The Mutational Spectrum of the HPRT Gene from Human T Cells in Vivo Shares a Significant Concordant Set of Hot Spots with MNNG-treated Human Cells


ABSTRACT

The preponderance of G:C to A:T transitions in inherited and somatic human mutations has led to the hypothesis that some of these mutations arise as a result of formation of O\textsubscript{6}-methylguanine in DNA. To test this hypothesis, the fine structure map of N-methyl-N\textsuperscript{-}nitro-N-nitrosoguanidine (MNNG)-induced mutations was determined in human lymphoblastoid cells in the human hypoxanthine-guanine phosphoribosyltransferase (HPRT) gene and compared with HPRT mutations observed in somatic T lymphocytes from normal individuals. Human TK6 cells, which are methylguanine methyltransferase deficient (MGMT\textsuperscript{-}), were treated with the methylating agent MNNG to create a level of O\textsubscript{6}-methylguanine in cellular DNA equal to that found in normal human tissues. A total of 676 bp of the HPRT gene was scanned using constant denaturing capillary electrophoresis and high-fidelity PCR. MNNG induced 14 predominant hot spots, all of which were G:C to A:T transitions. Thirteen of these 14 MNNG-induced hot spots were found among the in vivo set, and 10 of the MNNG-induced hot spots were among 75 putative in vivo hot spots (mutations observed two or more times in vivo). Using a hypergeometric test for concordance, the MNNG-induced hot spots were found to be a significant subset of the putative in vivo hot spots (P < 4 × 10\textsuperscript{-5}). The set of shared hot spots comprise some 18% of the HPRT in vivo hot spot spectrum and strongly suggest that MNNG-induced hot spots in vitro share a common mutational pathway with a significant subset of somatic mutations in vivo.

INTRODUCTION

Although inherited and somatic mutations are known to cause disease, the causes of these mutations are generally undefined. Mutational spectrometry, the study of quantitative patterns of mutations (1), permits the comparison of inherited and somatic mutations observed in vivo over identical DNA sequences to patterns of mutation induced in cells in vitro by suspect agents or conditions. Concordance of significant subsets of mutational hot spots is a form of evidence indicating shared mutational mechanisms.

The patterns of point mutation in the third exon of the HPRT gene have been observed after treatment of human lymphoblastoid cells by several mutagenic agents. These include benzo(a)pyrene (2), benzopyrene diol epoxides (3), chromium VI (4), UV light (5), X-rays (6), hyperbaric oxygen, hydrogen peroxide (7), the intercalating mutagen ICR-191 (8), and the SN\textsubscript{1} methylating agents MNNG and MNU (9, 10). Reports of human inherited and somatic point mutations in the HPRT gene (10–16) made it possible to discover how many, if any, of these are also among those induced by various mutagens in human cells. The results were straightforward in that all mutagenic agents tested, save the two methylating agents MNNG and MNU, and UV light, induced strong mutational hot spots that were unobserved in the human in vivo spectrum. UV light induced two G:C to A:T transitions found in the in vivo HPRT spectrum but also induced multiple hot spots not found in the in vivo spectrum (5).

MNNG and MNU mutational spectra in HPRT exon 3 were, however, identical and consisted of two major G:C to A:T transition hot spots at bp 208 and 209 found in both inherited and somatic human spectra and no hot spots not reported in vivo (8, 9). This concordance of MNNG- and MNU-induced spectra for HPRT exon 3 was consistent with, but not convincing statistical evidence for, a potential mutagenic pathway sharing mechanisms of mutation with the known sequelae after chemical alkylation of DNA (17, 18).

