Pol $\iota$ Is a Candidate for the Mouse Pulmonary Adenoma Resistance 2 Locus, a Major Modifier of Chemically Induced Lung Neoplasia

Min Wang,1 Theodora R. Devereux,2 Haris G. Vikis,4 Scott D. McCulloch,3 Wanda Holiday,2 Colleen Anna,2 Yian Wang,1 Katarzyna Bebenek,3 Thomas A. Kunkel,3 Kunliang Guan,4 and Ming You1

1Department of Surgery and The Alvin J. Siteman Cancer Center, Washington University School of Medicine, St. Louis, Missouri; 2Laboratory of Molecular Carcinogenesis and Laboratory of Molecular Genetics and Laboratory of Structural Biology, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, North Carolina; and 3Department of Biological Chemistry, University of Michigan, Ann Arbor, Michigan

Abstract

In this study, we performed systematic candidate gene analyses of the Pulmonary adenoma resistance 2 locus. Differential gene expression in lung tissues and nucleotide polymorphisms in coding regions between A/J and BALB/cJ mice were examined using reverse transcription-PCR and direct sequencing. Although not all genes in the interval were analyzed at this moment due to the recent database updating, we have found that the Pol $\iota$ gene, encoding the DNA polymerase $\iota$, contains 25 nucleotide polymorphisms in its coding region between A/J and BALB/cJ mice, resulting in a total of ten amino acid changes. Primer extension assays with purified BALB/cJ and A/J proteins in vitro demonstrate that both forms of Pol $\iota$ are active but that they may differ in substrate discrimination, which may affect the formation of Kras2 mutations in mouse lung tumors. Altered expression of Pol $\iota$ protein and an amino acid-changing nucleotide polymorphism were observed in human lung cancer cells, suggesting a possible role in the development of lung cancer. Thus, our data support the Pol $\iota$ gene as a modifier of lung tumorigenesis by altering DNA polymerase activity.

Introduction

Lung cancer is the leading cause of cancer-related death in the United States (1). Although cigarette smoke is implicated in ~90% of male and 75–80% of female lung cancer deaths, only ~15% of heavy smokers will ultimately develop lung cancer, which suggests that there is variation in individual susceptibility to lung cancer (2, 3). Inbred strains of mice vary markedly in their susceptibility to spontaneous and chemically induced lung tumorigenesis, thus representing a valuable model to study genetic susceptibility to lung cancer. On the basis of their mean tumor multiplicities induced by a lung carcinogen, the inbred mouse strains can be categorized into sensitive, intermediate, and resistant groups (4). The A strain is the most susceptible strain, whereas the C57BL/6J strain is the most resistant. Other strains such as the BALB/cJ strain belong to the intermediate group and are less susceptible to lung tumorigenesis than the A strain. Experimental crosses of inbred mouse strains have revealed Pulmonary adenoma susceptibility (Pas) loci, Pulmonary adenoma resistance (Par) loci, and Susceptibility to lung cancer (Sncs) loci (5).

A/J and BALB/cJ inbred strains of mice carry the same Pas1 allele but show different susceptibility to urethane-induction of lung tumors. In (A/J × BALB/c) F1 mice, the relatively resistant BALB/cJ phenotype was dominant over the high susceptibility of A/J mice (6). Additional analysis on F2 hybrids and backcross mice supported the hypothesis that a major locus named Par2 accounts for the difference in adenoma susceptibility between A/J mice and BALB/cJ mice (7). Par2 is mapped to the mouse chromosome 18 and accounts for 60% phenotype variance (7–9). The resistance of BALB/cJ mice appears due to the interaction between the Pas1 QTL and Par2 QTL in the BALB/cJ mouse genome (10). We previously used (A/J × BALB/cJ) × BALB/cJ congenic mice to successfully fine map the Par2 locus into a candidate region encompassed by the D18Mit103 and D18Mit162 markers (11). In a recent study, this region has been additionally narrowed down to a region encompassed by the marker D18Mit103 and D18Mit188 (12), which has resulted in a ~2.4-Mb candidate region based on the Celera and public mouse genome databases. Four known genes are located in this region, i.e., StarbD6, Pol $\iota$, Mbd2, and Dcc. Preliminary analysis of the polymorphisms in the Dcc and Pol $\iota$ genes were reported recently (11, 13).

