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Identification of a Novel Tumor Suppressor Gene *p34* on Human Chromosome 6q25.1

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Abstract

In this study, we observed loss of heterozygosity (LOH) in human chromosomal fragment 6q25.1 in sporadic lung cancer patients. LOH was observed in 65% of the 26 lung tumors examined and was narrowed down to a 2.2-Mb region. Single-nucleotide polymorphism (SNP) analysis of genes located within this region identified a candidate gene, termed *p34*. This gene, also designated as ZC3H12D, C6orf95, FLJ46041, or dJ281H8.1, carries an A/G nonsynonymous SNP at codon 106, which alters the amino acid from lysine to arginine. Nearly 73% of heterozygous lung cancer tissues with LOH and the A/G SNP also exhibited loss of the A allele. *In vitro* clonogenic and *in vivo* nude mouse studies showed that overexpression of the A allele exerts tumor suppressor function compared with the G allele. p34 is located within a recently mapped human lung cancer susceptibility locus, and association of the p34 A/G SNP was tested among these families. No significant association between the less frequent G allele and lung cancer susceptibility was found. Our results suggest that *p34* may be a novel tumor suppressor gene involved in sporadic lung cancer but it seems not to be the candidate familial lung cancer susceptibility gene linked to chromosomal region 6q23-25.

Introduction

Lung cancer is the leading cause of cancer deaths in the United States (1). Lung tumor development is a multistage process involving the accumulation of genetic alterations that affect many oncogenes and tumor suppressor genes (2). Several tumor suppressor genes, including *p16, p53*, and *RASSF1A*, are frequently inactivated during lung tumor

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development (2). Hypermethylation of *p16*, mutation of *p53*, and deletion of 3p, 9p, and 17p have been frequently detected in lung hyperplasia and dysplasia (2). Inactivation of *p16* by hypermethylation and homozygous deletion has been detected in most non–small cell lung carcinomas, whereas mutation of *p53* is observed in ~50% of non–small cell lung tumors. Furthermore, loss of heterozygosity (LOH) of several loci (in 3p, 5q, 9p, 13q, and 17p) has been observed in carcinomas of the lung (2). Deletions in 18q and 22q have been seen only in invasive carcinomas, suggesting that the genes in these two loci may be responsible for malignant progression of lung cancer (3).

Although 85% to 90% of lung cancer cases are attributable to cigarette smoking, several lines of evidence suggest that genetic factors also play an important role. For instance, epidemiologic studies have indicated that only ~10% of heavy smokers eventually develop lung cancer, suggesting individual variation in genetic predisposition to the disease (4). Differential susceptibilities to lung tumors have also been clearly shown in inbred strains of mice (5). More importantly, familial aggregation, a characteristic of genetic diseases, has been observed in lung cancer patients (6). Recently, we localized a putative human lung cancer susceptibility locus to chromosomal region 6q23-25 through whole-genome linkage analyses on lung cancer families (7). A maximum heterogeneity LOD score of 4.26 was achieved using 23 multigenerational pedigrees with five or more affected individuals (7).

Because LOH is a common somatic event for many previously identified familial cancer susceptibility genes, we conducted a deletion mapping study of chromosomal region 6q23-25 using DNA from tissues of sporadic lung cancer patients. We identified a gene (provisionally named *p34* based on its putative protein molecular weight) with *in vitro* and *in vivo* tumor suppressor function. However, through association analysis, the *p34* gene seems not to be the candidate familial lung cancer susceptibility gene in chromosomal region 6q23-25.

Materials and Methods

LOH assay

Polymorphic microsatellite markers were selected using the National Center for Biotechnology Information (NCBI) UniSTS database.[12] Depending on the marker polymorphism, either ^{32}P-labeled radioactive primers or unlabeled primers were used. Normal or tumor DNA (50 ng) was PCR amplified using the following conditions: 95°C for 2 min followed by 30 cycles at 94°C, 55°C, and 72°C each for 30 s. PCR products were resolved on either 3% MetaPhor agarose gels (Cambrex BioScience, Rockland, ME) for unlabeled PCR products or 6% polyacrylamide gels for ^{32}P-labeled products. LOH was scored if reduction in the signal of one allele from the tumor sample was noted in comparison with the allele signal from the corresponding normal sample. Where necessary, LOH was also assessed by densitometry. The allele ratio was calculated as (T1/T2)/(N1/N2), and LOH was defined as an allele ratio >2 or <0.5, indicating a reduction of ≥50% in one of the tumor sample alleles when compared with a heterozygous normal tissue control. The amount of normal tissue contamination in all tumor tissues used in this study is <30%. All experiments were done in duplicate. To determine which specific *p34* allele is lost by RFLP assay, a set of primers was designed to amplify a region of p34 containing the codon 106 single-nucleotide polymorphism (SNP): 5′-GTGTCTCTATGATTTCTTTGTTTTCCCATTGTAGCCATGGAA*CTA*-3′ (forward) and 5′-CTCTGATAGGGGTGTCAGCTCTTGGTGGGTCCTTC-3′ (reverse). A *Bfa*I (CTAG) restriction enzyme site is formed when the G allele is amplified. After purification with

[12] http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=unists.