G:C to A:T transitions overwhelmingly dominate the kinds of mutations induced by MNNG in cells of many species (8, 19–23). Although a variety of lesions are created during MNNG treatment, including N7-methylguanine, N3-methyladenine, O\textsubscript{6}-meG, and methylyphosphotriesters, there is a wealth of evidence supporting O\textsubscript{6}-meG as the principle mutagenic intermediate (reviewed in Ref. 24). Alkylation of DNA, capable of creating O\textsubscript{6}-meG adducts, has been detected in normal individuals at levels ranging from ~48 to 1000 adducts/cell in leukocytes (26–29). Strategies for protecting the genome from alkylation have been highly considered in nature and include O\textsubscript{6}-meG-DNA methyltransferases (MGMT), which directly and specifically repair O\textsubscript{6}-meG adducts (30, 31), and the mismatch repair heterodimer MutS\textalpha (composed of MSH2 and MSH6; Ref. 32).

To test the hypothesis that O\textsubscript{6}-meG is an important mutagenic intermediate in vivo, human cells (MGMT\textsuperscript{-}) were treated with MNNG to create O\textsubscript{6}-meG adducts at levels comparable with that observed in human tissues, and the size of the HPRT sequence scanned was extended to include 676 bp of coding regions and splice sites of exons 2–8 of the HPRT gene. This provided the opportunity to compare the MNNG-induced mutational spectrum with that observed in humans in vivo with greater statistical rigor. As a necessary control, the background mutational spectrum derived from untreated cells after 60 doublings was also determined.

MATERIALS AND METHODS

Cell Culture. TK6 B-lymphoblastoid cells were grown in suspension cultures using 7-liter spinner flasks in RPMI 1640 (Life Technologies, Inc., Grand Island, NY) supplemented with 5% horse serum (Life Technologies, Inc.) in a 37°C water jacket incubator with 5% CO\textsubscript{2}. Cells were passaged by daily dilutions to 2.5–5 × 10\textsuperscript{5} cells/ml (33).

Untreated Cultures. Untreated cultures were started from 10\textsuperscript{5} cells of a TK6 stock culture with an HPRT mutant fraction of 2 × 10\textsuperscript{-4}. Three cultures were then selected and expanded to 2.4 × 10\textsuperscript{6} cells in 7-liter tanks and maintained in exponential growth for 60 generations (48 days). Mutant fractions and plating efficiency were determined (34) by plating aliquots from each culture with and without 6TG (Sigma Chemical Co., St. Louis, MO) every 6 days. On day 48, 6TG was added to a final concentration of 1 μg/ml to select for HPRT mutants. These observations were used to calculate the background or “spontaneous” mutation rate (35).

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1 Supported by grants from the National Institute for Environmental Health, Sciences; PO1-ES07168-05, P42-ES04675, P01-ES01640, and the Department of Energy; and 10 of the 676 bp of the HPRT gene that were scanned was extended to include 676 bp of coding regions and splice sites of exons 2–8 of the HPRT gene. This provided the opportunity to compare the MNNG-induced mutational spectrum with that observed in humans in vivo with greater statistical rigor. As a necessary control, the background mutational spectrum derived from untreated cells after 60 doublings was also determined.

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MNNG-treated Cultures. Six cultures of TK6 cells (3.5 × 10⁹ cells each in 7-liter tanks) were grown for a day with 100 μl of 100% ethanol to ensure that doubling times were unaffected by the presence of ethanol. Twenty microliters of 14 μM MNNG (Sigma Chemical Co.) were added to four cultures of cells for a final concentration of 40 nM MNNG for 1.5 h. Two cultures were kept as untreated background controls. After incubation with MNNG for 1.5 h, cells were resuspended in fresh media. An aliquot of cells was drawn from each flask at the time of resuspension to determine cell survival for each MNNG-treated and control culture. After cultivation and analyzed using high-fidelity PCR coupled with constant denaturing CDCE or DGGE (3, 37, 38). Primers used for mutational spectrometry, CDCE electrophorograms of the PCR back-ground trace from 6TG R cell population at spontaneous hot spots (mutations present in the 6TG R cell population at spontaneous hot spots) were consistent with previous experiments using bulk cultures of exponentially growing TK6 cells (7, 8, 35, 41).