Pol $\iota$ represents an interesting candidate for the Par2 locus. It is the most error-prone DNA polymerase and preferentially incorporates G rather than A across from template T (14–16). Human Pol $\iota$ has at least two distinct catalytic activities including translesion DNA synthesis and 5'-deoxyribosyl phosphate lyase activity (17–19). Its 5'-deoxyribose phosphate lyase activity and capability for filling short gaps implicate that this polymerase may play a role in certain base excision repair (BER) reactions (19). It may also play a role in lung tumorigenesis by affecting DNA adduct repair and thus Kras2 mutations that are found in a high proportion of mouse and human lung tumors (13, 20).

In the present study, we identified multiple nucleotide polymorphisms and alternatively spliced transcript isoforms in the Pol $\iota$ gene between A/J and BALB/cJ mice. We provide initial biochemical support for the hypothesis that the amino acid differences between the two mouse isoforms may affect their substrate discrimination properties. An amino acid-changing nucleotide polymorphism and altered expression of Pol $\iota$ protein in human lung cancer cell lines was observed. These data support the Pol $\iota$ gene as a modifier of lung tumorigenesis by altering DNA polymerase and, possibly, repair activity.

Materials and Methods

Expression and Nucleotide Polymorphism Analyses of Genes in the Par2 Region. Inbred mouse strains were purchased from The Jackson Laboratory (Bar Harbor, ME). DNA was isolated from tail snips. For RNA isolation, 100 mg of lung tissue were pulverized and total RNA extracted using TRIzol reagent according to the manufacturer’s protocol (Life Technologies, Inc., Gaithersburg, MD). The quality of the isolated RNA was assayed by absorbance at 260 nm, the A260/A280 ratio (1.7–1.9), and electrophoresis on 1% agarose/formaldehyde gels that indicated the intensity and integrity of the 28S and 18S bands. Two µg of total RNA were used in a reverse transcription reaction to synthesize the first-strand cDNA using oligo-dT primer. Primers were designed for each gene based on their published sequences. The following PCR condition was used for each primer set: 95°C for 3 min, followed by
30 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 1 min, and finally 72°C for 6 min. Annealing temperature was optimized for each primer set. Electrophoreses on 1.5% agarose gels were performed to resolve PCR products. Genomic DNA- and DNase I-treated cDNA samples were used for those containing only one exon. Amplified PCR products were purified with Quick gel extraction kit (Qiagen, Valencia, CA) and subjected to direct sequencing.

To detect alternative transcripts and nucleotide polymorphisms in the Pol ε gene, five primer sets were used to cover the entire Pol ε coding region: primer set 1: forward, 5'-GAGGCGGATCCGGCGAGCCAGGCTTCGACC-3'; primer set 2: forward, 5'-GAAGCGCTTCTTGAC-3'; primer set 3: forward, 5'-GTAGTAAAGCTGCTGCTG-3'; and reverse, 5'-GAGCGGTCTTCTGCTGCTG-3'; primer set 3: forward, 5'-GTAGTAAAGCTGCTGCTG-3'; reverse, 5'-GACCCTCTACATCAGCTG-3' and primer set 4: forward, 5'-AGGTGGAACGCTTCTGCACGC-3' and reverse, 5'-ACTAGTATCTCCTGCTGCTG-3'. Alternative mRNA splicing was predicted by using primer sets 1 and 2.

A/J and BALB/cJ GST-Pol ε and Plasmid Constructs. A full length of Pol ε coding sequence was separately amplified using two sets of oligonucleotides: P1 set: forward, 5'-GAACCGGATCCCGCGAGCCAGGCTTCGACC-3'(A/J); forward, 5'-GAAGCGCTTCTTGACGAGCCAG-3'; and reverse, 5'-ATAAGGATATCAATCAGCGGAGGC-3'; and P2 set: forward, 5'-CCTCCCTTCTGATCTTCTTG-3' and reverse, 5'-CTCCCTCCATCGATGGACTTATCTGTGCGCCGAGG-3'. P1 and P2 PCR products were digested with BamHI/EcoRV and EcoRV/ClaI restriction enzymes. Digested P1 and P2 PCR products were separately cloned into the pBluescript II SK vector, and their sequences were confirmed by direct sequencing in both directions by the A/J and BALB/cI GST-Pol ε plasmids containing a full length of Pol ε open reading frame was produced by subcloning the P2 fragment into the pBluescript II SK vector.

