Autosomal mutation in somatic cells of the mouse

Mitchell S. Turker

Center for Research on Occupational and Environmental Toxicology and Department of Molecular and Medical Genetics, Oregon Health and Science University, Portland, OR 97239, USA

Tumor suppressor genes are located on autosomal chromosomes. Therefore, an understanding of how cancer-related mutations occur in somatic cells requires a detailed understanding of spontaneous and induced autosomal mutagenesis. This review will present recent advances in the study of how autosomal mutations form in somatic cells by focusing on the mouse Aprt and Tk model systems that have been developed to examine the formation of autosomal mutations in vivo. These loci can detect the entire spectrum of mutations known to inactivate tumor suppressor genes. Studies with these models have provided novel information on the frequencies and types of spontaneous autosomal mutations that occur in different cell types. They also show great promise for the screening of genotoxic effects resulting from environmental exposures and for the study of mutation when DNA repair pathways are compromised. Continued use of the mouse Aprt and Tk models will have a significant impact on our understanding of some of the earliest steps in the conversion of normal cells to those with malignant phenotypes.

Introduction

The chromosomal complement of each cell is divided into the autosomes and the sex chromosomes. Each autosomal gene is present in two copies per diploid cell and in most cases both genes are expressed. The exception to this rule is imprinted autosomal genes, for which expression is limited to only one of the two alleles (Brannan and Bartolomei, 1999). X chromosome-linked genes are present in only one copy per male cell and in only active copy per female cell. A comparison of mutational events that can affect autosomal loci versus those that can affect genes on the X chromosome reveals several significant and overlapping differences (Turker, 1998). One is that only a single mutational event is required to cause phenotypic loss of cellular expression for an X-linked gene. In contrast, both copies of an autosomal gene must be inactivated for the cell to become phenotypically null because retention of one active allele is sufficient to maintain a wild-type phenotype under most conditions (Wijnhoven et al., 2001b). A second difference is that mutational events that delete one or more critical loci on the X chromosome are lethal to the cell whereas multiple genes with critical functions can be lost from an autosomal chromosome as long as the remaining homolog retains functional copies of these genes. A third difference is that the spectrum of mutations that can alter expression of an X-linked gene is broader than the spectrum of mutations that can alter expression of X-linked genes. A comparison of mutational events that can alter expression for X-linked genes with second step autosomal mutation is shown in Table I. Significant events that can result in second step mutation, such as gene conversion, mitotic recombination, chromosome loss (with or without re-duplication of the remaining homolog) and large interstitial deletion, are limited to autosomal loci (Turker, 1998; Wijnhoven et al., 2001b).

Molecular techniques that are used to examine autosomal mutations rely on polymorphisms, such as microsatellite sequences, that can distinguish two homologs from each other. As shown in Figure 1, the patterns for loss of heterozygosity (LOH) for these polymorphic markers can be used to determine the types of mutations that have occurred.

Spontaneous mutation in wild type backgrounds

Knockout technology has allowed both the mouse Aprt (chromosome 8) (Stambrook et al., 1996; Van Sloun et al., 1998) and Tk (chromosome 11) (Dobrovolsky et al., 1999a) loci to be used to examine mutation formation in vivo. Based on the integrated linkage and physical maps (www.information.jax.org), Aprt is located ~10 cM from the chromosome 8 telomere and Tk is located ~6 cM from the chromosome 11 telomere. By starting with mice heterozygous for Aprt or Tk it has been possible to examine second step mutations. At the present time spontaneous mutants have been isolated from three cell types: T cells from the spleen (Van Sloun et al., 1998; Dobrovolsky et al., 1999a; Shao et al., 2000), mesenchymal cells from the ear (Shao et al., 1999) and epithelial cells from the kidney (Ponomareva et al., 2002). Aprt mutant cells from these three cell types have been reported, whereas Tk mutants have only been reported for T cells.

An analysis of spontaneous mutant frequencies has revealed several interesting observations. One is that significant animal-to-animal and tissue-to-tissue variation exists for autosomal mutant frequencies with as much as 50-fold differences observed in a given study (Van Sloun et al., 1998; Shao et al., 1999; Ponomareva et al., 2002). A second observation is that Hprt mutant frequencies for T cells are ~5- to 20-fold lower than the T cell Aprt and Tk mutant frequencies, with significantly less variation (Dobrovolsky et al., 1999a, 2000, 2002b; Wijnhoven et al., 2001b; Liang et al., 2002; VonTungeln et al., 2002). Lower Hprt mutant frequencies have also been reported for the kidney (Horn et al., 1984) when compared with Aprt mutant frequencies for this tissue (Turker et al., 1999b; Ponomareva et al., 2002). Taken together, these observations are consistent with the broader spectrum of mutational events for autosomal loci (Table I) and they suggest that this broader spectrum leads to higher autosomal mutant frequencies and a greater degree of variation.

