Cytogenetic Markers of Susceptibility: Influence of Polymorphic Carcinogen-metabolizing Enzymes

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Polymorphisms of xenobiotic-metabolizing enzymes, responsible for individual differences in metabolic activation and detoxification reactions, may profoundly modulate the effects of chemical carcinogens. In the case of genotoxic carcinogens, differences in biological effects due to genetic polymorphisms can be evaluated by cytogenetic methods such as the analysis of chromosomal aberrations (CAS), sister chromatid exchanges (SCEs), micronuclei (MN), and changes in chromosome number. These techniques can be applied to any exposure known to induce such alterations, without additional method development for each exposing agent. The influence of polymorphic genes on the cytogenetic effects of a carcinogen can quickly be tested in vitro using metabolically competent cells collected from donors representing different genotypes or phenotypes. For instance, erythrocytes from individuals positive for glutathione-S-transferase T1 (GSTT1) express GSTT1, whereas GSTT1-null donors, having a homozygous deletion of the GSTT1 gene, completely lack this detoxification enzyme. This deficiency results in highly increased sensitivity to SCE induction in whole-blood lymphocyte cultures by 1,2,3,4-tetrahydroxybutane, a reactive metabolite of 1,3-butadiene. The same cytogenetic techniques can also be applied as effect biomarkers in studies of human populations exposed to genotoxic carcinogens. For example, elevated rates of chromosome damage have been detected among smokers lacking glutathione-S-transferase M1 (GSTM1-null genotype), and the baseline level of SCEs seems to be increased in GSTT1-null individuals. Information obtained from cytogenetic studies of genetic polymorphisms can be used, for example, to recognize the genotoxically relevant substrates of the polymorphic enzymes, to identify genotypes that are susceptible to these genotoxins, to improve in vitro genotoxicity tests utilizing human cells, to increase the sensitivity of cytogenetic endpoints as biomarkers of genotoxic effects in humans, and to direct mechanistic studies and cancer epidemiology. — Environ Health Perspect 105(Suppl 4):829-835 (1997)

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Introduction

Susceptibility to the hazardous action of chemicals may derive from genetic or acquired characteristics of the individual. In the case of genotoxic carcinogens, susceptibility can be associated with individual differences, e.g., in the efficiency of carcinogen metabolism or in the ability to repair DNA lesions induced by the carcinogen. Certain recessive genes, which among homozygotes result in chromosome instability syndromes characterized by defects of DNA repair or recombination, increased baseline or induced chromosome damage, and high cancer incidence—may also increase cancer in heterozygotes (1,2). Besides such heterozygotes, sensitivity to genotoxic carcinogens could be associated, for example, with subtle genetic polymorphisms influencing DNA repair processes, although such traits have not yet been found in the human population. On the other hand, studies of individual differences in xenobiotic metabolism have revealed an increasing number of polymorphic enzymes that are involved in the metabolism of carcinogens (3,4).

Biotransformation, involving metabolic activation and detoxification, plays a central role in determining the ultimate effects of exposure to chemical carcinogens. Depending on the enzyme, there may be one or several mutant alleles that produce enzyme variants with reduced or increased efficiency in comparison with the wild-type form. In some cases, such as the null genotypes of glutathione S-transferases M1 (GSTM1) and T1 (GSTT1), the functional enzyme is completely missing (3).

Numerous epidemiological studies have indicated an over-representation of some metabolic genotypes or phenotypes in various forms of cancer, suggesting that those individuals are excessively prone to such diseases (3,4). With the possible exception of tobacco smoking in, for example, lung cancer associated with the GSTM1 and cytochrome P450 (CYP) polymorphisms, epidemiology is not usually able to exactly pinpoint the exposures that are responsible for the findings, as the critical influence of carcinogen-metabolizing enzymes occurs several years before exposure-associated cancer develops.

The question of individual sensitivity to genotoxic carcinogens can also be viewed by studying the involvement of genetic polymorphisms in determining genotoxic response after exposure to specific carcinogens. For this purpose, methods based on genotoxicity assays with human cells, such as cytogenetic techniques, are very useful as they can be applied to any exposures known to produce such alterations. Thus, no specific method development for each new exposing agent is required, and the net effect of complex exposures can be evaluated. Differences between genotypes can be assessed by comparing cytogenetic responses after in vitro exposure or in individuals exposed to the genotoxins of interest in vivo.

