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Mutational Specificity and Cancer Chemoprevention

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Abstract Mutational specificity describes the composite of all of the genetic alterations in a collection of mutations arising from a specific treatment. The information includes not only the nature of the genetic change (e.g., a base substitution or a frameshift), but also information about nucleotide position and hence the DNA context. As both the type of DNA damage and its position can be expected to reflect the nature of the chemical and physical mutagen, mutational specificity can be expected to provide insights into mechanisms of mutation. Conversely, mutational spectra should also provide insights into the identity of the mutagen. Indeed, the pioneering work on mutational specificity in *Escherichia coli* indicates that each physical or chemical treatment produces a unique spectrum of mutations.

With the application of biotechnology to the field of genotoxicology, the database of sequenced mutations has become quite substantial. Both in vitro and in vivo data has been obtained following exposure to a variety of agents. In this communication we will critically assess whether the reality of mutational specificity has fulfilled the expectations and to examine what potential remains to be explored, especially in the area of monitoring human populations. The usefulness of both mutational spectra analysis and population monitoring with regards to chemoprevention are discussed. J. Cell. Biochem. 25S:99–107. © 1997 Wiley-Liss, Inc.

Key words: mutational assays; mutational spectra; monitoring mutation in people; transgenic animals; *lacI;* hprt T-cell clonal assay; chemoprevention

INTRODUCTION

The Early History of Mutational Specificity

The earliest investigations to use mutational specificity to study mutational mechanisms preceded the development of effective DNA sequencing technologies. These studies were dependent on protein sequences and were made possible by the elucidation of the genetic code. In a classic example of such work, Streisinger et al. [1], using a bacteriophage lysozyme gene, first proposed the slippage mechanism for frameshift mutagenesis from the elegant use of amino acid data.

The first study of mutational specificity using direct DNA sequencing involved a bacteriophage M13 reversion assay, in which Brandenburger et al. [2] sequenced 125 mutants recovered after UV and ionizing radiation treatments. Despite the limited number of sites available, they concluded that most of the mutations induced by UV light occurred at pyrimidine dinucleotides indicating that pyrimidine dimers may be involved in the induction of mutations.

While a range of studies, particularly on protein structure and function produced some insight into the nature of mutation, by far the most important system was that of J.H. Miller [3] which used the suppression patterns of amber and ochre mutations to deduce single base pair substitutions in the *E. coli lacI* gene. Five of the six possible substitutions could be detected by this system. Only the A:T→G:C transition could not be detected since the TAA (ochre) to TAG (amber) or TGA (opal) changes represent the only codons available in which a single base substitution could produce a nonsense mutation. Using the *lacI* nonsense system, the mutational specificity of dozens of chemicals was determined. In addition, the impact of DNA repair has been studied by selecting the mutations in host strains of diverse genetic backgrounds. The observation that each physical or chemical agents examined demonstrate a unique spectrum of mutation was made first in this system, and remains one of the

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most important principles of mutational specificity [3].

This uniqueness of mutational specificity reflects two components: the nature and distribution of the initial DNA damage and the sequence specificity of DNA repair. As a consequence, the nature and the distribution of mutations (i.e., mutational specificity) is unique for each mutagen.

The next development in the study of mutational specificity arose from advances in DNA sequencing technology. Using these technologies many thousands of *lacI* mutants have been sequenced over the past decade [4]. One of the obvious advantages of direct DNA sequencing is that it permitted the direct detection of all six classes of base substitutions as well as frameshifts, deletions, duplications, and insertions. The sequencing data confirmed, for example, that most UV-induced mutations occurred at di-pyrimidine sites [4] as had been suggested from the analysis of *lacI* nonsense mutations recovered after UV irradiation [3]. The sequencing studies, however, also revealed something that had not been suspected: the occurrence of tandem double mutations. These generally took the form of CC→TT changes at CC sites and have become the hallmark of UV mutagenesis from studies in *E. coli* [3], mammalian cells [5], to human skin cancer [6]. In addition to suggesting a mutagenic role for CC cyclobutane dimers in targeting mutations, these studies, especially when coupled with the analysis of damage distribution, led to the conclusion that mutations could be targeted by both the originally suspected lesion, the cyclobutane dimer and the (6–4) pyrimidine-pyrymidone photolesion [7].