QIAquick PCR purification kit (Qiagen, Inc., Valencia, CA), PCR products were incubated with *Bfa*I at 37°C for 1 h and resolved on a 3% MetaPhor agarose gel. A PCR fragment with the G allele was cut by *Bfa*I, generating a fragment 43 bp shorter than the uncut A allele fragment.

p34 SNP identification

PCR primers were designed for SNP identification in the *p34* gene coding exons (exons 2–7): exon 2, gaaacacctgatggatgc (forward) and caaaccacaaggaagaggg (reverse); exon 3, ctcttgattatgagactg (forward) and caaacaactaagtagatatttg (reverse); exon 4, gggtggttctcaggg (forward) and caagctcctccacctggtag (reverse); exon 5, cttcctggtgttcttggg (forward) and ctgagcaccaggccagcgc (reverse); exon 6, ctcacgtcgcgccctctctg (forward) and aggctgtgcgcaaatggttc (reverse); and exon 7, actgggcgggcccgactgggtgt (forward) and agacggagcgcccagggaag (reverse). PCR amplification followed the following scheme: 95°C for 2 min followed by 30 cycles at 94°C, 55°C, and 72°C each for 1 min. Products were resolved using 1.2% agarose gels and purified for direct sequencing.

Cloning and *in vitro* clonogenic assay

The open reading frames of *p34* alleles A and G were amplified from cDNA samples prepared from human lung tumors and cloned into a hemagglutinin (HA)-tagged pcDNA3-based (Invitrogen, Carlsbad, CA) mammalian expression plasmid. Appropriate expression of *p34* was confirmed by transient transfection into HEK293T cells (data not shown) and Western blotting with anti-HA monoclonal antibody (Covance, Richmond, CA). H1299, a human non–small cell lung cancer cell line, was selected for *in vitro* cell culture–based and *in vivo* nude mouse–based cell growth analyses. The H1299 cells were cultured in RPMI 1640 with 2 mmol/L L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 10 mmol/L HEPES, and 1.0 mmol/L sodium pyruvate, supplemented with 10% fetal bovine serum, in the presence of antibiotic-antimycotic (10 μL/mL) and were grown at 37°C in a humidified 5% CO_2 atmosphere. For the colony formation assay, H1299 cells were seeded at 1.5×10^6 per 10-cm dish and transfected with 4 μg of linearized HA-p34-A allele, HA-p34-G allele, or empty expression vector using LipofectAMINE 2000 (Invitrogen). The transfected cells were cultured in the presence of 0.6 mg/mL G418 for 2 weeks. Cells that survived selection were fixed with 10% formalin and stained with 0.125% crystal violet. The numbers of colonies (≥1 mm) were counted from triplicate dishes.

Nude mouse assays

For *in vivo* nude mouse tumorigenesis studies, H1299 cells stably expressing the HA-*p34* A or G alleles were collected and pooled from multiple cell culture dishes after transfection and G418 selection for 3 weeks. Female athymic mice [Hsd:athymic nude-*nu* (*nu/nu*) ages 4–6 weeks] were purchased from Harlan (Indianapolis, IN) and housed in institutional animal facilities. Food and water were given *ad libitum*. All animal work was done under protocols approved by the Washington University School of Medicine Institutional Animal Care and Use Committee (St. Louis, MO). We injected 3 million cells s.c. into each flank of the nude mice. The health of the animals was monitored, and the size of tumors was measured weekly for 7 weeks.

DNA samples

LOH analysis was done on 26 matched normal/tumor samples from sporadic human lung cancer patients collected by the Tissue Procurement Core of Washington University. A set of 68 cancer-free control DNA samples was purchased from the Coriell Institute for Medical Research (Camden, NJ), and 196 cancer-free control DNA samples were collected for an independent nicotine addiction study. DNA samples from 276 familial lung cancer probands

were also analyzed, and 94 DNA samples of spousal controls were provided by the Center of Inherited Disease Research (CIDR) or other Genetic Epidemiology of Lung Cancer Consortium (GELCC) sources. A total of 204 sporadic lung cancer samples was analyzed. Approximately 50% were adenocarcinomas, 40% were squamous cell carcinomas, and 5% were large cell carcinomas or unknown. DNA samples of NCI-60 cell lines were kindly provided by the National Cancer Institute (Rockville, MD).