The present review will concentrate on the application of cytogenetic biomarkers—chromosomal aberrations (CAS), sister chromatid exchanges (SCEs), micronuclei (MN), and numerical chromosome changes—in studies of individual susceptibility related to polymorphisms of carcinogen-metabolizing enzymes.
Cytogenetic Parameters

The four cytogenetic parameters already mentioned—CAs, SCEs, MN, and numerical chromosome alterations—are all based on microscopic analyses of cell specimens, but each of them measures a different genotoxic phenomenon. In humans, cytogenetic alterations are most often scored from peripheral lymphocytes after they have been stimulated to divide in culture by a mitogen, e.g., phytohemagglutinin (5).

CAs are structural alterations, breaks and rearrangements, in chromosomes, usually observed in metaphase-blocked cells using conventional microscopy (5). In principle, chromosomal aberrations can be divided into chromosome-type lesions—produced by ionizing radiations and some chemical clastogens in G0-G1-stage cells—and chromatid-type lesions that ionizing radiation causes in S-G2-stage cells and that are the main type of aberrations induced by most (S-phase dependent) chemical clastogens. Chromosome-type rearrangements, such as translocations and dicentrics inspected in biological dosimetry of radiation, can also be analyzed using chromosome painting based on fluorescence in situ hybridization (FISH) with chromosome-specific DNA probe libraries (6). Recently, a simplified FISH method to score chromosome breakage and alterations of chromosome number, using tandem DNA probes specific for a region in chromosome number 1, was reported (7).

Polymerase chain reaction-based methods for the analysis of lymphocyte-specific illegitimate chromosome recombination involving human immunoglobulin or immune receptor loci and considered to depict genetic instability have also been described (8,9). In two recent independent reports, increased rates of CAs in peripheral lymphocytes were shown to be associated with later development of cancer (10,11). Thus, the analysis of CAs is presently considered to be the cytogenetic method of choice in studies of human exposure to genotoxic carcinogens. A wide range of genotoxins have shown CA induction in vitro and in vivo. Known in vivo inducers of CAs in humans include ionizing radiations, alkylating cytostatics, tobacco smoking, benzene, and styrene.

SCEs represent symmetrical exchanges of DNA segments between the sister chromatids of a duplicated metaphase chromosome and (because the sister chromatids are supposed to be equal) are not themselves mutations. SCEs are formed during the S-phase of the cell cycle and are efficiently induced by, for example, UV light and many chemical genotoxins, especially the S-phase dependent clastogens (12). The fact that each cell examined yields an SCE score makes the technique a very powerful tool for the assessment of differences between genotypes, particularly in vitro. In human lymphocytes in vivo, tobacco smoking, alkylating cytostatics, and ethylene oxide are well-documented SCE inducers.

Micronuclei are small additional nuclei observable in interphase cells. They are formed fromacentric chromosome- or chromatid-type fragments and whole chromosomes that have lagged behind in cell division, being left outside both daughter nuclei (13). Because of their dual origin (fragments or whole chromosomes), MN induction can be triggered by either clastogens or agents influencing the mitotic apparatus, such as spindle poisons. As cell division is a prerequisite for MN formation, MN in cultured peripheral lymphocytes are usually analyzed from cytokinesis-blocked cells, to identify cells that have divided once in culture (14). MN can also be scored in exfoliated cells of buccal, nasal, esophageal, bronchial, or urothelial mucosa (15). The presence of whole chromosomes in MN can be checked by identifying centromeric DNA sequences or kinetochore proteins in the MN (16,17). MN analysis appears to be a good in vitro tool to investigate the effects of clastogens and agents (aneuploidogens) that induce numerical abnormalities of chromosomes. In vivo, increased MN frequencies have been associated with exposure to ionizing radiation, aging, and gender (18). In buccal or nasal mucosa, MN induction has clearly been shown for various ethnic tobacco-chewing habits (15) and exposure to formaldehyde (19).

The development of FISH techniques has made it possible to detect, in a very simple manner, the copy numbers of any specific human chromosome for which a centromeric DNA probe is available (20). Both aneuploid and polyplloid cells are identified, although hyperdiploids cannot usually be distinguished from polyploids unless probes for more than two chromosomes are used. The tandem FISH assay mentioned above provides information on both structural and numerical chromosomal aberrations (7). Such analyses have thus far been used as biomarkers in humans only in a few cases, although they can be applied, besides peripheral lymphocytes, to any human tissue available.

