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REGULATORY RESEARCH PERSPECTIVES

Impact on Public Health

Developing Methods of Genetic Analysis to Improve Cancer Risk Assessment

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Abstract: Cancer risk assessment is currently based mainly on the two year rodent cancer bioassay. Advances in the scientific understanding of the genetic basis of cancer are now making it possible to improve risk assessment by obtaining and applying information about a chemical's mode of action. However, routine methods of monitoring the genetic damage that leads to cancer are not yet available. At the FDA National Center for Toxicological Research (NCTR), genotypic selection methods are being developed as an approach for obtaining such information. Genotypic selection refers to the DNA-based detection of rare mutation. Using genotypic selection, the oncogene and tumor suppressor gene mutations that are the earliest persistent changes in tumor development can be measured and used as biomarkers for cancer risk. Three different genotypic selection methods have been developed. The first assay (MutEx enrichment) uses the mismatch binding protein, MutS to shield mutant DNA while wild-type DNA is selectively degraded. The second assay is an allele-specific amplification technique called ACB-PCR. By coupling these two techniques, the third assay, MutEx/ACB-PCR was developed. This assay has the sensitivity to detect mutant DNA in the presence of a 10^7-fold excess of wild-type DNA.

Introduction

Acquiring the capability to precisely assess the risk associated with chemical exposure is vitally important in terms of human health. Many different public health issues revolve around whether or not a chemical exposure causes cancer. In terms of drugs, for instance, the therapeutic value of a particular drug must be weighed against that drug's potential to induce cancer. In this context, precision in assessing cancer risk could translate into better healthcare decisionmaking. In terms of potentially carcinogenic environmental exposures, the wisdom of reducing or eliminating an exposure can only be judged if the cancer risks associated with the different levels of exposure are reliably known. The approaches used to show that a chemical is, or is not, a cancer hazard must be robust and credible if they are to attach public confidence. Unfortunately, our risk assessment capabilities are not yet viewed as having the level of scientific rigor warranted by the degree to which cancer risk assessment impacts human health (1).

Many of the regulatory responsibilities of the US Food and Drug Administration (FDA) involve assessing the cancer risk associated with chemical exposures (2). Of the five FDA product centers, three of them have regulatory responsibilities that involve cancer risk assessment. The Center for Food Safety and Applied Nutrition (CFSAN) is responsible for determining whether food additives and contaminants of food additives are carcinogenic. The Center for Veterinary Medicine (CVM) is responsible for ensuring that veterinary drugs used in food-producing animals do not leave carcinogenic food residues. The Center for Drug Evaluation and Research (CDER) must determine whether new drugs entering the drug ap-

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proval process are carcinogenic. Clearly, improving available methods for assessing chemical associated cancer risk is an issue of great importance to the FDA. Because the National Center for Toxicological Research (NCTR) performs fundamental research to support the regulatory needs of the other FDA centers, an important part of the NCTR's mission is to develop, characterize, and validate new cancer risk assessment approaches.

Current Cancer Risk Assessment Practices

Cancer risk assessment can be described as a three-step process: hazard identification, carcinogenicity determination, and dose-response assessment. In terms of hazard identification, the genotoxicity of a chemical is examined using a battery of tests. Because chemicals that cause DNA damage can be carcinogens, these tests identify chemicals that cause various types of DNA damage, such as point mutations, DNA strand breaks, loss of heterozygosity, or aneuploidy. The test battery includes a bacterial gene mutation assay, an *in vivo* test for chromosome damage in rodent hematopoietic cells, and either an *in vitro* cytogenetic test with mammalian cells or the *in vitro* mouse lymphoma mutation assay that selects for inactivation or loss of the thymidine kinase gene (3). Although short-term transgenic cancer assays are presently being assessed, at the present time carcinogenicity is evaluated using the two species rodent tumor bioassay. In a classic lifetime tumor bioassay, the maximum tolerated dose (MTD) for a particular chemical is determined in a dose-range finding study, then doses close to the MTD are administered to male and female rats and mice for two years (4). A statistically significant, chemical-associated increase in tumor count is taken as evidence of carcinogenicity. Next, a dose-response assessment is used to determine whether there is any dose of the chemical that is likely to be safe for humans. Using a particular mathematical approach, an extrapolation is made from the dose(s) that gave a reliable tumor response to the generally much lower doses relevant to human exposures. For cancer risk assessment, the conservative approach of a linear low dose extrapolation is used (5). Ultimately, the regulatory action taken based on the genotoxicity test battery, the tumor outcome, and the dose-response assessment depends in large part on the intended use of the chemical.

The rodent tumor bioassay has been used for carcinogenicity testing for many years, primarily for four reasons. First, the rodent tumor bioassay fulfills an obvious need for an experimental system. Treating rodents with largely uncharacterized chemicals is a necessary step in drug development; it assures some minimum level of safety even in the use of experimental drugs. Second, *in vivo* exposure and tumor development are considered more relevant endpoints for assessing human cancer risk than *in vitro* tests with bacterial or mammalian cells or non-tumor endpoints. Third, the rodent tumor bioassay has been used for many years without disastrous human health consequences. And finally, at the present time there is no other "ideal" assay with which to replace the rodent tumor bioassay.