Mutational spectra differ depending upon the mutagenic treatment. Table I summarizes the nature of mutation in the NC+ or DNA binding region of the *lacI* gene, as characterized in *E. coli* without exposure and after exposure to dimethylsulfate (DMS), and ethylmethanesulfonate (EMS). Note that the most common events in the spontaneous spectrum are single base-pair transitions (33.2%), particularly G:C→A:T. The spectra of induced mutations demonstrate a dramatic shift away from the spontaneous spectrum. Both the EMS and the DMS induced spectra show a large increase of G:C→A:T transitions (98 and 74%, respectively).

The entire *lacI* gene includes a hotspot for spontaneous mutations consisting of 3 repeated TGGC sequences (position 620–632). When this hotspot is included in the analysis, the majority of spontaneous mutations occur at this site (duplications or deletions of one of these repeats at a ratio of 4:1, respectively). Clearly different mutagenic treatments yield strikingly different mutational spectra. It should be pointed out that mutational specificity depends not only upon the genetic target and mutagen, but also upon the biological system. For example, the spontaneous spectrum in *lacI* in bacteriophage M13, lacks the spontaneous frameshift hotspot that predominates in the bacterial spectrum [13].

TABLE I. Mutational Spectra of Bacterial lacI Mutations for the NC+ Region Only*

	Spontaneous	DMS	EMS
Transitions			
G:C → A:T	137/33.3	90/74.4	1241/98.0
A:T → G:C	38/9.2	3/2.5	8/0.6
Transversions			
G:C → T:A	23/5.6	14/11.6	3/0.2
G:C → C:G	12/2.9	2/1.6	2/0.2
A:T → T:A	35/8.5	4/3.3	3/0.2
A:T → C:G	48/11.7	4/3.3	2/0.2
Others			
+1 Frameshift	0/0.0	0/0.0	0/0.0
−1 Frameshift	18/4.4	4/3.3	4/0.3
Deletions	69/16.8	0/0.0	1/0.1
Insertions	32/7.8	0/0.0	2/0.2
Complex	0/0.0	0/0.0	0/0.0
Tandem	0/0.0	0/0.0	0/0.0
TOTAL	412/100%	121/100%	1266/100%

*Spontaneous Collection Was Collected by Schaaper et al. (8), the Dimethylsulfate (DMS) Collection Zielenska et al. (9), While the Ethyl Methanesulfonate (EMS) Collection Was Compiled from Pienkowska et al. (10),Halliday et al. (11), and Burns et al. (12)

The Study of Mutational Specificity in Mammalian Cells in Culture

The study of mutation has been facilitated by the development of selection systems that permit the assessment of mutagenic potential at a range of targets in a variety of cell types [14]. Some of the genetic targets examined are amenable to analysis at the DNA sequence level. The most important of these are the *aprt*, *hprt*, TK, *dhfr*, and the ouabain systems. In addition, an artificial retroviral shuttle vector construct containing the *E. coli* xanthine (guanine) phosphoribosyltransferase (*gpt*) gene integrated into

the chromosomal DNA of mouse cells, permitting the recovery of mutant genes, has been used. An important aspect of this system is that the mutational target being characterised is integrated into the chromosomal DNA rather than existing as part of autonomously replicating plasmids. Databases of DNA sequence alterations are available for several of these gene targets each with a number of mutagenic treatments [14].

The Development of Transgenic Animals for the Study of Mutational Specificity

The development of transgenic animals carrying target genes on recoverable shuttle vectors has provided important novel ways to assess mutagenicity and mutational specificity in animals in vivo. For the first time questions of strain, species, sex, age, and tissue and organ specificity of mutation can be examined in vivo in a short-term assay. Our discussion specifically focuses on BigBlue® transgenic mice and rats which carry the *E. coli lacI* gene as the mutational target [15]. Such targets can be recovered from mouse genomic DNA with γ packaging extracts and plating onto an *E. coli* host. A DNA fragment of 1,254 base pairs, containing the *lacI* gene can then be amplified by PCR and directly sequenced. The availability of a BigBlue® rat allows important inter-species comparison yielding essential information for extrapolation to humans. For in vitro experiments, a transgenic rat cell line, based on the same *lacI* construct, has also been established.