RESULTS

Spontaneous Cell Cultures. The doubling times for the three cultures were 17.4 ± 0.5 h (mean ± SD). Untreated cultures reached a mutant fraction of 1.8 ± 0.3 × 10⁻⁵ (mean ± SD) after 60 doublings. The background or spontaneous mutation rate, determined by least squares linear regression by plotting the mutant fraction as a function of doublings, was 2.7 × 10⁻⁷ ± 0.3 × 10⁻⁷ (mean ± SD) per cell doubling (33).

Cell survival for MNNG-treated cultures was 67 ± 3% (mean ± SD), and the MNNG-induced mutant fraction was 2.4 ± 0.2 × 10⁻⁵ (mean ± SD). The outcomes of both spontaneous and MNNG-treated cultures were consistent with previous experiments using bulk cultures of exponentially growing TK6 cells (7, 8, 35, 41).

Mutational Spectrometry. As an illustration of the results of mutational spectrometry, CDCE electrophorograms of the PCR background trace from HPRT™ cells (negative control) and the sets of the MNNG-induced mutant peaks from two independent cultures [a known copy number of mutant exon 8 sequence eluting at 16 min (data not shown) was included as an internal standard to permit calculation of mutant fractions for each peak observed].

Mutational Hot Spots of the Human HPRT Gene

Spontaneous Hot Spots in Vitro. Fourteen reproducible spontaneous hot spots (mutations present in the 6TG R cell population at a level of ≥0.4%) within the 676-bp sequences scanned accounted
for ~10% of all spontaneous HPRT\(^{-}\) mutants (Fig. 2, Table 1). Large deletions account for an additional 40% of spontaneous mutations in TK6 cells (42). Combining these studies, we estimate that about half of all background in vitro HPRT\(^{-}\) mutants are accounted. For a mutant hot spot present at 0.4%, the number of independent mutations that arose in each 6-liter culture is 173\(^{a}\). About half of all background cultures, totaling 508 spots were reproducibly detected and sequenced in MNNG-treated cultures (2%). It is possible that the 508 transition arose spontaneously (i.e., via deamination and/or misincorporation) and was diluted by MNNG-induced mutants before 6TG selection. However, this mutation was not present at a level > 0.4% in the untreated background controls carried alongside the MNNG-treated cultures. Because the total MNNG-induced mutant fraction is almost seven times higher than the untreated background controls, it is assumed that all four hot spots were induced by MNNG.

### MNNG-induced Hot Spots in Vitro

Fourteen mutational hot spots were reproducibly detected and sequenced in MNNG-treated cultures, totaling ~45% of all MNNG-induced 6TG\(^{R}\) mutants (Fig. 2, Table 2). For an observed mutant fraction of 0.4%, the number of independently induced mutations was 203 ± mutations (mean ± SD). All hot spot sequences from MNNG-treated cultures were G:C to A:T transitions mutations.

### Somatic Hot Spots in Vivo

The human HPRT mutational database contains both somatic mutations of 6TG-resistant peripheral

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### Table 1: Summary of spontaneous HPRT hot spots in human TK6 cells after 60 in vitro doublings