Genetic Polymorphisms and Cytogenetic Assays in Vitro

The involvement of a polymorphism in determining the cytogenetic effects of a specific genotoxin can easily be tested in vitro in cultured cells collected from donors representing different genotypes or phenotypes. A requirement is, of course, that the cells used adequately express the polymorphic condition, i.e., in the case of metabolic genes, the enzyme in question. In the examples available, peripheral lymphocytes have been used, which are also the most convenient choice, because of easy availability and well-established culture techniques (Table 1).

The first example of the utilization of the in vitro approach was the study of Wiencek et al. (21) in which the effects of two isomeric epoxides, trans-stilbene oxide and cis-stilbene oxide, on SCEs were studied in cultured lymphocytes from persons able or unable to conjugate trans-stilbene oxide with glutathione. This trait reflects the genetic polymorphism of GSTM1 that is expressed in the leukocytes of the conjugators (corresponding to the GSTM1-positive genotype, with at least one copy of the intact gene) but is totally absent from the nonconjugators (the GSTM1-null genotype having a homozygous deletion of the gene). The nonconjugators were clearly more sensitive than the conjugators to SCE induction by trans-stilbene oxide, a substrate of GSTM1; no clear difference was observed between the phenotypes in tests with cis-stilbene oxide, which is not a good substrate for GSTM1.

GSTM1-mediated glutathione conjugation also appeared to be involved in the case of 1,2-epoxy-3-buten (22), as lymphocytes of GSTM1-null donors were more sensitive than GSTM1-positive donors to SCE induction by this epoxide metabolite of 1,3-butadiene. Another epoxide, styrene 7,8-oxide, a metabolite of styrene, showed the same SCE induction in both genotypes, and appeared, therefore, not to be dependent on the GSTM1-catalyzed detoxification (22). The GSTM1 genotype (or phenotype) did not influence SCE induction by two other reactive metabolites of 1,3-butadiene—1,2,3,4-diepoxybutane and 3,4-epoxybutene-1,2-diol (23-25).

Haller et al. (26) described another polymorphism, identified by the presence or absence of methyl bromide glutathione conjugation in erythrocytes. After treatment of whole-blood samples, the nonconjugators' lymphocytes showed SCE induction by methyl bromide, methylene chloride,
Table 1. Results of in vitro studies on the influence of genetic polymorphisms of carcinogen-metabolizing enzymes on the induction of chromosomal aberrations, sister chromatid exchanges, and micronuclei in cultured human lymphocytes.

| Genotype or phenotype studied | Chemical                    | Cytogenetic end point | Risk genotype or phenotype<sup>*</sup> | Reference                  |
|------------------------------|-----------------------------|-----------------------|-----------------------------------------|----------------------------|
| GSTM1 activity               | trans-Stilbene oxide        | SCEs                  | GSTM1-deficient                        | Wiencke et al. (21)       |
| ciss-Stilbene oxide          | SCEs                        | None                  |                                         | Wiencke et al. (21)       |
| DEB                          | SCEs                        | None                  |                                         | Wiencke and Kelsey (24)   |
| GSTM1 genotype               | 1,2-Epoxy-3-butene          | SCEs                  | GSTM1-null                             | Uusikivi et al. (22)      |
| Styrene 7,8-oxide             | SCEs                        | None                  |                                         | Uusikivi et al. (22)      |
| DEB                          | SCEs                        | None                  |                                         | Norppa et al. (23)        |
| GSTT1 activity               | EBD                         | SCEs                  | Nonconjugator                          | Bernadini et al. (25)     |
| Methyl bromide               | SCEs                        | Nonconjugator         |                                         | Hallier et al. (26)       |
| Ethylene oxide               | SCEs                        | Nonconjugator         |                                         | Hallier et al. (26)       |
| Dichloromethane              | SCEs                        | Nonconjugator         |                                         | Norppa et al. (23)        |
| DEB                          | SCEs                        | Nonconjugator         |                                         |                          |
| DEB-sensitivity              | DEB                         | SCEs, CAs             | DEB-sensitive                          | Norppa et al. (23); Wiencke and Kelsey (24); Wiencke et al. (27,28,32); Kelsey et al. (29,30); Landi et al. (31) |
| GSTT1 genotype               | 1,2-Epoxy-3-butene          | SCEs                  | DEB-sensitive                          | Wiencke and Kelsey (24)   |
| Nitrogen mustard             | SCEs                        | None                  |                                         |                           |
| DEB                          | SCEs, MN                    | GSTT1-null            |                                         | Norppa et al. (23,33); Kelsey et al. (30); Landi et al. (31) |
| GSTT1 genotype               | EBD                         | SCEs                  | GSTT1-null                             | Norppa et al. (23,33); Kelsey et al. (30); Landi et al. (31) |
| Styrene 7,8-oxide             | SCEs                        | None                  |                                         | Bernadini et al. (25)     |
| ALDH2 genotype               | Hydroquinone                | SCEs                  | ALDH2-deficient                        | Morimoto and Takeshita (41) |