The GST-Pol ε plasmid was generated by subcloning the ~2.2-kb BamHI to Clal fragment from Pol ε-Bluescript II SK plasmid into BamHI/ClaI double-digested glutathione S-transferase (GST)-tagged expression vector pEBG-3X-HV. The in-frame open reading frame of GST-Pol ε fusion was confirmed by direct sequencing.

Purification of GST-Tagged Pol ε Proteins. The GST-Pol ε expression plasmids were transfected into HEK293 cells using Lipofectamine (Invitrogen, Carlsbad, CA), and 48 h after transfection, cells were lysed in NP40 buffer (20 mM Tris (pH 7.5), 100 mM NaCl, 1% NP40, 1 mM EDTA, 1 mM DTT, 0.1 mM phenylmethylsulfonyl fluoride, 5 µg/ml aprotinin, and 5 µg/ml leupeptin). Lysates were cleared by centrifugation, and NaCl was added to 1 x before the addition of glutathione-agarose beads and incubation for 2 h. Beads were washed with NP40 buffer and eluted in glutathione elution buffer (50 mM Tris (pH 8), 100 mM NaCl, 10 mM glutathione, and 1 mM DTT). Eluted GST-Pol ε proteins were resolved by SDS-PAGE to determine purity and concentration. In addition, protein concentrations were estimated by Western blot analysis using human GST-i as a standard. The antibody to full-length human POL ε was used to confirm the even loading of protein.

Results

Expression and Nucleotide Polymorphism Analyses of Genes in the Par2 Region. The present minimum Par2 physical region is encompassed by the D18Mit103 and D18Mit188 markers. We obtained the marker sequence information from the Whitehead Institute/MIT Center for Genome Research. Using the marker sequences to blast against the Celera and National Center for Biotechnology Information (NCBI) mouse genome databases, we localized these two markers on both maps. The physical distance between the D18Mit103 and D18Mit188 markers was ~2.4 Mb in the Celera map with both markers located on the scaffold GA_x6K02T2R3. In the NCBI mouse map, these two markers were localized to the contig NT_082383 and encompassed a ~2.6-Mb region (D18Mit103 at the nucleotide position 2932731-2932845 and D18Mit188 at the nucleotide position 5505960-5506058). The chromosome position for each gene in the candidate region is illustrated in Fig. 1. Generally, the gene annotations on both maps are consistent to each other. For example, four known genes, namely StarD6, Pol ε, Mbd2, and Dcc, are located in both Par2 regions. Sequence comparison revealed that the mCG9249 gene in the Celera map represents the same gene as the 2310002L13Rik does in the NCBI map. Both the mCG9258 and Loc225699 genes encode a protein similar to 60S ribosomal protein L5 and should represent a same gene. However, there are also some discrepancies between the Celera and NCBI mouse Par2 region. The Celera Par2 region has 7 annotated genes, whereas the NCBI Par2 region has 11, which may reflect different gene prediction tools and prediction stringency used by annotators. Another apparent discrep-

acy is the position of Mbd2 (Fig. 1). For the four known genes, their order in the NCBI mouse genome map is Mbd2-Stard6-Pol τ-Dcc, which is different from their order (Stard6-Poli-Mbd2-Dcc) in the other three maps, i.e., the NCBI human, Celera mouse, and Celera human genome maps. Because of the high homology between human and mice Par2 region, the Mbd2 gene apparently has been somehow mistakenly placed during the NCBI sequence assembling. We used reverse transcription-PCR (RT-PCR) and direct sequencing to examine differential expression in lung tissues and coding-region nucleotide polymorphisms for the annotated genes. The result is presented in Table 1. Because the 4930503Rik, Loc383391, Loc383392, and Loc381176 genes were annotated in the Par2 region after this work was submitted, relevant work is still ongoing in our laboratory. A previous study using Northern blot suggested that the Stard6 gene is expressed exclusively in the mouse testis, and its function has been suggested to be specific for fertility (21). However, our RT-PCR results indicate that Stard6 is expressed in mouse lung tissues, and has no differential expression or nucleotide polymorphism between A/J and BALB/cJ inbred strains. Our previous study has revealed neither differential expression nor nucleotide polymorphism between A/J and BALB/cJ in more than one exon and were not detected by DNase I-treated RT-PCR in lung. We could not detect 2310002L13Rik/mCG9249 in either A/J or BALB/cJ lung tissues.