While the strengths of the rodent tumor bioassay are clear, its drawbacks are equally well known. The use of the rodent tumor bioassay has led to quantitative risk assessment based on tumor counts. This is actually quite problematic because high doses of chemical are needed to induce statistically significant tumor responses in treated rodents (4). This in turn necessitates extrapolating from the measurable tumor response down to the doses relevant to humans but for which there is no actual tumor data (2). Treatment of rodents at the levels of exposure relevant to humans is not done because the absence of an induced tumor response gives no assurance of safety in humans, particularly considering the relatively high background tumor rate of the tumor-sensitive rodent strains used. In other words, the rodent tumor response is not sensitive enough to detect low-dose effects. At the same time, high doses of chemical often lead to a cytotoxic effect in animal tissues. This cytotoxicity may lead to cell proliferation and other promoter-like events (6-8). Many "high-dose" rodent carcinogens have been identified, but the relevance of those findings to human disease is unclear.

A basic premise of risk assessment has been that genotoxic and non-genotoxic chemical carcinogens should be regulated differently. Genotoxic carcinogens are those that interact with DNA and cause DNA damage, while non-genotoxic carcinogens operate through other mechanisms. Although challenged recently, it has long been assumed that if a chemical is genotoxic, then even a single molecule of that chemical might cause a tumor and, therefore, linear low dose extrapolation is warranted (5,9). Carcinogens that have non-genotoxic modes of action may be considered to have a threshold below which the chemical is non-carcinogenic. The genotoxicity test battery gives an indication of a chemical's genotoxicity; but there is currently no clear-cut approach for determining whether a chemical is operating through a genotoxic or non-genotoxic mode *in vivo* and at low doses. Consequently, the inability to determine when linear low dose extrapolation is warranted, when a different type of dose response extrapolation is warranted (J- or U-shaped), or if a chemical's effects have a threshold are major deficiencies in the science of cancer risk assessment (10,11).

After the dose-response assessment has been completed, the rodent tumor data must be used to estimate human risk and/or assign a safe level. The rodent data are first transformed using a scaling factor

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to account for the gross difference in body weight between rodents and humans. Then safety margins or uncertainty factors may be invoked. These are usually ten-fold reductions in allowable exposures that are considered necessary given the lack of scientific rigor in species extrapolation or because of the possible existence of sensitive subpopulations (12). Thus, the lack of scientific rigor in rodent to human extrapolation is another area of risk assessment that could be improved.

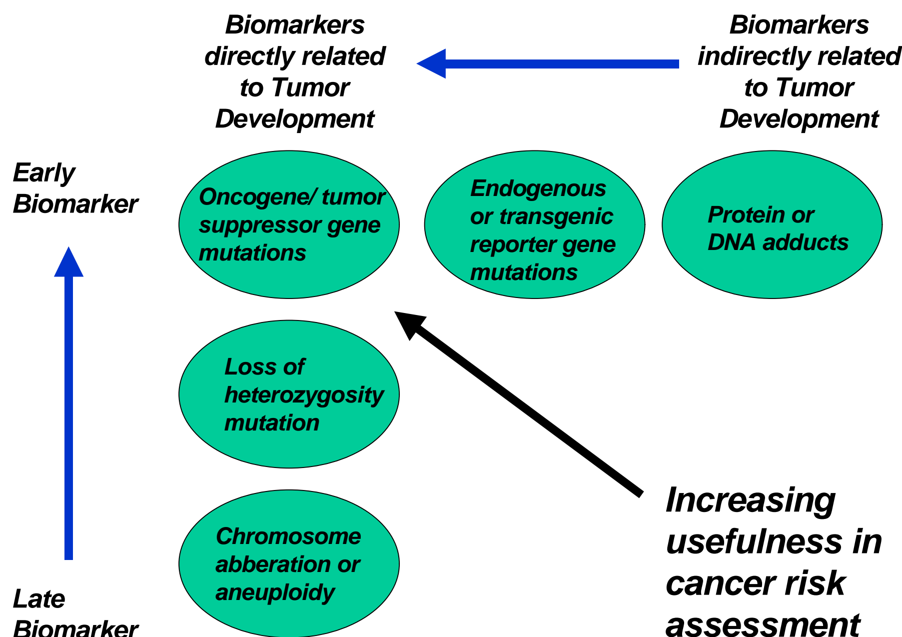

Figure 1. The potential usefulness of different types of biomarkers for cancer risk assessment.