To whom correspondence should be addressed at: CROET, L606, Oregon Health and Science University, 3181 SW Sam Jackson Park Road, Portland, OR 97239, USA. Tel: +1 503 494 2168; Fax: +1 503 494 3849; Email: turkerm@ohsu.edu

© The Article Author(s). Published by Oxford University Press 2003
potentially more mutant cells, this turnover will also lead to continuous loss of the mutant cells. In contrast, lower turnover of cells in solid tissue could lead to the gradual accumulation and/or retention of mutant cells.

The ability to expand mutant Aprt and Tk clones has allowed analyses of the spectrum of spontaneous autosomal mutations. The predominant observation for both loci is that second step loss of gene expression is often accompanied by large mutational events that cause loss of the entire wild-type gene (Van Sloun et al., 1998; Dobrovolsky et al., 1999a; Shao et al., 1999; Ponomareva et al., 2002). Such loss is observed in ~35–80% of mutant cells, with higher percentages observed more often for Aprt mutations. As shown in Table I, there are four distinct mutational mechanisms that can remove an entire copy of an autosomal gene: mitotic recombination, chromosome loss, gene conversion and interstitial deletion. However, to detect these events by molecular analysis it is necessary to examine multiple syntenic (i.e. linked) loci, which can best be achieved by creating a situation in which heterozygosity exists for these loci (Figure 1). This can be accomplished by breeding mouse strains that are heterozygous for a microsatellite sequence on a given chromosome (Shao et al., 1999; Ponomareva et al., 2002).

Several reports of Aprt mutation in vivo have suggested unexpected complexity in mutational pathways. Spontaneous mutations occurring in kidney and ear epithelial cells revealed tissue-specific differences (Ponomareva et al., 2002). Molecular evidence for chromosome loss was observed in ~41–43% of kidney cell mutants, but in only 6% of ear cell mutants. In contrast, apparent deletional events were observed in ~20% of ear mutants, but were not observed in kidney cell mutants. Approximately 35% of mutational events were due to mitotic recombination in both cell types. This latter observation is not in agreement with other reports showing that ~80% of ear cell Aprt mutants (and T cell Aprt mutants) are due to mitotic recombination (Shao et al., 1999).

The discordant results for the prevalence of mitotic recombination in the ear cells observed by different groups is potentially explained by a separate observation showing that the frequency of mitotic recombination is significantly influenced by the degree of homology between homologous chromosomes (Shao et al., 2001). When the degree of homology falls too low, as will happen when homologs from different mouse species are present in the same cell, mitotic recombination is essentially eliminated. Tissue-specific differences were also observed in this study because in certain hybrid strains mitotic recombination was eliminated in ear cells, but still occurred commonly in T cells.

The presence of more than one mutant cell in a given solid tissue can have two potential sources. One is that all mutant cells are derived from a progenitor cell in which the mutational event occurred. The prediction for this possibility is that all mutant cells isolated from a given tissue will exhibit the same mutational event. Alternatively, multiple mutational events can occur within a given tissue leading to the observation of mutant cells from a given tissue exhibiting different types of mutations. Studies with Aprt have suggested that both possibilities can occur, sometimes in the same tissue (Shao et al., 1999; Ponomareva et al., 2002).

An additional observation that has been made regarding spontaneous loss of gene expression in vivo is that many small events are not actually mutations, but instead appear to be caused by epigenetic silencing. Evidence for epigenetic events

<p>| Table I. Comparison of mutational events that can lead to inactivation of genes on the X chromosome versus those on autosomes |</p>
<table>
<thead>
<tr>
<th>Mutational event</th>
<th>X chromosome</th>
<th>Autosome</th>
<th>Loss of wild-type allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene silencing</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Basepair substitution</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Gene deletion</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Megabase deletion&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Yes/No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Gene conversion</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Mitotic recombination</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Chromosome non-disjunction (loss)</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

<sup>a</sup>For autosomal mutations, this comparison assumes that one of the two alleles is inactivated and the second step mutational event is being analyzed.

<sup>b</sup>Upper limit for HPRT gene region in human cells is ~1.3–1.7 Mb (Nelson et al., 1995).