**Nucleotide Polymorphisms and Alternative mRNA Splicing in the Pol τ Gene.** The remaining known gene in the Par2 region, Pol τ, has a 2154-bp open reading frame and encodes a 717-amino acid protein. By sequencing its entire coding region, we found a total of 25 nucleotide polymorphisms in the Pol τ coding region between A/J and BALB/cJ mice. As shown in Fig. 2A, we have identified 10 amino acid-changing polymorphisms and two alternatively spliced exons in Pol τ between A/J and BALB/cJ mice. Among them, 9 amino acid polymorphisms and 1 alternative splicing variant were consistent with those reported previously (13). The newly observed amino acid changing polymorphism at codon 606 encodes a positively charged arginine (CGA) in BALB/cJ but a neutral Glutamine (CAA) in A/J (Fig. 2B).

**Pol τ exon 4a transcript isoform was reported as a product of alternative splicing on exon 4 (13). In our study, we have found that in addition to this isoform (detected by our primer set 2; data not shown), another isoform also exists in mouse lung tissues. The new alternative transcript was detected by primer set 1 and has a shorter nucleotide sequence compared with the regular one (Fig. 2C, a). Directly sequencing this shorter PCR product has revealed that the exon 2 in the full-length Pol τ mRNA is spliced out without changing open reading frame (Fig. 2C, b). We present this isoform as exon 2d (“d” means “deleted”). In C57BL/6j mice, the exon 2d isoform seems to be more abundant than the regular isoform that contains exon 2, whereas A/J, BALB/cJ, 129/SvJ, and C3H/HeJ lung tissues have more of the regular-length isoform transcript than the exon 2d isoform. We did not observe differential expression of the new exon 2d transcript isoform between A/J and BALB/cJ mice.

**Initial Biochemical Characterization of Pol τ Protein.** To investigate the properties of the Pol τ isoforms encoded in A/J and BALB/cJ mice, we expressed and purified both enzymes as full-length GST fusion proteins and measured their polymerization activity. Both enzymes were active in extending a correctly paired primer-template through the addition of dAMP opposite template T (Fig. 3A, a). Thus, the amino acid differences between the two polymerases do not result in loss of polymerization activity by either protein. At concentrations of Pol τ estimated to be equivalent, more extension of the correctly paired primer was ob-

---

Table 1: Expression and nucleotide polymorphism analyses for genes in the Par2 candidate region

<table>
<thead>
<tr>
<th>Gene ID</th>
<th>Description</th>
<th>Lung expression</th>
<th>Nucleotide polymorphisms between A/J and BALB/c</th>
</tr>
</thead>
<tbody>
<tr>
<td>mCG9249/2310002L13</td>
<td>Riken transcript</td>
<td>No</td>
<td>Ten amino acid polymorphisms</td>
</tr>
<tr>
<td>4930503L19</td>
<td>Riken transcript</td>
<td>ND*</td>
<td></td>
</tr>
<tr>
<td>Stard6</td>
<td>START domain-containing protein 6</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Pol τ</td>
<td>DNA polymerase 1</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>A430085C19</td>
<td></td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Mbd2</td>
<td>Methyl-CpG binding domain protein</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Loc225699/mCG9258</td>
<td>Similar to 60S ribosomal protein L5</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Dcc</td>
<td>Tumor suppressor protein</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Loc383391</td>
<td>Similar to DCC tumor suppressor protein</td>
<td>ND*</td>
<td>ND*</td>
</tr>
<tr>
<td>Loc382292</td>
<td></td>
<td>ND*</td>
<td>ND*</td>
</tr>
<tr>
<td>Loc381176</td>
<td></td>
<td>ND*</td>
<td>ND*</td>
</tr>
</tbody>
</table>

*ND, not determined because of recent database update.*
served for the BALB/cJ enzyme than for the A/J enzyme. However, in parallel reactions using somewhat higher enzyme concentrations, a difference between the two isoforms was not apparent for extension of a primer containing a terminal T-G mismatch (Fig. 3 A, b). These data suggested that the two isoforms of Pol /H9259 may differ in their ability to use aberrant substrates. To further test this hypothesis, we examined a property that distinguishes Pol /H9259 from all other DNA polymerases studied to date—the preferential incorporation of incorrect dGMP over correct dAMP opposite template thymine (14–17). The results show that both mouse isoforms of Pol /H9259 do exhibit this noncanonical behavior (Fig. 3 B). Thus, when examined at two different concentrations of dGTP or dATP (0.1 and 1.0 mM), incorporation of dGTP is preferred by both enzymes (compare squares to circles). However, at the higher deoxynucleoside triphosphate concentration (black symbols), the A/J Pol /H9259 prefers dGTP by a factor of 3–4-fold, whereas the BALB/cJ Pol /H9259 prefers dGTP by a factor of <2-fold. This observation additionally supports the hypothesis that the amino acid differences between the two mouse isoforms may alter their substrate discrimination properties.