The nature of spontaneous mutations recovered from the animal is extremely similar for most tissues [de Boer et al. in preparation] with the largest single class of events being G:C→A:T transitions. The majority (approximately 75%) of these transitions are recovered at 5′-CpG-3′ dinucleotide sequences, which suggests that they are the result of spontaneous deamination at methylated cytosines. The mutagenic response in these systems has been evaluated for a number of carcinogens, including DMBA [16]. The importance of mutational specificity is especially evident when the increase in mutant frequency is small. For example, we have recently determined the DNA sequence changes in mutants recovered after treatment with the flame retardant tris(2,3-dibromopropyl)phosphate (TDBP). TDBP causes tumours preferentially in the kidney of experimental animals. It is in this tissue specifically that a small increase (40%) in mutant frequency was observed; other tissues, notably stomach and liver, did not demonstrate an increase in mutant frequency. A clear shift in the nature of the mutations was evident only in mutants recovered from kidney tissue (unpublished results). Both the fraction of G:C→A:T transitions and the proportion recovered at 5′-CpG-3′ sequences is reduced in a dose responsive manner, while the loss of G:C base pairs increased significantly.

In a similar fashion, Recio et al. [17] determined the sequence of alterations in the *lacI* gene in BigBlue® mice after the inhalation of 1,3-butadiene. Mutant frequencies were increased in 2–3.5-fold in spleen and bone marrow. DNA sequence analysis demonstrated that substitutions at A:T base pairs were enhanced 3–4-fold. These substitutions are found only rarely in spontaneously arising mutants. It is clear that DNA sequence analysis increases the information provided by these studies as well as increasing the power of mutant detection when the overall increase in mutant frequency is so slight as to be statistically uncertain.

Although the transgenic animal models have only recently become available, the results from studies in these systems suggest that monitoring mutations in human populations when accompanied by DNA sequence analysis, should provide insights into the genotoxic effects of diet, lifestyles, and occupational and domestic exposures.

Monitoring Mutation in Human Populations Using the HPRT system

The T-cell hypoxanthine guanine phosphoribosyltransferase (*hprt*) clonal assay has been widely employed to monitor in vivo mutations in people [18]. The assay which permits the enumeration of 6-thioguanine resistant (TG^R) T-lymphocytes [19], also permits mutant characterization at the DNA sequence level [20]. The gene is relatively large, 44 kb, but the processed mRNA is relatively small, 1.6 kb, permitting the 647 bps of actual coding sequence to be easily sequenced. Mutations that occur in the noncoding regions such as the introns are detectable when the characterization of *hprt* cDNA yields specifically truncated species. These species are termed exon loss events and indicate the presence of a mutation that affects the

proper splicing of *hprt* mRNA. Thus the mutational target size extends beyond the coding sequence.

T-lymphocytes undergo in vivo clonal expansion as part of their role in the immune system. As a consequence independent mutational events can be over-represented should a mutant undergo division in vivo. Moreover, the actual act of cell division may be a critical step for mutation fixation. An inherent advantage of the T-cell system is that the mutant frequency can be adjusted for clonality. This is facilitated as each T-cell possesses a unique rearrangement of the T-cell receptor (TCR), which makes it possible to adjust mutant frequencies into actual mutation frequencies [20].

As the *hprt* clonal assay depends upon the peripheral T-lymphocytes population, the status of that pool has direct consequences for the assay. The peripheral lymphocyte pool, about 5×10^9 cells, is only a small fraction of the total T-lymphocyte population (3×10^{12} cells) which is largely sequestered in the marginal pool [21]. Most T-lymphocyte subsets are tissue specific and come from the gut, skin, or lymph nodes via the lymphatic system, and then preferentially return to those tissues [22]. Migration of the "naive" subset to the lymph nodes is observed to increase after antigen challenge [22]. In addition, there is a daily influx of approximately 10^9 "naive" T-lymphocytes from the thymus. Despite the complexity and heterogeneity of the T-lymphocyte peripheral pools, the ability to culture peripheral T-cells and to select mutants, makes this a convenient tissue for the study of in vivo mutations with the currently available protocols.

An additional complication with the use of T-cells for monitoring populations is related to their natural turnover rates in the peripheral blood. Attempts have been made to establish the half-life of T-lymphocytes by determining the rate of elimination of chromosome aberrations from these populations. However, the conclusions reported are highly variable. Estimates of half-lives range considerably: 0.3 years; 1.45 years; 3 years, to approximately 4 years [reviewed in 23]. Based upon observations of the reduction in *hprt* mutant frequency over time we have used the Buckton model and calculated a T-cell half-life of 2.1 years [23]. We believe that the *hprt* mutant half-life in peripheral T-cells is relatively short. Such a short half-life limits the effective use of the *hprt* assay as a method of choice to monitor exposures occurring in the distant past (>5 years).