How to Improve Cancer Risk Assessment

A generally accepted strategy for improving cancer risk is to incorporate chemical-specific, mechanistic, and biological information. The logic behind this strategy is that specific mechanistic information will define a chemical's mode of action; information that is essential if "biological realism" is to be incorporated into a scientific judgement of risk (1,13,14). Operationally, the idea is to identify biomarkers that are relevant to mode-of-action and can be used to support a particular low-dose extrapolation method or determine if a threshold exists and relate what takes place in a rodent to the biology of a human. While there is consensus in terms of using this general strategy to improve cancer risk assessment, there is little consensus regarding what specific information should be sought to determine mode-of-action or what biomarkers would be most informative for assessing cancer risk.

In theory, the ideal biomarker(s) to use in evaluating cancer risk would have several characteristics. First, the biomarker should have a direct relationship to cancer; the more direct the relationship between the biomarker and tumor development the more credible that biomarker will be in predicting cancer risk (see Figure 1). Second, the ideal biomarker would be measurable and have the same relationship to tumor development in both rodents and humans: such circumstances would facilitate translating the cancer risk defined in an experimental rodent model to human risk. And third, a biomarker that appears relatively early in tumor development will be more useful than one that appears later because of the possibility of interceding in tumor development.

At the NCTR a variety of different endpoints are being developed and evaluated for their potential use in predicting chemical associated cancer risk. These include measurement of DNA adducts, mutation in endogenous or transgenic reporter genes, and the identification of chemically induced changes in gene expression using gene arrays (15-17). Another promising approach for improving chemical risk assessment that is being developed at NCTR is genotypic selection.

Genotypic selection refers to the DNA-based detection of specific, rare mutations. The potential strength of applying genotypic selection to cancer risk assessment is that the actual oncogene and tumor suppressor gene mutations known to be involved in cancer causation could be measured. Figure 1 illustrates why the measurement of oncogene and tumor suppressor gene mutations may represent the ideal metric to use for cancer risk assessment. Such point mutations are known to occur early in the tumorigenic process; they are the initiating mutations detected in tumors and sometimes in pre-neoplastic lesions. Most importantly, these mutations themselves are directly and mechanistically related to carcinogenesis; i.e., cells accumulate these permanent genetic changes that confer the new phenotypes and capabilities of a tumor cell (18). Developing genotypic selection methods to detect these specific and rare mutational events is technically challenging. Also, the idea of using the measurement of specific oncogene or tumor suppressor gene mutations as biomarkers of cancer risk is relatively new. Consequently, the NCTR is taking the lead in investigating the potential use of genotypic selection methods for improving cancer risk assessment.

Genotypic Selection Methods

Genotypic selection can be used

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to detect a variety of mutations including translocation, deletion, expansion of microsatellites, and point mutation. The value of techniques that can detect specific mutations in pools of DNA molecules has been recognized for almost 20 years. The first genotypic selection methods were developed in order to detect oncogene mutations in tumor tissues. More recently, genotypic selection has also been used in the diagnosis and management of cancer patients, for the identification of specific microbes, and in pool screening for genetic polymorphisms (19).

A variety of different molecular approaches have been developed for the detection of rare point mutation. These approaches span a large range in terms of their sensitivity. In the context of genotypic selection, sensitivity refers to the level of mutant DNA sequence that can be detected amongst wild-type DNA; the lower the mutant fraction detected by a particular genotypic selection, the more sensitive the assay. The sensitivity of a genotypic selection method determines the applications for which they will be useful (19). Measurement of the rare chemically induced mutations in oncogene and tumor suppressor gene targets is an application that requires a great deal of sensitivity. Fortunately, the development of new tools and methodologies has improved the sensitivity of genotypic selection to a point where the potential use of these methods for cancer risk assessment has become an important avenue of research.

Development of Genotypic Selection Methods in the Division of Genetic and Reproductive Toxicology at the National Center for Toxicological Research

Information from a number of different research areas had to be analyzed before this research could proceed in an effective manner. Specifically, a theoretical framework was needed in terms of what experimental designs should be used (see Figure 2), what mutational targets should be investigated, and what sensitivity might be required. The necessary information was recently assembled in the form of a review article (20). It was concluded that eventually a small battery of genotypic selection assays should be developed. Initially, development of genotypic selection methods should focus on the most common mutations that occur in human tumors, and it should be determined empirically which of those behave similarly between rodent and human. The most important mutational specificities to include in such a battery are G:C to T:A, G:C to A:T, and A:T to T:A because analysis of 31 different carcinogens showed that the mutagenic effects of 29 of these were encompassed by these three mutational specificities. It was also concluded that the information provided by genotypic selection would be most valuable for risk assessment if the ability to detect background levels of spontaneous mutation was possible (Figure 2). It was determined that a spontaneous mutation frequency of ~10^{-7} per basepair is expected based on data from phenotypic selection assays (spontaneous mutation frequency per locus divided by target size in basepairs gives a value of ~10^{-7} per basepair). This information was used to define the goal of this research; to develop a genotypic selection method that could detect mutant DNA sequence in the presence of a 10^{7}-fold excess of wild-type DNA sequence.