**Human POL /h Mutation and Expression in Lung Cancer Cell Lines.** To explore the possible role of POL /h in human lung cancer development, we examined 11 human lung cancer cell lines for mutations in the coding region of the human POL /h gene. Two silent polymorphisms at codon 12 (TCG to TCT, Ser) and codon 293 (GTG to GTC, Val) were identified in the CaLu-1 cells, and a common polymorphism at position 2180, which changes the amino acid at codon 706 (ACA to GCA, Thr to Ala), was identified in A427, A549, CaLu-3, CaLu-6, NCI-H460, and NCI-H596 cells. These polymorphisms in the lung cancer cell lines all appeared to be heterozygous with the published sequence. Fig. 4 A shows a sample without and one with the polymorphism at codon 706.

**Fig. 2.** Amino acid alterations and transcript isoforms of the mouse Pol /h gene. A, schematic illustration of amino acid-changing nucleotide polymorphisms and alternative splicing. The full length of Pol /h mRNA consists of 10 exons (E1-E10) with a total of 2497 bp (GenBank accession no. NM_011972). Exon 2d isoform is produced by alternatively splicing out exon 2 without changing the entire open reading frame. The asterisk sign ( ¦ ) indicates that the exon 4a isoform has extra 32 bp (C) on regular exon 4, which produces a truncated protein caused by an early termination codon (tga) in exon 5. Ten amino acid changing codons are located in exon 1, exon 8, exon 9 (1), and exon 10 (6). The start codon (atg) is at 95 bp position, and the stop codon in the regular isoform is at 2246 bp position. B, amino acid-changing nucleotide polymorphism on codon 606 of the Pol /h full-length transcript. Direct sequencing results revealed that the codon 606 of the Pol /h full-length transcript encodes a positive-charged arginine (CGA) in BALB/cJ but a neutral glutamine (CAA) in A/J mice. C, alternative mRNA splicing on exon 2. a, reverse transcription-PCR result using primer set 1 (see “Materials and Method” for primer sequences). Two transcript isoforms were detected in A/J and BALB/cJ mouse lung. The longer one is regular Pol /h transcript with exon 2. The shorter one is a new isoform without exon 2. b, sequencing result revealed that the new Pol /h isoform is caused by in-frame exon 2 skip.
In addition to the sequence analyses, an assessment of POL \( \beta \)/H9259 protein expression in the human lung cancer cell lines was made. Western analysis demonstrated that POL \( \beta \)/H9259 expression varied among the cell lines from low expression in the normal MRC-5 and the A427, SK-LU-1, and NCI-H460 cancer cells to very high in the CaLu-1 and NCI-H520 cell lines with the other cells in between (Fig. 4B). These expression differences did not correlate with the amino acid change at codon 706 observed in certain cell lines or with the tumor morpho...

Fig. 3. Primer extension assays. A, differential activities in incorporation of dATP opposite template T at high enzyme concentration. Incorporations of dATP extend matched (a) and G/T mismatched (b) primer-template T. Pol \( \beta \) enzymatic activity is reflected by percentage of primers being converted. B, preferential incorporation of dGTP versus dATP opposite template T. Two ng of A/J and BALB/cJ full-length Pol \( \beta \) proteins were used in each reaction. At 0.1 and 1 mM deoxynucleoside triphosphate (dNTP) concentrations, both proteins exhibit classical Pol \( \beta \) enzymatic property, preferring incorporation of dGTP opposite template T. At 1 mM dNTP concentration, the BALB/cJ Pol \( \beta \) protein is less efficient (~2-fold) at incorporating dGTP opposite template T than the A/J protein.
Among all of the annotated genes in the Par2 region, Pol ε was identified as a candidate mouse lung resistance gene based on two lines of evidence: (a) genetic variants were identified between susceptible A/J and resistant BALB/cJ mice, and changes including genetic polymorphisms and altered expression of POL ε were also observed in human lung cancer cells; (b) primer extension assays with purified BALB/cJ and A/J proteins in vitro found that both forms of Pol ε are active but that they may differ in substrate discrimination. This result strongly supports the hypothesis that the amino acid differences between the two mouse isoforms may alter their substrate discrimination properties, which may affect the formation of Kras2 mutations in mouse lung tumors. A major characteristic of the highly error-prone human POL ε is its preference for inserting wobble base G rather than A opposite template T. Primer extension assays demonstrated that the full-length BALB/cJ Pol ε protein is less efficient than the A/J protein in incorporating G compared with A opposite a template T. At higher concentrations, the full-length BALB/cJ Pol ε protein incorporates Watson-Crick base A opposite template T more efficiently than the A/J protein. These results are consistent with a genetic modifier role for the Pol ε variant in mouse and, possibly, human lung cancer susceptibility.