One significant advantage of the HPRT system is the extensive database for both in vivo and in vitro mutations (Mutabase Software, Durham, NC). The current release of the human HPRT data base includes the sequences of more than 2,000 independent mutations. Despite the size of this collection, the mutational spectrum is by no means saturated. We have recently reported four novel (not previously reported in the database) single base pair substitutions out of 20 independent mutations from a single male subject [20] and have a much more striking example from a larger collection of independent mutations collected from Russian monozygotic twins. In this case, 32 novel single base pair substitutions were identified from a total of only 62 mutations.

In order to demonstrate the mutational specificity of an exposure or treatment, the spontaneous mutational spectrum must first be revealed. We have recently analyzed the *hprt* database and extracted mutants from both smokers (n = 161) and nonsmokers (n = 290) and have failed to demonstrate that the mutational spectrum of smokers differs, even though sufficient numbers of mutants were available for a reliable comparison. Either the numbers of mutants that where available to make this comparison were insufficient or the system does not have the sensitivity to reveal a smoking effect at the *hprt* gene in T-cells.

Monitoring Mutations Induced by Chemotherapy: The case of VP-16

Etoposide (VP-16), a semisynthetic derivative of epipodophyllotoxins, is widely used as an anticancer drug and is a known mutagen [25]. VP-16 has been implicated as a causative agent of secondary leukemia [26]. Such epipodophyllotoxin-related leukemia are often (>50%) associated with abnormalities at chromosome band 11q23, particularly translocations [27] . It is feasible that the achievable plasma concentrations of etoposide during cancer chemotherapy can lead to the accumulation of DNA mutations and rearrangements in the blood cells which may become a cause of secondary leukemia. To investigate the possibility of etoposide induced mutation following chemotherapy in cancer patients, we employed the *hprt* T-cell cloning assay [18] to study 12 individuals with small cell lung cancer. No increase in MF has been ob-

served after as many as four monthly treatment cycles [Karnaoukhova et al., in preparation].

We are currently pursuing the hypothesis that the failure to detect the induced mutation reflects the induction of apoptosis in the target cells. Etoposide has been shown to induce apoptosis in a variety of systems in vitro [28]. Matsubara et al. [29] showed that leukemia cells freshly obtained from patients before therapy undergo apoptosis within 6 h following in vitro etoposide-prednisolone treatment. However, no apoptotic cells or fragmentation of DNA derived from peripheral blast cells were detected at any preparation following etoposide-prednisolone chemotherapy, suggesting that apoptotic cells cannot be detected due to rapid removal from the circulation [29]. Cytotoxic drugs, such as etoposide, primarily target rapidly dividing cells. The circulating lymphocytes with etoposide-induced DNA damage and mutation are the likely candidates to undergo apoptosis. As a result it is possible that the *hprt* and other assays are not able to detect etoposide-induced mutants.

Spectra of Mutations in the *p53* Gene From Human Cancers

As a consequence of efforts toward understanding the molecular events occurring during carcinogenesis, a wealth of mutational data related to genes involved in cancer has been generated. The prime example and largest database concerns the human tumour suppressor gene, *p53* [30]. The *p53* mutational database contains over 3,000 entries [31]. The *p53* gene is well suited for mutational analysis. More than 100 different codons that have been recovered as mutated from diverse human cancers (Hollstein *et al.* 199431) and the large number of mutable sites available in the coding sequence makes the *p53* gene very attractive for the study of mutational specificity.

Our current understanding of the etiology of cancer implies that the process leading to clinical disease is characterised by a series of genetic alterations, including point mutations in *p53* as an important step. The cause of these mutations can be endogenous (e.g., deamination, replication errors, DNA repair defects) or exogenous (exposure to environmental agents). The precise nature of these changes at the DNA sequence level offers insights into the nature of the mutagen.

The most striking example is probably found in human skin cancers. The spectrum of *p53* mutations found in invasive squamous cell carcinomas demonstrated the mutational specificity consistent with previous exposure to UV-light [32]. Specifically, *p53* mutations occurred preferentially at dipyrimidines and included tandem double base substitutions, often CC→TT. These tandem double base substitutions accounted for 18% of the *p53* gene mutations characterised in skin cancer. In contrast, less then 0.1% of the *p53* mutations (in all other cancer sites combined) are tandem base substitutions [31]. This feature is considered the hallmark of UV-induced mutations [5].