Identifying the most useful mutational targets is a key issue in successfully applying genotypic selection to cancer risk assessment and is the first step in assay development. *Ras* was identified as a prototype for oncogene targets. The ras family of proteins (H-*ras*, N-*ras*, and K-*ras*) function as molecular switches in signal transduction pathways. The ras protein is a small (21 kDa) GTPase, which is in its active conformation when bound to GTP. The most common mutations

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What type of data should be collected	How to use the data in risk assessment
Human, age-related range of spontaneous mutation	**Identify background mutant frequencies with no adverse effect**
Human, age-related range of mutation after exposure; occupational, environmental or therapeutic	**Using epidemiological data, establish the relative risk associated with a particular mutant frequency**
Rodent, age-related range of spontaneous mutation	**Compare to human data for species extrapolation**
Rodent, age-related range of mutation frequency after mimicked human exposure	**Define how rodent mutation induction relates to human risk, eventually predict risk in the absence of human data**

Figure 2. Experimental strategies for applying genotypic selection methods to cancer risk assessment.

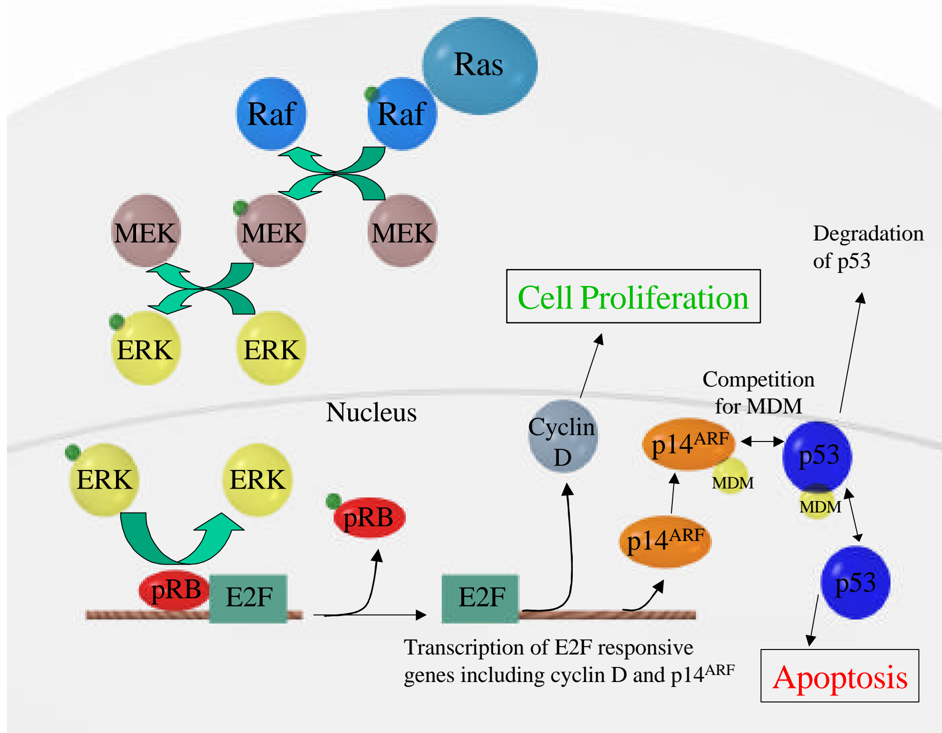

Figure 3. Consequences of ras activation are dependent on cell-type specific signal transduction effector molecules. Two different outcomes of ras activation are depicted. In one pathway, active ras recruits raf to the membrane, facilitating the phosphorylation (activation) of raf. Raf then phosphorylates MEK (mitogen activated and extracellular response kinase), which phosphorylates ERK (extracellular response kinase), which translocates into the nucleus where it can activate transcription factors to increase cyclin D1 levels in the cell. Cyclin D1 (with its cofactors) phosphorylates pRB, which releases E2F, allowing E2F regulated proteins to be transcribed. At this point, the cell is committed to the S phase and cell proliferation (44). However, ras can also activate the expression of p16ARF (alternative reading frame), which is considered a tumor suppressor. P16ARF indirectly affects the activity of p53 by sequestering Mdm2 (an inhibitor of p53). In this case, the ras initiated pathway can lead to apoptosis (45).

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in the *ras* gene result in protein species that bind GTP, but not GDP, and are, therefore, continuously active. Constitutive ras activity can cause continuous cell proliferation via the raf-MAPK pathway (Figure 3). However, this is not the only pathway affected by ras activity. It is becoming clear that there is extensive cross-talk between GTPase signaling pathways. Thus, the effect of continuous ras activity will depend on cell background and sometimes results in apoptosis.

Ras mutations frequently occur in both spontaneous and chemically induced rodent tumors and are localized to a few specific DNA regions, codons 12, 13, and 61 (21). Similar patterns of *ras* mutation are present in a number of different human tumor types. *Ras* mutation is found in 90% of pancreatic tumors, 50% of colon and thyroid tumors, and 30% of lung tumors and leukemias (22). The frequencies with which the most commonly occurring *ras* gene base substitutions have been detected in human tumors are given in Figure 4 (22-38). Thus, *ras* mutations are valuable targets to use in the development and application of genotypic selection methods.