<table>
<thead>
<tr>
<th>bp Position(^{a})</th>
<th>Exon/intron(^{b})</th>
<th>Sequence context(^{c})</th>
<th>Mutation type(^{d})</th>
<th>Point mutant fraction(^{e})</th>
<th>Phenotypic Δ(^{f})</th>
<th>Somatic mutations(^{g})</th>
<th>Germ-line mutations(^{h})</th>
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<tr>
<td>258-9 or 257-8</td>
<td>Exon 3</td>
<td>G AA(\triangleright)A/GA AA(\triangleright)AG</td>
<td>del 2</td>
<td>5.0 × 10(^{-3})  Frameshift</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>265</td>
<td>Exon 3</td>
<td>GA A(\triangleright)A/GA TT(\triangleright)GA</td>
<td>del 1</td>
<td>4.0 × 10(^{-3})  Frameshift</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>401 or 403</td>
<td>Exon 5</td>
<td>GTG G(A(\triangleright)G)Gac(\triangleright)tc</td>
<td>del 4</td>
<td>2.0 × 10(^{-2})  Splice site loss</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>435-437</td>
<td>Exon 6</td>
<td>A(G(\triangleright)T)(T)TG TTT</td>
<td>ins T</td>
<td>5.0 × 10(^{-3})  Frameshift</td>
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<td>0</td>
<td>0</td>
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<tr>
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<td>0</td>
</tr>
<tr>
<td>IVS6 + (2-5)</td>
<td>Intron 6</td>
<td>AG gta(\triangleright)gta aca</td>
<td>del 4</td>
<td>4.0 × 10(^{-3})  Splice site loss</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
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<td>Exon 7</td>
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<td>C &gt; T</td>
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<td>6</td>
<td>0</td>
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<tr>
<td>538 or 539</td>
<td>Exon 8</td>
<td>GAA TAC TCC</td>
<td>C &gt; A</td>
<td>7.0 × 10(^{-2})  Frameshift</td>
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### Table 2: Summary of MNNG-induced (40 nM) HPRT hot spots in human TK6 cells

<table>
<thead>
<tr>
<th>bp Position(^{i})</th>
<th>Exon/intron(^{j})</th>
<th>Sequence context(^{k})</th>
<th>Mutation type(^{l})</th>
<th>Point mutant fraction(^{m})</th>
<th>Phenotypic Δ(^{n})</th>
<th>Somatic mutations(^{o})</th>
<th>Germ-line mutations(^{p})</th>
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</thead>
<tbody>
<tr>
<td>208</td>
<td>Exon 3</td>
<td>AAG GGG GCC</td>
<td>G &gt; A</td>
<td>1.0 × 10(^{-1})  Gly. - Arg</td>
<td>6</td>
<td>2</td>
<td>0</td>
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<tr>
<td>209</td>
<td>Exon 3</td>
<td>AAG GGG GCC</td>
<td>G &gt; A</td>
<td>1.0 × 10(^{-1})  Gly. - Glu</td>
<td>5</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>IVS4-1</td>
<td>Intron 4</td>
<td>catg AAT</td>
<td>G &gt; A</td>
<td>1.0 × 10(^{-1})  Splice site</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>400</td>
<td>Exon 5</td>
<td>GTG AAG ata</td>
<td>G &gt; A</td>
<td>2.0 × 10(^{-1})  Glu. - Lys</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IVS5 + 1</td>
<td>Intron 3</td>
<td>GAA gta</td>
<td>G &gt; A</td>
<td>1.0 × 10(^{-1})  Splice site</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>IVS5 + 5</td>
<td>Intron 5</td>
<td>taugg</td>
<td>G &gt; A</td>
<td>4.0 × 10(^{-1})  Splice site</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IVS6 + 1</td>
<td>Intron 6</td>
<td>A Agggat</td>
<td>G &gt; A</td>
<td>1.0 × 10(^{-1})  Splice site</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>508</td>
<td>Exon 7</td>
<td>CCA CGA AGT</td>
<td>C &gt; T</td>
<td>7.0 × 10(^{-1})  Arg. - stop</td>
<td>15</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>IVS7 + 5</td>
<td>Intron 7</td>
<td>taugg</td>
<td>G &gt; A</td>
<td>1.0 × 10(^{-1})  Splice site</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>538</td>
<td>Exon 8</td>
<td>GTG GAA TTT</td>
<td>G &gt; A</td>
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<td>1</td>
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<td>569</td>
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<td>1</td>
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<td>0</td>
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<tr>
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<td>Exon 8</td>
<td>GAT GAC TAT</td>
<td>G &gt; A</td>
<td>4.0 × 10(^{-1})  Asp. - Gln</td>
<td>3</td>
<td>1</td>
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<tr>
<td>599</td>
<td>Exon 8</td>
<td>TTC AGG GAT</td>
<td>G &gt; A</td>
<td>1.0 × 10(^{-1})  Arg. - Lys</td>
<td>3</td>
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<tr>
<td>IVS8 + 1</td>
<td>Intron 8</td>
<td>AAT gta</td>
<td>G &gt; A</td>
<td>1.0 × 10(^{-1})  Splice site</td>
<td>4</td>
<td>0</td>
<td>0</td>
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</tbody>
</table>