Human chromosome 18q21.1 locus is deleted in many human cancers, including squamous cell carcinoma, osteosarcoma, colon cancer, and breast cancer (23–26). Recent studies have indicated that deleted segments of 18q21 contained a lung cancer tumor suppressor gene (27, 28). Because the mouse Par2 region is highly homologous to human chromosome 18p21.1 region, positional cloning of the Par2 gene may facilitate the identification of the human lung cancer suppressor gene. To date, there are three known tumor suppressors (DCC, SMAD2, and SMAD4) located in the human chromosome18q21.1 region, although there is no evidence that these genes play a role in lung cancer. Because the mouse Smad2 and Smad4 genes are located outside of the refined Par2 candidate region, they can be excluded as Par2 candidates. The status of the Dcc gene as a Par2 candidate has been challenged by the fact that neither nucleotide polymorphism nor significant lung expression difference has been found between A/J and BALB/c mice (11). The MBD2 gene encodes methyl-CpG binding proteins that suppress transcription from methylated promoters and is a candidate tumor suppressor. However, one recent mutation study suggests that the MBD2 gene may only have a limited role, if any, in lung tumorigenesis (29).

Multiple nucleotide polymorphisms and functional testing results (i.e., different enzymatic activities) provide evidence that the Pol ε gene is a strong candidate for the mouse Par2 gene. As with tumor suppressors DCC, SMAD2, and SMAD4, the human POL ε was also mapped to the chromosome 18q21.1 (22). The gene belongs to the Rad30 branch of the recently described UmuC/DinB/Rev1/Rad30 family of DNA polymerases and encodes a 715-amino acid DNA-dependent polymerase POL ε in human and a 717-amino acid Pol ε protein in mouse (30). On the basis of in vitro studies, human POL ε has the lowest fidelity of any eukaryotic polymerase studied to date, which suggests that its activities must be highly specialized. The fact that POL ε orthologues are evolutionarily conserved in higher eukaryotes from Drosophila to humans also suggests that it provides some selective advantage. Several studies show that the POL ε gene may play a role in somatic hypermutation (31–33) by which specific mutations occur as part of antibody diversity. Human POL ε protein has also been shown carrying an intrinsic 5′-deoxyribose phosphate lyase activity and can substitute for POL β during BER reactions in vitro (19).

Twenty-five nucleotide polymorphisms in the Pol ε coding region were observed between A/J and BALB/cJ strains that resulted in 10 amino acid alterations. Although none of these amino acid polymorphisms has been located in the conserved enzymatic regions, recent studies have shown that the COOH-terminal region of human POL ε protein may also have additional critical functions (34–36). For ex-
ample, the motif SRGVLSSF (in which the conserved residues are indicated in bold) is present in residues 540–547 of human POLH9259 and may contribute to PCNA binding (34). Another study has shown that the localization of POLH9259 protein in the nucleus requires sequences within amino acids 219–451, and sequences within amino acids 492–539 and 636–715 are critical for interaction between POLH9257 and POLH9259 proteins (35, 36). The results in Fig. 3 are nonetheless consistent with the possibility that the BALB/cJ and A/J isoforms of PolH9259 differ in substrate discrimination properties. The higher apparent activity for incorporation of dAMP (Fig. 3A) and the lower ratio of dGTP to dATP incorporation (Fig. 3B, 1.0 mM) by the BALB/cJ enzyme are both consistent with the possibility that this enzyme may be more accurate than the A/J isoform, at least for T-dGMP mismatches that would lead to T-A to C-G transition mutations. A more comprehensive test of this hypothesis for this and other mismatches is currently underway.