MutEx and ACB-PCR Genotypic Selection

Some of the most powerful tools that have been used for genotypic selection are restriction enzymes. Digestion of DNA with a restriction enzyme is used to selectively destroy wild-type DNA sequences. This effectively enriches for sequences not digested because they carried a mutation in the restriction enzyme cleavage site (19). A report by Ellis *et al.* suggested that the *E. coli* mismatch binding protein, MutS could be used similarly as a tool for the selective destruction of wild-type DNA sequence while avoiding the limitation of only being able to analyze restriction enzyme cleavage sites (39). In their "MutEx" assay, PCR products were synthesized, denatured, and reannealed; thereby creating heteroduplex molecules in the DNA from individuals that were heterozygous for a germline mutation. The *E. coli* MutS protein was incubated with the PCR products and the 3' - 5' exonuclease activity of T7 DNA polymerase was used to digest the heteroduplex DNA. The bound MutS protein blocked this digestion so that the length of the protected DNA fragment defined the position of the germline mutation in the PCR product being analyzed. Because homoduplex DNA would be degraded in such an assay, it was realized that this approach might also be used for genotypic selection; to selectively degrade a large excess of wild-type sequence while preserving mutant sequence. Consequently, this type of MutS selection was the basis for the first genotypic selection method developed at the NCTR.

The H-*ras* codon 61 CAA to AAA mutation was used as the model system in the development of the MutEx approach as a genotypic selection method. This mutation was selected because: 1) It is the most frequent mutation detected in mouse liver tumors, 2) its occurrence in tumors can be increased by chemical treatment, and 3) mouse strain differences in the frequency of this mutation might eventually be used for method validation (21). Therefore, mutant and wild-type mouse H-*ras* sequences were cloned and restriction fragments corresponding to each were isolated and quantified. These restriction fragments were used in reconstruc-

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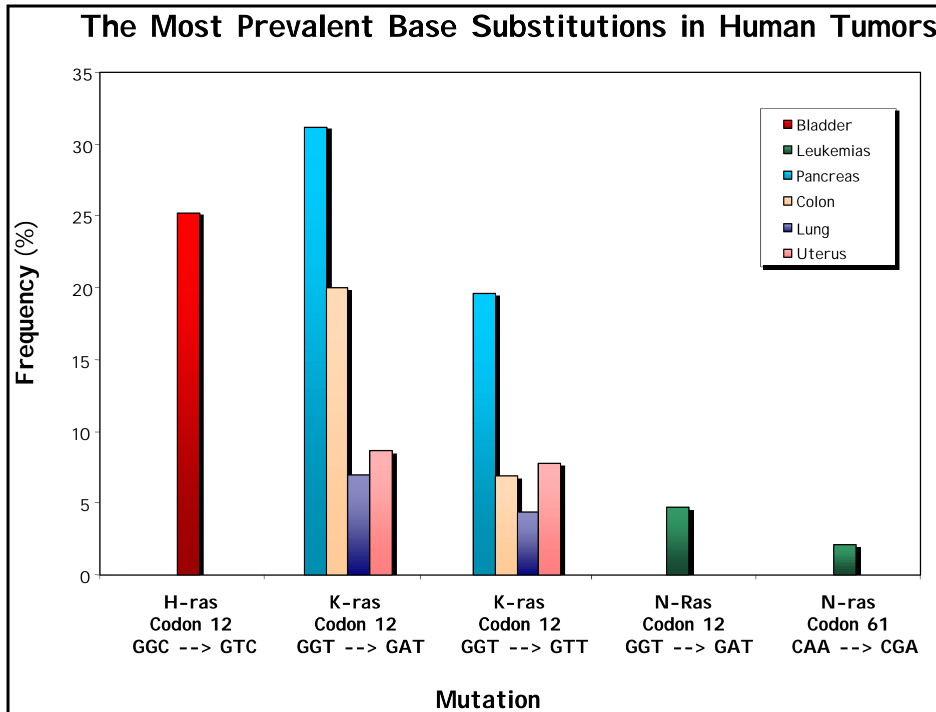

Figure 4. Frequency of the most common *ras* mutations in human tumors. The frequency of each particular basepair substitution mutation was plotted. Only mutations that were detectable in less than or equal to 2% of the tumors of a particular tissue origin are included.