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\(^{a}\) Column 1, mutational hot spot position (A of ATG is nucleotide 1 for mutations occurring in coding sequences.

\(^{b}\) Column 2, the affected exon/intron.

\(^{c}\) Column 3, sequence context on the untranscribed DNA strand (uppercase letters indicate coding sequences; lowercase letters indicate intron sequences). Bold characters indicate the changed bp or sequence; underlining highlights local repeat sequences.

\(^{d}\) Column 4, mutation type.

\(^{e}\) Column 5, observed mutant fraction (mf\(_{i}\)).

\(^{f}\) Column 6, expected phenotypic change.

\(^{g}\) Column 7, number of times the mutation was observed out of 458 6TG\(^{R}\) mutants from peripheral T-lymphocytes of normal individuals.

\(^{h}\) Column 8, number of times the mutation was observed out of 108 Lesch-Nyhan patients.

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**Note:** This text is a simplified representation of the provided content. For a more detailed analysis, please refer to the original source. The table and figure references are also included to provide context for the data presented.
blood T-lymphocytes (10–14, 16) and inherited mutations (10, 15). 458 somatic point mutations (bp substitutions and ≤20 bp insertion/deletions) from normal, unexposed individuals and 108 inherited point mutations from Lesch-Nyhan patients have been reported in this database for the 676 bp scanned by CDCE/DGGE. [Gout patients were excluded because even 1% of normal HPRT activity can lead to toxic effects with the addition of 6TG (43), and inherited mutations related to Lesch-Nyhan syndrome may be more comparable with 6TG selected mutants than those associated with familial gout.] The somatic set contained 75 putative in vivo hot spots (Table 3), herein defined by two or more independent occurrences (2 of 458 somatic mutations or the equivalent of 0.4%). We estimated that the probability for any point mutation to be observed twice was 8.4% for a distribution across 676 bp × five possible point mutations (3 bp changes and two possible framenshifts +/− 1 bp). Our definition of a hot spot requires that of the 75 point mutations so designated, perhaps 6 would have been expected by chance as opposed to resulting from a hot spot requires that of the 75 point mutations so designated, perhaps 6 would have been expected by chance as opposed to resulting from a hot spot.

<table>
<thead>
<tr>
<th>Position</th>
<th>Mutation type</th>
<th>Somatic mutations in vivo</th>
<th>Sequence context</th>
<th>Position</th>
<th>Mutation type</th>
<th>Somatic mutations in vivo</th>
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<td>g &gt; a</td>
<td>3</td>
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<tr>
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<td>c &gt; t</td>
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<tr>
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<td>2</td>
<td>GAG GTT</td>
<td></td>
</tr>
<tr>
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<td>IVS8 + 1</td>
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<td>AAT gtaa</td>
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<td>g &gt; a</td>
<td>5</td>
<td>AAT gtaa</td>
<td></td>
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</tbody>
</table>

* Column 1, hot spot position (A of ATG is nucleotide 1 for mutations occurring in coding sequences; intron mutations are represented by intron number and donor or acceptor site).
* Column 2, mutation type on the untranscribed DNA strand.
* Column 3, number of times the hot spot mutation was observed out of 458 6TG mutants from peripheral T lymphocytes of normal individuals.
* Column 4, sequence position. Bold characters indicate the changed bp or sequence.
* Columns 5–8, Columns 1–4 repeated.