An amino acid polymorphism at the codon 706 of human POLH9259 gene was identified in human lung cancer and normal cell lines. It is unclear at this time if this polymorphism will alter its function. The Environmental Genome Project sampled 178 chromosomes during its resequencing of POLH9259 and identified A in position 2180 (codon 706) at a proportion of 0.758 and G at 0.242. The estimated heterozygosity was 0.366 for this SNP. 8 This frequency is not statistically different from our finding. We also found that the human lung cancer cell lines exhibit varied expression of POLH9259 protein, which may suggest a role in the development of lung cancers. Although these expression differences did not correlate with the amino acid change at codon 706 observed in certain cell lines or with the tumor morphological subtypes, it is noteworthy 8 Internet address: http://www.genome.utah.edu/genesnps/cgi-bin/frame.cgi_gene_id/H11005.

Fig. 5. Clustalw alignment of human, mouse, and fruit fly Pol proteins. Mouse Pol protein is represented by the A/J sequence. “c” indicates the identical residues in that column in the alignment. “.” indicates conserved substitutions. The codon 706 is highlighted.
that there are a number of polymorphisms in the 5′-end flanking region of the gene identified by Environmental Genome Project that may affect expression or function. Thus, additional analysis of the POL γ promoter region should be pursued.

The Kras2 gene plays an important role in mouse lung tumor development, with most adenomas and adenocarcinomas containing Kras2 mutations (5, 20). After treatment with urethane, nearly all Kras2 mutations arise in codon 61. Kras2 codon 61 mutations were very frequent (90.9% for CAA→CGA and 61.1% for CAA→CTA) in lung tumors following urethane treatment from BALB.B6-Par2 congenic mice and slightly lower (86% for CAA→CGA) in tumors from BALB/cJ mice (13). After treatment with another carcinogen, diethylthiolsulfamine, the CAA→CGA mutation rates in tumors were 46% for parental BALB/cJ mice and 79% for BALB.B6-Par2 congenic mice, respectively (13). It was unclear whether these differences were because of differential protein expression in lungs of BALB/cJ and A/J mice (e.g., parental BALB/cJ mice producing less than half amount of full-length Pol γ protein as congenic mice) or qualitative (i.e., enzymatic activity) factors. Our primer extension assays present, for the first time, the evidence for the hypothesis that enzymatic activity differences between the BALB/cJ and A/J (or C57BL/6J) Pol γ proteins may contribute to differences in Kras2 mutation frequency. Indeed, preferred DNA damage and poor repair have been demonstrated to be responsible for the mutation hotspot at codon 12 of Kras gene in human lung cancer (20). The urethane-induced DNA adduct 1-N2-ethenodeoxyadenosine, has been shown to be a strong mutagenic DNA adduct when replicated in mammalian cells (37). A recent study has revealed that the level of 1,N2-ethenodeoxyadenosine and 3,N2-ethenodeoxycytidine were ~70% higher in A/J mice than in C57BL/6J mice (38). Because of the conservation between mouse Pol γ and human Pol γ proteins, we speculate that mouse Pol γ proteins are also involved in BER and may affect Kras2 mutation during BER reactions. On the basis of our results, the BALB/cJ Pol γ protein would less efficiently incorporate dGTP and might more efficiently incorporate correct dATP base into the damaged DNA strand than the A/J Pol γ protein during BER. Thus, the probability of forming a CAA→CGA mutation at codon 61 in BALB/cJ Kras2 gene will be less than in A/J Kras2 gene. Further in vitro enzymatic studies, e.g., BER test using Kras2 gene sequence as template, are needed to confirm this hypothesis. Additionally, it has been revealed recently that the functional Pol γ protein is deficient in 129 strains of mice, which is consistent with its susceptibility to chemically induced lung tumorigenesis (39, 40). Thus, 129 strains of mice provide an ideal model to evaluate the relationship between Kras2 mutation and Pol γ proteins in vivo.

References
Pol1 Is a Candidate for the Mouse Pulmonary Adenoma Resistance 2 Locus, a Major Modifier of Chemically Induced Lung Neoplasia


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/64/6/1924

Cited articles
This article cites 35 articles, 12 of which you can access for free at:
http://cancerres.aacrjournals.org/content/64/6/1924.full#ref-list-1

Citing articles
This article has been cited by 9 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/64/6/1924.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.