Abbreviations: ALDH2, aldehyde dehydrogenase 2; CAs, chromosomal aberrations; DEB, 1,2,3,4-diepoxybutane; DEB sensitivity, enhanced SCE response to 1,2,3,4-diepoxybutane; EBD, 3,4-epoxy-1,2-butanol; GSTM1, glutathione S-transferase M1; GSTT1, glutathione S-transferase T1; MN, micronuclei; SCEs, sister chromatid exchanges. *Genotype or phenotype giving an enhanced cytogenetic response.

It is presently unclear what the DEB-sensitive GSTT1-positive individuals described by Wiencke et al. (32) actually represent. A point mutation resulting in a loss of GSTT1 activity, which would not be detected by usual GSTT1 genotyping, has been described by Warholm et al. (35); this mutation was, however, very rare (2 among 270 donors) in the Swedish population studied. In cultures of purified lymphocytes—without any erythrocytes and no GSTT1—the DEB-resistant (GSTT1-positive) donors showed very similar SCE induction as the DEB-sensitive (GSTT1-null) donors (34), and the addition of erythrocytes from a resistant person to the purified lymphocytes of a sensitive person removed the DEB sensitivity (36).

In Salmonella typhimurium transfected with human GSTT1, the expression of GSTT1 activity increased the mutagenicity of DEB, thus functioning as a metabolic activation route (37). Therefore, it would appear that glutathione conjugation of DEB leads to enhanced genotoxicity in (bacterial) cells, where the target DNA is present, while the same reaction in erythrocytes of whole-blood lymphocyte cultures prevents DEB from reacting with DNA. Although epidemiologic studies have shown several examples of increased disease risk in GSTT1-null but not in GSTT1-positive individuals (3) it may also be possible that GSTT1 activates some tissue- or cell-specific mutagens in humans (38).

Our recent findings suggested that the GSTT1 nulls also show higher SCE induction by an in vitro treatment with styrene 7,8-oxide, although the effect was not nearly as dramatic as with DEB (39). This result appears to agree with the fact that glutathione conjugation is a minor pathway in styrene 7,8-oxide detoxification. However, the proposed main detoxification enzyme of styrene 7,8-oxide, microsomal epoxide hydrolase, is also polymorphic (3,40), albeit the significance of this polymorphism in determining epoxide hydrolase levels in humans is still unclear. It must be kept in mind in all studies correlating biological effects with genetic polymorphisms that differences in mean biological responses between genotypes are not necessarily attributable to that particular genotype, but may also be due to such uncontrolled factors as other unknown or unchecked polymorphisms, acquired differences in enzyme levels, or experimental variation.

The deficiency of low Km aldehyde dehydrogenase (ALDH2), resulting in impaired detoxification of acetaldehyde, is
common in Oriental populations. About one-half of all Japanese are either heterozygotes (ALDH2/ALDH2) or homozygotes (ALDH2/ALDH2) for a mutation in the ALDH2 gene (41). Morimoto and Takeshita (41) observed that lymphocytes of ALDH2-deficient donors are more sensitive than those of ALDH2-proficient individuals (ALDH2/ALDH2) to SCE induction by an in vitro treatment with hydroquinone, a metabolite of benzene. The genotype effect was higher in habitual daily alcohol drinkers than in those who consumed alcohol less frequently. The authors did not provide an explanation for these findings.