Table 3 Table of putative somatic HPRT in vivo hotspots

The possibility that the hot spot concordance was not determined by the mutagenic pathway but was instead attributable to the kind of mutation created was also tested. To determine whether the concordance held within the set of G:C to A:T transitions in the HPRT gene, the population number was reduced to 86 phenotypically observable...
HPRF MUTATIONS SHARE HOT SPOTS WITH MNGN-TREATED CELLS

Table 4 Table of putative in vivo G:C to A:T hotspots

<table>
<thead>
<tr>
<th>Position of Mutation</th>
<th>Somatic mutations in vivo</th>
<th>Sequence context</th>
<th>CpG or CNG site</th>
<th>MNNG hotspot</th>
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<tbody>
<tr>
<td>bp</td>
<td>type</td>
<td></td>
<td></td>
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<td>47</td>
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<td>CCA GGT TAT</td>
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<td>13</td>
<td>GAA GGT CTT</td>
<td>Cpg</td>
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<td>GGT TCT GCT</td>
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<td>197</td>
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<td>AAG GGG GCC</td>
<td>MNNG</td>
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<td>212</td>
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<td>GGG GCC TAT</td>
<td></td>
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<tr>
<td>325</td>
<td>c &gt; t</td>
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<td>GAC GAC TCA</td>
<td>CAG</td>
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<td>2</td>
<td>GAAaat</td>
<td></td>
</tr>
</tbody>
</table>

* Column 1, hotspot position (A of ATG is nucleotide 1 for mutations occurring in coding sequences; intron mutations are represented by intron number and donor or acceptor site).
* Column 2, mutation type on the untranscribed DNA strand.
* Column 3, number of times the hotspot mutation was observed out of 458 G:Ts.
* Column 4, sequence position. Bold characters indicate the changed basepair or sequence.
* Column 5, hotspots at CpG or CNG sites.
* Column 6, hotspots induced by MNNG in vitro.

G:C to A:T mutations that have been reported previously and fell within the CDCE/DGGE scanned regions. The null hypothesis that of 86 possible G:C to A:T mutations, 10 of 14 MNNG-induced hot spots were concordant with 10 of 29 in vivo hot spots (Table 4) by chance was still rejected ($P < 0.002$). The concordance suggested that the MNNG-induced hot spots comprised, therefore, a considerable non-random and reproducible subset of the 29 reported G:C to A:T hot spots of the in vivo somatic set.

Inherited Mutations in Vivo. Because of the small number of reported inherited mutations (currently only 108 Lesch-Nyhan patients), identification of germ-line hot spots could not be determined. However, 3 of 14 spontaneous hot spots and 9 of 14 MNNG-induced hot spots were observed at least once in the inherited set (Tables 1 and 2). It is interesting to note that the three spontaneous hot spots (bp 508, 538, and 580) observed in the germ line were also among the 4 hot spots shared by the in vitro set.

DISCUSSION

Mutational hot spots have been demonstrated to be dependent on the duration and concentration of mutagen exposure (2). Thus, special effort was made to simulate human in vivo conditions in human cells in vitro, with regard to the level of 6-O-methyleneguanine in cellular DNA. It was determined previously that treatment with 40 nM MNNG in MGMT-deficient TK6 cells would create ~400 6-O-methyleneguanine adducts per cell (35), within the midrange of 6-O-methyleneguanine steady-state levels observed in normal human organs (26–29).

The Concordant Set. The 10 in vivo T-cell mutational hot spots found in the MNNG-induced spectrum support the hypothesis that a significant subset of somatic in vivo hot spots share a mutational pathway with MNNG-induced mutations. These 10 G:C to A:T hotspots account for 18% (48 of 265) of all point mutations occurring as putative hot spots in T cells and 33% (48 of 145) of the set of G:C to A:T hot spots in vivo (see Table 4). Because there is strong evidence that MNNG acts via 6-O-methyleneguanine, the shared mutational pathway could involve reaction with one or more SN1 methyleneguanine agents that would be expected to create a distribution of 6-O-methyleneguanine in the HPRT gene similar to that created after exposure to MNNG.