A third interesting observation is that average Aprt mutant frequencies for the solid tissues (i.e. ear and kidney) (Turker et al., 1999b; Shao et al., 2000, 2002; Ponomareva et al., 2002) are ~2– to 4-fold higher than those observed for splenic T cells (Langlois et al., 1990; Van Sloun et al., 1998; Wijnhoven et al., 1998; Shao et al., 2002). A similar elevation for Hprt mutant frequencies in solid tissues can be discerned when comparing T cell Hprt mutant frequencies (Dobrovolsky et al., 1999a, 2000, 2002b; VonTungeln et al., 2002) with those reported for mouse kidney and skeletal muscle tissues (Horn et al., 1984). Reduced mutant frequencies for the T cells could be due to a selective disadvantage resulting from loss of purine salvage enzymes, as has been shown for Hpult-deficient T cells in the mouse (Deubel et al., 1996). Alternatively, it is possible that mutant cells can accumulate in the solid tissues because they do not turn over as rapidly as blood cells. Although rapid turnover will lead to more cell divisions and, therefore,
includes a lack of detectable point mutations when many Aptr alleles (Shao et al., 1999; Rose et al., 2000) or Tk cDNAs (Dobrovolsky et al., 2000) were sequenced. Reversibility of inactivation for cultured Aptr-deficient cells derived from the kidney (Rose et al., 2000) is additional evidence for gene silencing in vivo.

Induced mutagenesis

It is important to describe the types of mutations induced by various carcinogens to better assess how these agents can trigger cancer. This information will also provide signature mutations that can allow carcinogenic exposures to be determined after they have occurred. For example, C→T and CC→TT mutations are induced by UV light in a number of experimental systems. These mutations are commonly found in mutant p53 and Patched genes in human skin cancers providing molecular confirmation of the link between some types of skin cancer and exposure to the sun (Dumaz et al., 1994; Daya-Grosjean et al., 1995; Brash, 1997; Bodak et al., 1999). The majority of studies describing the types of mutations induced by various genotoxins in vivo have focused on small events such as base pair substitutions because bacterial transgenes are most often used as the mutational target (Gossen et al., 1994; Dean et al., 1999). However, large events that trigger LOH for chromosomal segments are more common in cancers (Lasko and Cavenee, 1991; Wijnhoven et al., 2001b; Thiagalingam et al., 2002). Therefore, a great deal of information is still needed to understand how carcinogens induce large mutational events in mammalian cells.

Ionizing radiation has been shown to cause large mutational events, including interstitial deletions, in cultured cells (Grosovsky et al., 1988; Hutchinson, 1995; McGuinness et al., 1995; Turker et al., 1997; Giver and Grosovsky, 2000; Gauny et al., 2001; Kraemer et al., 2001). Therefore, it should provide an ideal genotoxin to induce large mutational events in vivo. However, studies with single exposures to 1–4 Gy X-rays for Aptr (Wijnhoven et al., 1998; Liang et al., 2002) and to 2–4 Gy 60Co γ-radiation for Tk (Dobrovolsky et al., 2002b) failed to demonstrate statistically significant increases in mutant frequencies for T cells. In contrast, a single exposure to 7.5 Gy 137Cs γ-radiation caused a statistically significant increase in mutant cells for both the ear (−3-fold) and kidney (−4.5-fold) (Ponomareva et al., 2002). Statistical power was increased for this study by only irradiating one side of each animal and then comparing results from the exposed and non-exposed sides on an animal-by-animal basis. Moreover, the induction of apparent deletions by ionizing radiation was observed in kidney cells. A second molecular observation from this study was that discontinuous LOH (Figure 1) on chromosome 8 was induced by ionizing radiation in both ear and kidney cells. Discontinuous LOH represents mutational patterns in which LOH events are observed on a chromosome that is apparently unlinked to the mutational event causing loss of gene expression. Interestingly, the discontinuous LOH mutational patterns were observed in ionizing radiation-induced Aptr mutants ≥8 months after exposure. This result suggested a delayed mutagenic effect in vivo. Consistent with this possibility, discontinuous LOH on chromosome 8 was observed as a delayed effect of ionizing radiation in cultured kidney cells (Ponomareva et al., 2002).