Another aspect, aside from the use of the in vitro cytogenetic assays to identify dependence on genetic polymorphisms, is the influence of genotype data on routine genotoxicity testing using human cells. For instance, lymphocytes from GSTM1-null donors will respond higher to certain treatments than individuals with the GSTM1-positive genotype, and differential responses obtained, e.g., between human whole-blood and isolated lymphocyte cultures, may partly be explained by the GSTT1 activity of red blood cells not present in purified lymphocyte cultures. In critical cases, such as those shown by Hallier et al. (26) for methyl bromide, methylene chloride, and ethylene oxide, one phenotype (conjugators) will give a negative result and another (nonconjugators) positive.

Individual Susceptibility and Cytogenetic Markers in Vivo

The few studies available on genetic polymorphism and the rate of cyto genetic alterations in humans in vivo have almost exclusively been performed using peripheral lymphocytes. Thus far, the studies have addressed induced or baseline cytogenetic damage in association with polymorphisms of GSTM1, GSTT1, cytochrome P450IA1 (CYP1A1), cytochrome P4502D6 (CYP2D6) and N-acetyltransferase 2 (NAT2).

The first investigation describing an association between genetic polymorphisms and cytogenetic response to a genotoxic exposure in man in vivo was the report of van Poppel et al. (42) on a small increase in SCEs in heavy smokers not expressing GSTM1, as compared with GSTM1-proficient heavy smokers; the effect was not observed in moderate smokers or nonsmokers. MN in sputum cells were not influenced by smoking or the GSTM1 phenotype (43).

Cheng et al. (44–46) observed a significant association between the GSTM1-null genotype and elevated SCE frequencies (but not MN) in lymphocytes of healthy control persons (but not of lung cancer patients). However, the finding appeared not to be associated with smoking. CYP1A1MspI and 11e-Val mutations and CYP2D6G to A mutations did not influence SCE frequencies among the controls (44).

The influence of the GSTM1 genotype on smoking-induced chromosome damage was further supported by our recent studies, which show a statistically significant association between the GSTM1-null genotype and elevated CA rates (but not SCE or MN) in lymphocytes of smoking Italian greenhouse workers and controls (47,48). The effect particularly concerned chromatic-type aberrations. The pesticide exposure of the greenhouse workers had no significant influence on CAs, SCEs, or MN. No dependence of the cytogenetic biomarkers on GSTTI or NAT2 genotypes was observed, except for a slightly higher baseline SCE level among GSTTI-positive donors in comparison with GSTTI-null individuals; this finding was possibly due to chance, as there were only four GSTTI-null subjects (47).

The preliminary results of another recent study among Danish bus drivers indicated an increasing gradient of CA frequencies from GSTM1-positive/NAT2 fast acetylator genotype to GSTM1-null/NAT2 slow acetylator genotype (39). This finding suggested that these genotypes modulate the genotoxic effects of exposures (diesel exhaust) experienced by the bus drivers in their work.

Carstensen et al. (49) and Ichiba et al. (50) studied MN in T- and B-lymphocytes of chimney sweeps, but could not observe any significant association between MN frequency and GSTM1 and CYP1A1 genotypes, in connection with the occupational exposure or smoking. Although the GSTM1 genotype itself was not significantly associated with variations in MN frequency, correlation between the frequency of MN in T-lymphocytes and aromatic DNA adducts in total white blood cells was statistically significant among GSTM1-null individuals (sweeps and controls combined) (50). CYP1A1MspI and 11e-Val polymorphisms did not influence MN frequencies, although the common “noninducible” MspI genotype m1/m1 showed significantly higher DNA adduct levels than the tentative high-inducible m1/m2 genotype (50).

There are also studies suggesting no influence of GSTM1 genotype on cytogenetic damage in human lymphocytes, but these data derive from control cultures established for in vitro studies not designed to reveal differences in baseline rate of cytogenetic damage (21–23,28).

The clear difference in sensitivity to DEB (metabolite of 1,3-butadiene) in vitro in association with the GSTTI genotype (above) has also triggered in vivo cytogenetic studies on a possible genotype effect among 1,3-butadiene production workers. The preliminary results of a European study showed an increase in CA in lymphocytes of GSTTI-null workers in comparison with GSTTI-positive workers (51). A study in the United States on SCEs in 1,3-butadiene producers, however, failed to show any association with the GSTTI genotype (30). Due to automated processes, exposure to butadiene in the facilities studied was fairly low. The positive European study suggested that genotype determination may improve the sensitivity of cytogenetic assays in detecting the effects of low-level genotoxic exposures.