The mutations in *p53* from colon cancer are characterised by G:C<A:T transitions (63%, n = 960), and most of these (47% of the total *p53* mutations in colon cancer) occur at CpG sites [31]. Transitions at CpG sites are characteristic of spontaneous mutational events [33] and are thought to derive from deamination of 5-methylcytosine. The high incidence of G:C→A:T transitions suggests that mutational events in colon cells might be dominated by spontaneous mutations including deamination and replication errors. This is further supported by the finding that a defect in mismatch repair has been shown to be the cause of hereditary, nonpolyposis colon cancer (HNPCC) [34].

A correlation has also been observed between exposure to aflatoxin B1, the excretion of aflatoxin-B1-N^7-guanine in urine [35], and the incidence of liver cancer [36] in geographic regions with aflatoxin exposure from exogenous sources. One such region is Qidong Province in China, where the mutational spectrum of *p53* mutations recovered from liver tumours has been investigated. The mutational spectrum shows G:C→T:A transversions at codon 249 of *p53*, accounting for over 50% (12/21) of the mutations [36]. Aflatoxin targets G:C base pairs and the predominance of the transversion event and the appearance of a hotspot is consistent with induction by this exposure. Aflatoxin B1 induces mostly G:C→T:A transversions and the mutational spectrum from *hprt* shows a distinct G:C→T:A transversion hotspot at position 209 [37].

The spectrum of *p53* mutations recovered from bladder tumours also shows unique features. *p53* mutations show a high proportion of G:C→C:G transversions and double mutations both in smokers and nonsmokers [38,39]. The

G:C→C:G transversion is a relatively rare mutational event. This transversion represents only 8% of all the *p53* mutations recovered (3% in colon cancer, n = 960). However, in bladder cancer G:C→C:G transversions account for 21% of the sequenced mutations. Oxidative DNA damage has been suggested as an underlying cause [38], and indeed this rare event has been induced by the singlet oxygen generating mutagen methylene blue plus light [40]. Other factors need to be considered as well: 4-aminobiphenyl derived adducts at G:C base pairs have been found as a major DNA lesion in bladder biopsy samples from smokers [41] and G:C base pairs are the major targets for 4-aminobiphenyl induced base substitution mutations [42]. Metabolism may also contribute, as allelic differences in the carcinogen-metabolizing genes glutathione-S-transferase (GSTM1) and N-acetyltransferase (NAT2) and have been identified as bladder cancer risk factors [43]. The presence of a second *p53* mutation in a single tumour may be related to a general genetic instability of the tumour cell.

Future insights will depend on the accumulation of specificity data from patients having better defined occupational and lifestyle based exposures. Such conditions are difficult to achieve in real people and the human cancer database may have to be complemented by mutational analyses using transgenic animals. The *p53*-null mouse model [44] and other transgenic systems seem ideally suited to elucidate the molecular fingerprints of carcinogens in specific genes and tissues.

The Future: Monitoring Mutation and Chemoprevention

Our ability to monitor mutational specificity in populations is severely limited by current technologies. These limitations include both biological factors, such as the gene targets and tissue types to be used for selection, and the labor intensive nature of the existing systems. We stress our belief that the problems identified with the *hprt* system largely reflect the target tissue and not the sensitivity of the target gene. Nevertheless, *hprt* is a surrogate endpoint for a biological effect, which we hope to use to assess carcinogenic potential. As a surrogate endpoint, it permits the detection of mutation much earlier than the appearance of malignancy. On the other hand, *p53* is an intermediate endpoint rather than a surrogate endpoint and thus more directly related to the development of cancer.

Both markers provide important information, but it is after the fact, since damage is already present. It would be of great value to monitor mutation in an earlier genetic marker for carcinogenesis. However, such a genetic marker/s remains to be identified. From a therapeutic standpoint knowing the progression of disease is of interest. These markers may offer advantages in drug trials for chemoprevention studies, since they can be used as early markers for disease recurrence, particularly *p53*, which has already been used for this purpose [45]. When a specific exposure is suspected and mutational specificity data available, it is possible to estimate *p53* mutation frequencies in vivo [46]. The approach involves the screening for the loss of restriction sites at mutational hotspots. The nature of the mutation is then confirmed by DNA sequencing. While exceedingly elegant, it is unlikely to provide a practical approach for monitoring populations. However, with future advances, it may ultimately be possible to use mutational specificity in humans to specifically identify exposure.