A comparison of two carcinogens known to produce base pair substitutions in bacterial transgenes and at the Hprt locus in mice (Gorelick et al., 1995; Walker et al., 1996) revealed interesting differences when they were tested for their abilities to induce Aptr and Tk T cell mutants. Most N-ethyl-N-nitrosourea (ENU) DNA lesions are non-bulky (Wijnhoven et al., 1998) and therefore they do not interfere with DNA replication, whereas 7,12-dimethylbenz[a]anthracene (DMBA) causes bulky lesions (Hollander et al., 2001) that interfere with replication. This difference is reflected in the predominant types of autosomal mutations that these carcinogens induced at the Tk and Aptr loci (Wijnhoven et al., 1998; Dobrovolsky et al., 1999b, 2000). ENU induced predominantly small events consistent with base pair substitution events, which was demonstrated directly at the sequence level for Tk mutants. DMBA also induced intragenic events at Aptr; however, its predominant mutagenic effect was to induce large mutational events that were shown with a cytogenetic analysis for Aptr to be the result of mitotic recombination or chromosome loss. These observations suggest that replication blocks occurring as a result of bulky adduct formation caused by DMBA, but not the smaller lesions caused by ENU, can trigger large mutational events.

Etoposide, a topoisomerase II inhibitor that is used to treat a variety of human cancers (da Rocha et al., 2001), was also tested for its ability to induce Tk (Dobrovolsky et al., 2002b) and Aprt (Turner et al., 2001) mutations in T cells. For Aprr, a statistically significant 3-fold increase in the mutant frequency was observed when the mice were treated with a 1 mg/kg dose. Most of the mutant cells examined exhibited loss of the wild-type Aprr allele, again consistent with the induction of large events. At the same dose, a 2-fold increase was observed for Tk, but this number was not statistically significant for the number of animals tested. Interestingly, when a 10-fold higher dose of etoposide was tested the mutant frequencies decreased in both systems. These results were most likely due to increased T cell killing by etoposide at this higher dose.

The Tk locus was also used to examine the mutagenic potential of two drugs used to prevent mother-to-child transmission of HIV, 3′-azido-2′,3′-dideoxythymidine (AZT) and 3′-thia-2′,3′-dideoxycytidine (3TC) (VonTungeln et al., 2002). When tested singly, a 2- to 3-fold increase in the Tk mutant frequency was observed for AZT, but no increase was observed for 3TC. However, a synergistic interaction was observed when the drugs were used in combination, with a 4- to 6-fold induction of Tk mutant cells. Although AZT and 3TC are base analogs, most of the induced mutations were large events that resulted in loss of the wild-type Tk locus. AZT induced deletional events at the Hprt locus in a cultured lymphoblastoid cell line (Sussman et al., 1999), suggesting that incorporation of this genotoxin caused strand breaks. The cross-linking agent mitomycin C was also shown to induce large mutational events causing loss of Tk expression in T cells (Dobrovolsky et al., 2002b).

Spontaneous and induced mutagenesis in DNA repair-deficient cells

A significant advantage of using in vivo systems is that it is possible to breed mice with DNA repair deficiencies to those with Aprr or Tk deficiencies to examine spontaneous and induced mutagenesis in different DNA repair-deficient backgrounds. This approach also provides a relatively easy mechanism to create DNA repair-deficient cell lines for mutagenesis work with cultured cells. At the present time the
effects of four DNA repair deficiencies on Aprt mutations have been published. (Similar experiments with Tk have not yet been published.)

Two published studies reported mutagenic effects resulting from loss of the PMS2 or MLH1 mismatch repair proteins. These two proteins form a functional heterodimer, MutLα, and loss of either protein has been associated with increased frequencies of frameshift mutations at mono- and dinucleotide repeat regions and increased frequencies of base pair substitutions (Buermeyer et al., 1999). Because mouse Aprt does not contain repeat sequences that are prone to mutations, it is useful for the study of mutagenic effects of mismatch repair deficiency, specifically for base pair substitutions. One interesting observation made with Pms2 and Mlh1 null cultured kidney cells is that the presence of two and occasionally three well-separated base pair substitution events is common at mouse Aprt after the cells were exposed to UV radiation or hydrogen peroxide (Shin et al., 2002). Multiple mutations at Aprt were not observed when wild-type cultured cells were exposed to these genotoxins (Khattar and Turker, 1997; Turker et al., 1999a). This observation provides multiple mutations as an additional marker for genomic instability in mismatch repair-deficient cells (Ceccotti et al., 2000; Zhang et al., 2002).