The American study did confirm the higher background SCE frequency of GSTTI-null subjects as compared with GSTTI-positive individuals (30). Wiencie et al. (32) calculated that the DEB sensitivity and the GSTTI genotype were the most important factors influencing baseline SCE frequency, explaining 37 and 27%, respectively, of the variation observed. Smoking, for instance, was found to explain only 6 to 16% of the variation. The genotype effect was independent of smoking and was thought to reflect exposure to internal or ubiquitous external factors dependent on glutathione conjugation (32). One candidate could be ethylene oxide, a tentative substrate of GSTT1 (26), formed by xenobiotic metabolism from endogenous ethylene. CA count was not significantly elevated in the lymphocytes of the DEB-sensitive subjects (28).

Morimoto and Takeshita (41) observed that ALDH2-deficient habitual alcohol drinkers had higher mean frequency of SCEs in lymphocytes than ALDH2-proficient individuals who consumed alcohol daily. This effect probably reflected the higher acetaldehyde levels found in the peripheral blood of the ALDH2-deficient subjects who also showed an elevated level of acetaldehyde adducts in hemoglobin (52). Other lifestyle factors may also play a role, since a significant genotype effect could be shown in smokers but not in nonsmokers (41).
Conclusions

The relationship between human genetic polymorphisms and genotoxicity is a new field of research and only a limited amount of information is presently available. Already, the data available suggest that donor genotype can have dramatic influence on induced or baseline levels of cyto genetic alterations both in vitro and in vivo.

The use of in vitro assays to test the impact of specific genotypes or phenotypes on the cyto genetic effects of genotoxins is highly recommended as it will give an idea about the biological significance of genotypic differences, which is not revealed, for example, by enzyme activity measurements alone. In this way, in vitro experiments can serve as a guideline for selecting exposures and genotypes to be studied in vivo. A prerequisite is, of course, that the polymorphisms of interest are expressed in the cells. Adequate expression has been shown at least for GST M1 in human lymphocytes and GST T1 in erythrocytes. The relationship between the GST T1 genotype and sensitivity to SCE induction by DEB seems to be a good example of an exceptionally clear association.

Understanding the significance of genetic polymorphisms in determining genotoxic response will also have an important influence on requirements concerning the use of human cells in genotoxicity testing. Some discrepancies between negative and positive findings or dramatically different active concentration ranges will be explained by different genotypes of the donors.

Studies of cyto genetic biomarkers among exposed humans show that the determination of polymorphisms is becoming an increasingly important aspect that may make the assays more sensitive and more specific in identifying the effect and the sensitive subgroups. At present, it seems that at least GST M1 and GST T1 genotypes of the donors should be studied on a routine basis when in vivo cyto genetic effects are concerned. Rarer genotypes can be evaluated only in large populations—even the 10 to 20% prevalence of the GST T1-null genotype among Caucasians may be too low to allow evaluation if the exposure groups are small. Besides good knowledge in genetic toxicology and molecular genetics, expertise in epidemiology is becoming quite useful in planning such exercises, and statistical treatment will not be valid without multivariate analyses in which all of the variables can be duly taken into account.

There will also be much to be learned in risk assessment. Restricted still in most cases to surrogate tissues, such as peripheral lymphocytes, instead of the real targets of carcinogenesis, many things can go wrong in assessing the risk. For instance, the expression or role of the critical metabolizing enzymes may vary in different tissues. From these considerations, one arrives at the ethical issues that are dealt with more thoroughly elsewhere in this issue (53). It is obvious that the cyto geneticist is faced with ethical questions both in sample collection and when the results of the research are used in risk estimation. It can be gathered from what has been presented above that sound conclusions about the role of genetic polymorphisms in determining genotoxic risks can be achieved only when the complex issue has been studied in depth.

Information obtained from cyto genetic studies of genetic polymorphisms can be used to recognize the genotoxically relevant substrates of the polymorphic enzymes, to identify susceptible genotypes, and to improve the sensitivity of cyto genetic assays both in vitro and in vivo. Such knowledge can be utilized in mechanistic studies and cancer epidemiology. In search for the role of genetic polymorphisms in carcinogenesis, the cyto genetic markers form a link in the chain of evidence which stretches from experimental in vitro work, through analyses of exposed humans, to studies on cancer. Genetic polymorphisms can be followed in all of these steps.

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