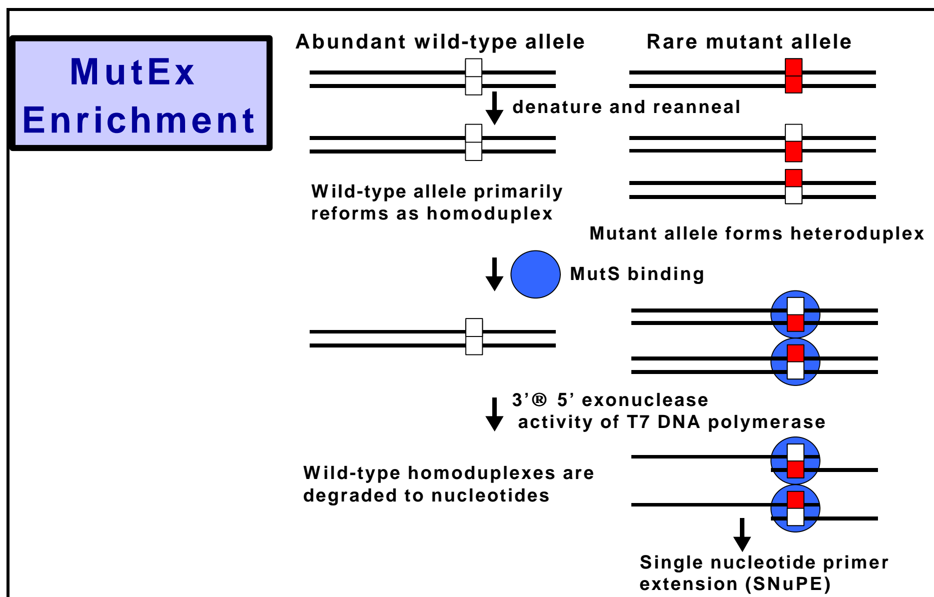

Figure 5. The MutEx/SNuPE genotypic selection method.

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tion experiments; meaning that DNA mixtures with known mutant fractions were prepared and used in the analysis of different experimental procedures. The molecular events occurring in each step of the MutEx genotypic selection that was developed are depicted in Figure 5. The result of this genotypic selection is that a large proportion of the wild-type DNA molecules is destroyed while the mutant sequences are preserved. Mutant sequence was then detected using single nucleotide primer extension (SNuPE) (40). In SNuPE, the extension of a primer adjacent to the base being interrogated is carried out in the presence of a single nucleotide complementary to either the mutant or wild-type base. Using this approach it was determined that mutant fractions between 0.5 and 2 x 10^{-5} were detectable (41). In addition, it was determined that SNuPE alone had a sensitivity of less than or equal to 2 x 10^{-2}. From this information it was concluded that the MutEx assay was providing an ~1,000-fold enrichment of mutant DNA sequences.

The second genotypic selection method that was developed at the NCTR was based on a completely different type of selection, allele-specific amplification. In an allele-specific amplification, a PCR primer that has more mismatches to the wild-type sequence than the mutant sequence is used to selectively amplify mutant DNA (19). Allele-specific competitive blocker PCR (ACB-PCR) is an allele-specific amplification method that was reported to have a sensitivity of 10^{-4} (42). The assay uses three different primers, a mutant-specific primer that amplifies the mutant sequence, a blocker primer that obstructs PCR amplification from the wild-type sequence, and an upstream PCR primer (Figure 6). At the NCTR, this approach was adapted to the detection of the H-*ras* codon 61 CAA to AAA mutation (43). The assay was modified in a number of ways, including the use of the Stoeffel fragment of *Taq* DNA polymerase and PerfectMatch PCR Enhancer. These modifications resulted in an increase in the assay sensitivity with mutant fractions as low as 10^{-5} being detectable.

Keeping in mind that the goal of this work was to develop an assay that could detect spontaneous mutation (estimated at 10^{-7}) neither the

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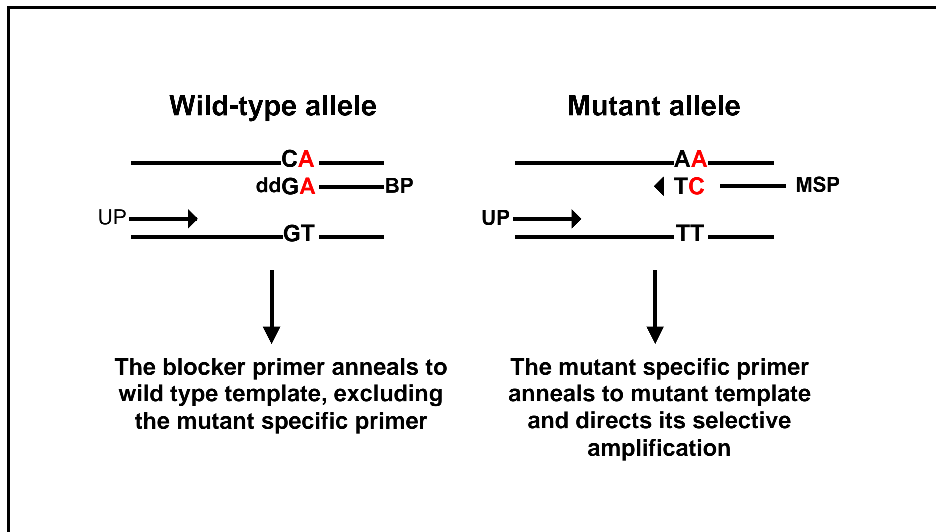

