrates. Immunohistochemical analysis of sections of breast tissue will generate information on localization of enzyme expression within the tissue. With the evidence that the human mammary gland is potentially exposed to a wide variety of genotoxic agents and the advent of sensitive methods for determining enzyme activity in human tissue, investigation of xenobiotic metabolizing gene expression and enzyme activity should be integrated into molecular epidemiological studies of breast cancer.

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MAMMARY EXPRESSION OF METABOLIZING ENZYMES AND BREAST CANCER


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