The mutational systems described in this article could be used to determine the effectiveness of chemopreventive agents, in that they would permit the changes in mutational specificity to be determined. In vitro studies employing human cell lines could be used to determine the spontaneous spectrum, an induced spectrum (exposed), and an induced spectrum in cells treated with a chemopreventive agent. Comparisons then of the mutation frequencies, and mutational spectra would permit an assessment of the chemopreventive agent. It is, however, important to realize that extrapolation of any in vitro testing results to the human situation will prove considerably difficult. Should a specific chemopreventive agent work to reduce the mutation rate in a cell line, then the in vitro system may hold promise. However, if the chemopreventive agent operates in a different manner, such as to reduce inflammation, the efficacy of the agent may not be revealed in these simple in vitro systems.

To explore the effectiveness of chemopreventive agents directly in humans with existing monitoring systems will prove exceedingly difficult for several reasons. With the current *hprt* system, the wide range of mutant frequencies values observed for human populations re-

quires that a large population be examined for any possible shift in mutant frequencies alone. In addition, our understanding of the in vivo spontaneous mutational spectrum is still limited such that any comparison to an exposed spectrum is difficult. Indeed any comparisons will require large numbers of mutants collected from suitable populations. Induced human spectra with numbers less than 100 are not useful, as the spontaneous spectrum can potentially mask any induced mutants.

Recently, the chemopreventive properties of oltipraz, an inducer of GST in mice and rats [47], and conjugated linoleic acid (CLA), have been investigated. Treatment of rats with oltipraz results in induction of GST Yc gene expression. This is of importance in the detoxification of aflatoxin-B_1 in the liver. Humans can hypothetically be made relatively resistant to the effects of AFB_1 through dietary supplementation with oltipraz; however, this hypothesis assumes that human liver, like rat liver, contains a GST isozyme that can be induced and which can detoxify AFB_1. Human clinical trials are currently underway to evaluate the chemoprotective effect of oltipraz in the diet. CLA, a mixture of positional and geometric isomers of linoleic acid which occurs naturally in dairy products and cooked meat demonstrates chemopreventive properties in rodents (48), and anticancer activity in human skin, colorectal, and mammary cells in vitro (49). Prevention is manifest at concentrations which are close to the levels consumed by humans in the diet. One percent of CLA in the diet suppresses mammary gland carcinogenesis in rats given a high dose (10 mg) of dimethylbenzanthracene (48). The use of transgenic animals to determine the modulation of mutation induction will prove to be an attractive method to investigate the properties of chemopreventive agents. In addition the determination of chemically induced mutation spectra in the absence and presence of chemopreventive compounds may elucidate some of the mechanisms by which these chemicals act.

Advances in DNA sequencing technology may make the direct detection of mutants possible without phenotypic selection. This would offer the advantage that cells would not need to be grown. Systems such as gradient denaturing gels [50], constant denaturing gels [51], or constant denaturing capillary electrophoresis [52] present some promise, but currently fall short of the sensitivity required to detect mutation at frequencies below one per 10^5 copies. It should be recognized that the accuracy required is greater than that provided by most available DNA polymerases utilized for PCR reactions.

TABLE II. Desireable Characteristics of a Mutational Monitoring System

1: Tissue does not need to be cultured.
2: No phenotypic selection.
3: Multiple tissue choices.
4: High sensitivity.
5: Univeral endpoints—deletions and base substutions.
6: Transcribed gene, expressed in all tissues in a similar manner.
7: Limited size for convenience (small cDNA) but reasonably large as a gene target (large gDNA).
8: Not sex linked.
9: Two homologs present so LOH can be studied. (Another reason for non-phenotypic selection, genetics neutral. Do not need a het or hemi state of dominant gene mutation.
10: Copy number stable in normal cells.
11: Well mapped chromosomal locations of polymorphisms for LOH and inheritance studies.

Future systems to monitor mutations in human populations can be expected to fulfill certain practical and scientific demands. Table II presents the wish list for an ideal system. Foremost, the system should avoid the need for in vitro culturing. Any tissue that can be painlessly obtained could then be used. This would include blood, buccal, hair follicles, skin as well as exfoliated bladder cells. One way to achieve freedom from the need to culture the cells, is to use a nonphenotypic method of mutant selection. This could also broaden the spectrum of recoverable mutants from a target gene. The utility of a system would also be enhanced if different classes of genetic alterations could be detected. In the near future, the human genome project will likely provide novel mutational targets, and mutational specificity studies can be expected to become more sophisticated. As each genetic target will offer both unique advantages and technical challenges, the system of the future may not rely upon a single target or single method of analysis.

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Joyce succumbed October 23, 1996 after a brave battle against pancreatic cancer.

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