Figure 6. Primer design used in allele-specific competitive blocker PCR (ACB-PCR). Three PCR primers are shown; the blocker primer (BP), mutant specific primer (MSP), and upstream primer (UP). The selective annealing of the MSP to mutant sequence and BP to wild-type sequence is depicted. These primer-template pairings result in single 3'-penultimate mismatches. These pairings are favored over annealing of the MSP to wild-type template or BP to mutant template, which would result in double 3'-terminal mismatches. The blocker primer carries a 3'-terminal dideoxy nucleotide and cannot be extended

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MutEx/SNuPE assay nor ACB-PCR alone had sufficient sensitivity. In an attempt to reach this sensitivity, the relatively insensitive mutation detection step of the MutEx/SNuPE assay was replaced by ACB-PCR. In other words, the MutEx mutant DNA enrichment was coupled with the sensitive ACB-PCR mutation detection method. This combined assay, named MutEx/ACB-PCR, was found to have a sensitivity of 10^{-7} in reconstruction experiments (Figure 7) (40). As a means of validating the use of this assay in the measurement of very low mutant fractions, the level of H-*ras* mutation induced by *Pfu* DNA polymerase during PCR amplification was determined. *Pfu* DNA polymerase was selected because it has the highest fidelity in replicating DNA sequences of any known thermostable polymerase and the most common error it produces is C to A transversion. The results from three replicate MutEx/ACB-PCR experiments measured the *Pfu* DNA polymerase-generated mutant fractions as $10 \pm 3 \times 10^{-7}$, from which a polymerase error rate of $8 \pm 3 \times 10^{-7}$ was calculated (40). This value is in good agreement with the published reports regarding *Pfu* DNA polymerase error rate and, therefore, substantiates the accuracy of the MutEx/ACB-PCR assay in the measurement of low mutant fractions.

Summary

Genotypic selection is being developed as an approach for improving chemical risk assessment. This is primarily because the biomarkers that will be measured by genotypic selection, oncogene and tumor suppressor gene mutations, have a direct relationship to cancer. At the NCTR, work toward this goal has proceeded in two areas: 1) identifying the theoretical issues that should be considered in the development of new assays and 2) the assay development itself. For example, evidence that an average spontaneous mutation frequency of

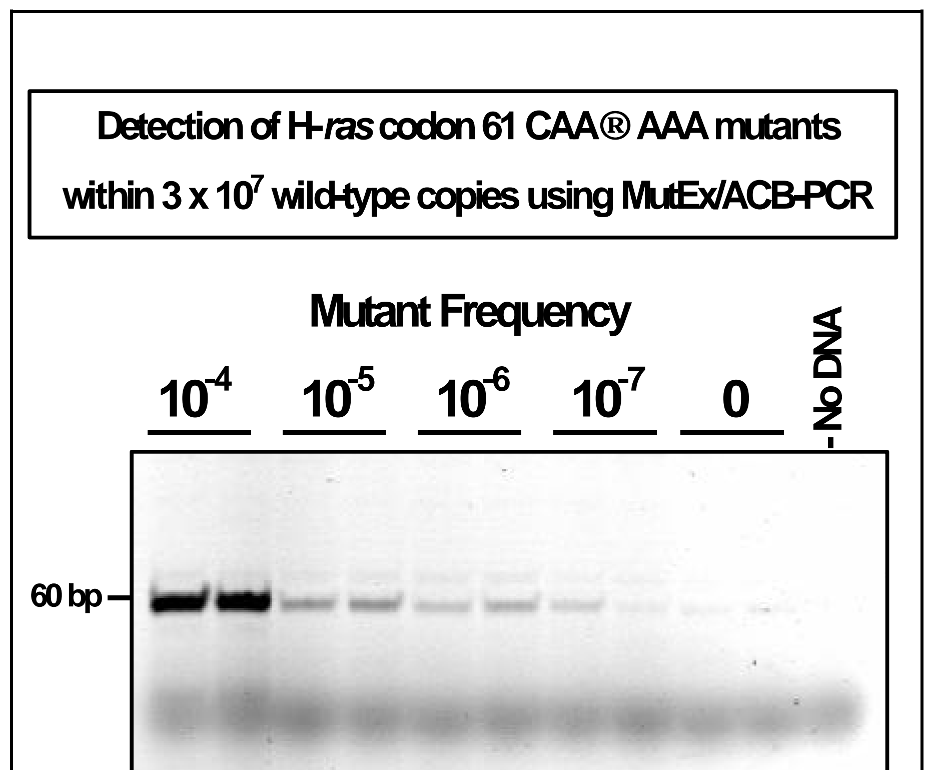

Figure 7. The MutEx/ACB-PCR assay developed at NCTR has a sensitivity of 10^{-7}. In the reconstruction experiment shown, each reaction contained 300 nanograms of genomic DNA and 3×10^7 copies of wild-type H-*ras* restriction fragment. Addition of different amounts of mutant restriction fragment was used to generate the mutant fraction standards analyzed (10^{-4} – 10^{-7}). The signal in the 10^{-7} lanes corresponds to the detection of three mutant H-*ras* molecules in the presence of 3×10^7 wild-type alleles.