Although no endogenous chemical has yet been demonstrated to create 6-O-methyleneguanine in DNA (45–47), there is little doubt that it occurs at measurable levels in human tissues or DNA (26–29). The source of the in vivo 6-O-methyleneguanine is unknown. Many DNA methyleneguanine agents can be formed by reaction of endogenous amines with N2O3 (reviewed in Ref. 48). Exogenous methyleneguanine agents include a variety of methyl-N-nitrosamines in tobacco smoke, nitrate-cured meat, and red wine (49, 50). The observations of this study do not, however, distinguish among the possibilities that methyleneguanine agents are of exogenous or endogenous origin or both. Although only normal individuals (i.e., no known chemical or radiation exposure) were used in the somatic set of in vivo mutations, smokers were also included (~200 individuals were considered smokers). The smokers did not appear to bias the concordant hot spots either positively or negatively.

The Discordant Set

MNNG-induced Hot Spots not Found in the in Vivo Spectrum. Four of 14 MNNG-induced hotspots were not observed two or more times among reported peripheral T-cell mutations. However, three of these were reported once among T-cell mutations, and of these, one was reported twice among inherited HPRT mutations, suggesting their absence from the somatic in vivo hot spot list results from the relatively small number of persons assayed. The single example of an MNNG-induced in vitro hot spot without a somatic or inherited mutation reported occurred at the final bp of the intron preceding exon 4. We note in passing that such splice site mutations are numerically underrepresented in the reported HPRT in vivo mutations because most reports involve cDNA sequencing, which would not identify specific splice site mutations. Other concordant hot spots may exist in unscanned exon sequences of the HPRT gene (119 bp) and sequences affecting gene expression or mRNA splice sites (>100 bp). Concordant mutations may also occur as less frequent events that were not detected by our mode of analysis.

In Vivo Hot Spots not Found in the MNNG Spectrum-CpG and CpNpG Sites. The set of 19 in vivo G:C to A:T hot spots not found within the MNNG-induced set contains those at CpG or CpNpG sites, as well as others of unknown etiology (Table 4). Almost 23% (33 of 145; G:C to A:T mutations) of the in vivo G:C to A:T hot spots occurred at CpG dinucleotide sites, which are believed to be sites of enzymatic cytosine methylation and are thus susceptible to deamination and subsequent mutation (51, 52). Another 25% (36 of 145 somatic G:C to A:T mutations) occurred at CpNpG trinucleotide sites, which are also thought to be potential sites for enzymatic cytosine methylation.

The Remaining in Vivo Hot Spots not Found in the MNNG Spectrum. Sixty in vivo HPRT hot spot mutations (two or more reported somatic mutations) were not concordant with the MNNG-induced spectrum nor with the set of CpG/CpNpG sequences. A small set of independent mutational pathways could account for them. If the single MNNG-like pathway could account for 10 of 75 hot spots, perhaps an additional half-dozen mutational pathways could account for the remainder. Among these could be unedited DNA polymerase misincorporation events during DNA replication, such as those created by the β DNA repair polymerase (53) during damage-induced DNA repair or metabolic DNA turnover (54).
In conclusion, it appears that MNNG treatment mimics a human T cell in vivo mutational pathway, accounting for 18% of somatic HPRT mutations. Whether DNA methylation is actually involved, as hypothesized, or whether endogenous or exogenous methylating agents are involved can not be determined from these data (55).

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The Mutational Spectrum of the HPRT Gene from Human T Cells in Vivo Shares a Significant Concordant Set of Hot Spots with MNNG-treated Human Cells


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