Although the PMS2 and MLH1 proteins form the MutLα heterodimer (Buermeyer et al., 1999), distinct mutational spectra were associated with loss of each protein. A:T→G:C transversions were the predominant small mutational event (~50% of all small events) in Pms2 null kidney cells, whereas a more broad distribution of base pair substitution events were observed in Mlh1-deficient cells (Shin et al., 2002). A:T→G:C base pair substitutions were also the most common small mutational events at Aprt in T cells in vivo (Shao et al., 2002). A second difference in the mutational spectra was that loss of the wild-type Aprt allele was common in Pms2-deficient kidney cells (~70% of all mutational events), but was rare in the Mlh1-deficient background (2% of events) (Shin et al., 2002). Loss of the wild-type allele was also common in vivo in Pms2-deficient mice in ear fibroblast mutants (65% of events), but was less common in the T cell mutants (35% of events) (Shao et al., 2002). This latter observation provides additional evidence that cell type specificity exists for mutation formation. A preliminary report of Tk mutation in T cells of Pms2 null mice has appeared recently (Dobrovolsky et al., 2002b).

The effect of p53 deficiency on mutagenesis was examined in ear fibroblasts and splenic T cells in vivo. Once again, a tissue-specific difference was observed because p53 deficiency increased the spontaneous frequency of Aprt ear fibroblast mutants ~3-fold but had no significant effect on the Aprt mutant frequency for T cells (Shao et al., 2000). However, a single dose of 4 Gy X-rays increased the frequency of Aprt mutant T cells ~8-fold in the p53 null mice, despite having little or no effect in wild-type mice (Liang et al., 2002). At the molecular level it was shown that much of this increase was due to the formation of deletional events. These results demonstrate nicely that a repair deficiency with little or no effect by itself on spontaneous mutant frequency can act in synergy with genotoxin exposure to produce a dramatic induction of mutations.

In a third study, the effect of deficiency of Atm (ataxia telangiectasia mutation), which is responsible for the repair response in cells exposed to ionizing radiation, was examined in kidney and ear cells in vivo (Turker et al., 1999b). Although Atm deficiency is grouped with a variety of chromosomal instability syndromes (Meyn, 1999), complete loss of expression did not increase the Aprt mutant frequency in either tissue relative to mice with Atm heterozygous deficiencies and to wild-type mice (Turker et al., 1999b). This negative result could reflect the relatively short lifespan of Atm null mice (~2–5 months) (Barlow et al., 1996) due to the formation of lymphomas and/or the relatively benign conditions in which they were maintained. Spontaneous genomic instability for Atm null kidney cells in culture was observed in the form of the discontinuous LOH mutational pattern (Figure 1; Gage et al., 2001). This mutational pattern has not been observed to occur spontaneously in wild-type cells (Turker et al., 1999a).

A fourth study examined the effect of deficiency for the nucleotide excision repair gene Xpc on spontaneous and DMBA-induced mutations in T cells with the Aprt model (Wijnhoven et al., 2001a). The spontaneous Aprt mutant frequency increased 3-fold in Xpc null null mice relative to mice that were heterozygous for Xpc, with increases being observed for both LOH events and intragenic mutations. As mentioned in the previous section, DMBA induced LOH events in wild-type mice (Wijnhoven et al., 1998). While it also did so in the Xpc null mice, a proportionally larger increase was observed for intragenic mutations, suggesting that NER normally lessens the induction of base pair substitutions by DMBA bulky lesions (Wijnhoven et al., 2001a).

Conclusions

The mouse Aprt and Tk systems allow the entire spectrum of autosomal mutations to be detected. As described in this review, an increasing number of studies are being performed with these systems to characterize the types of spontaneous and induced mutations that can occur in wild-type mouse cells and in those with impaired DNA repair pathways. One of the more interesting observations made has been that tissue-specific differences exist for the spectrum of autosomal mutations. Whether these differences represent intrinsic cellular properties or different environmental exposures remains to be determined. It is anticipated that the Aprt and Tk models will be used increasingly to test the mutagenic potential of a variety of genotoxic agents because they can detect very wide spectra of mutational events. Equally important, they will be used to elucidate basic mechanisms of mutagenesis in a variety of cell types, including those that have developed mutator phenotypes and/or that are malignant. Elevated rates of mutation have been reported in a variety of cancers (Jackson and Loeb, 1998). The availability of the Aprt and Tk mouse models should provide an abundance of novel information for the foreseeable future.

Acknowledgements

I would like to thank Drs. Jay Tischfield, Peter Stambrook, Robert Heflich, Vasily Dobrovolsky and Frederick Beland for making unpublished data available while this review was being written. This work was supported by NIH grants CA56383 and CA76528.

References


mice treated neonatally with zidovudine and lamivudine. Carcinogenesis, 23, 1427–1432.

Received on July 8, 2002; revised on August 2, 2002; accepted on August 8, 2002