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10^{-7} might be expected was important for setting a goal for assay sensitivity. That goal, the detection of mutant allele in the presence of a 10^{7}-fold excess of wild-type allele is now achievable using the MutEx/ ACB-PCR assay. However, additional challenges remain. In order to take advantage of MutEx/ACB-PCR sensitivity, a pool containing $>10^{7}$ molecules must be analyzed. This corresponds to a genomic DNA sample of >100 micrograms, a mass that would inhibit the sensitive MutEx/ACB-PCR approach. PCR amplification of target DNA cannot be used to generate the necessary DNA pool because the error rate of even the most reliable thermostable polymerase is higher than the level of mutation that needs to be detected (10^{-7}). Thus, the development of gene-specific enrichment techniques is viewed as necessary and development of such techniques is currently underway at the NCTR. Ultimately, the measurement of a small battery of oncogene and tumor suppressor gene mutations will be necessary to understand chemical-specific effects. Therefore, the genotypic selection assays already developed are being adapted to new mutational targets, namely human and rodent K-*ras* mutations. Eventually, the information provided by these sensitive assays should support a more scientifically rigorous approach to cancer risk assessment.

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The Authors

Pictured left to right:
Page B. McKinzie, Ph.D.
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Barbara L. Parsons, Ph.D.,
September 18, 2001
(NCTR Photo: Virginia B. Taylor)

Barbara L. Parsons is a FDA Staff Fellow in the Division of Genetic and Reproductive Toxicology at the National Center for Toxicological Research (NCTR), Jefferson, Arkansas. Dr. Parsons began her scientific career as a technician at Cold Spring Harbor Laboratory, Cold Spring Harbor, NY where she was involved in sequencing the *Adenovirus* genome. She entered the Department of Microbiology and Immunology and Interdisciplinary Program in Genetics at Duke University in 1982 and received her Ph.D. in 1988. During this time, her research was focused on animal virology; investigating the structure and function of *Orthopoxvirus* telomeres. Her first post-doctoral position was at the Beltsville Agricultural Research Center in Beltsville, Maryland where she studied changes in tomato fruit gene expression induced by wounding and the plant hormone, ethylene. Dr. Parsons began her work at the NCTR in 1994 when she was hired as a post-doctoral fellow through the Oak Ridge Institute for Science and Engineering (ORISE); work she continues now as an FDA Staff Fellow. Dr. Parsons was recently elected as a Councilor of the Environmental Mutagen Society. Her research interests lie in the development of DNA-based mutation detection methods and their application to cancer risk assessment.

Page B. McKinzie is an ORISE post-doctoral fellow in the Division of Genetic and Reproductive Toxicology at NCTR. Dr. McKinzie entered the graduate program of the Department of Biochemistry and Molecular Genetics in 1987 at the University of Alabama at Birmingham (UAB) and received her Ph.D. degree in 1993. Her graduate work was on the use of liposomes that are sensitive to low pH as a delivery vehicle for superoxide dismutase into fetal lung epithelial cells as an approach for relieving symptoms of bronchopulmonary dysplasia. Her subsequent post-doctoral position was with the Gene Therapy Program at UAB, with work focusing on: 1) using single-chain antibodies to knock out protein functions associated with carcinogenesis and 2) using replication deficient adenovirus as a vehicle for delivery of DNA into HPV-18 infected cells, with the goal of killing those cells. She began her work with the NCTR in 1999 and is currently adapting the DNA-based mutation detection methods developed at the NCTR to the detection of human and rat K-*ras* mutations.

Glossary

allele – one of a series of possible forms of a gene that differ in DNA sequence
aneuploidy – having an abnormal set of chromosomes
deletion – loss of a segment of DNA
expansion of microsattelites – an increase in the length of a cluster of repetitive DNA where each repeating unit is a particular DNA sequence and the increase in length corresponds to the addition of repeat units
loss of heterozygosity – only a single allele of a gene is present in a cell when previously two distinguishable alleles had been present
mutation – a heritable change in the DNA sequence of a gene
oncogene – a gene that causes uncontrolled cell proliferation
PCR primer – a short segment of single stranded DNA that binds to a longer, complementary DNA strand. A PCR primer is used to direct the *in vitro* replication of DNA that occurs in a Polymerase Chain Reaction (PCR)
phenotype – a manifest trait or ability exhibited by a cell or organism that is determined by that cell or organism's genes
restriction enzymes –proteins that cleave DNA at positions determined by the specific order or sequence of DNA bases
tumor suppressor gene – a gene that controls normal growth and cell division; loss or inactivation of such genes are known to contribute to tumor development
translocation – transfer of a segment of one chromosome to a different position within the same chromosome or to a different chromosome

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