Statistical analysis

Two-tailed Student's *t* tests were used to evaluate differential cell growth-suppressing effects of the p34 A and G alleles. χ^2 tests were done to examine the association between p34 and lung cancer susceptibility using the case and control populations.

Results

LOH at *p34* locus

The classic "two-hit" theory suggests that LOH may be a common genetic event for tumor suppressor genes (8). It is well known that many familial cancer susceptibility genes, such as the tumor suppressors *p53, RB*, and *BRCA1*, also show frequent LOH in sporadic cancer patients. In the present study, we initiated an investigation on LOH status in sporadic lung cancer patients focusing on the 6q23-25 region. Our intent was to use the LOH data in sporadics to potentially identify the gene responsible for familial lung cancer susceptibility. Of the 204 sporadic lung tumor DNAs that were screened, we selected 26 pairs of sporadic lung tumors (12 without the A/G SNP and 14 with the A/G SNP) and matched normal lung tissues to do LOH analysis. We identified a region of frequent LOH between microsatellite markers D6S1003 (144.6 Mb) and D6S473 (155.9 Mb). Overall, 17 of 26 (65%) exhibited LOH in this region (Fig. 1*A* and *B*). Furthermore, our analysis revealed a minimal 2.2-Mb region of LOH between microsatellite markers *D6S1637* (148.9 Mb) and *D6S1687* (151.1 Mb) that was common to all 17 tumors with LOH (Fig. 1*B*).

p34 nonsynonymous SNP at codon 106

Twenty genes have been annotated in the minimal LOH region as defined by markers D6S1003 and D6S473 (NCBI human genome, Build 36.1). We hypothesized that if a tumor suppressor gene is found in this LOH region defined by our analysis in sporadic tumors, then it is also possible that this tumor suppressor gene is the 6q familial susceptibility gene. Hence, we conducted direct nucleotide sequencing of genes in the region using genomic DNAs from 14 lung cancer probands and 16 spousal controls selected from GELCC familial lung cancer pedigrees showing strong genetic linkage to the 6q region (7). Note that identification of 6q familial lung cancer susceptibility genes with mutations is likely under-powered by screening such small numbers of probands and controls, especially when tobacco smoking or other environmental cofactors may also play a role in familial lung tumorigenesis. However, we believe that through screening these 6q-linked lung cancer probands, we might identify some interesting candidate genes for further association and functional tests. Indeed, our analysis identified a functionally unknown gene (renamed as *p34* for its putative protein molecular weight) harboring a nonsynonymous, heterozygous A/G SNP at codon 106 in one of the 14 lung cancer probands but not in any of 16 spousal controls. The *p34* gene is located at the 149.8-Mb position in the current NCBI human genome map (Build 36.1) and encodes three predicted mRNA transcripts (Genbank accession nos. *XM_291154, XM_933939*, and *XM_933941*). The largest transcript *XM_291154* consists of six coding exons and two untranslated exons, encoding a 321-amino acid protein (Fig. 2*A*). The nonsynonymous A/G SNP we identified is at the second nucleotide of codon 106 and alters the amino acid from lysine (AAA) to arginine (AGA; Fig. 2*B*).

Determination of *p34* allele loss in sporadic lung cancers with LOH

We selected 14 sporadic lung cancer samples with the p34 codon 106 A/G SNP and 12 sporadic lung cancer samples without (i.e., A/A genotype). Interestingly, 12 of the 14 (86%) tumors with the A/G SNP also exhibited LOH at the *p34* locus (Fig. 1*B*). This is in comparison with only 5 of the 12 (42%) tumors with the A/A genotype that exhibited LOH at the p34 locus. Because LOH was observed more frequently in the *p34* locus when the codon 106 SNP (i.e., G allele) was present, we asked whether one of the alleles was lost in lung tumors displaying LOH. For this purpose, we designed a set of PCR primers, which introduce an artificial *Bfa*I restriction enzyme site at the SNP position (Fig. 2*C*). After PCR and product purification, the amplicon from the G allele was digested by *Bfa*I, whereas the amplicon from the A allele remained intact (Fig. 2*D*). We tested all 12 tumors carrying the A/G SNP and showing LOH, and our results indicated that the A allele is lost in 8 of 11 (73%) lung tumors (the assay failed on sample 196; Fig. 1*B*). This suggests that the G allele is retained in the majority of tumors.

Overexpression of the p34 A allele inhibits tumor cell growth

Because the majority of the tested sporadic lung tumors with LOH at the *p34* locus exhibit loss of the A allele, we speculated that a functional difference exists between the A and G alleles. We evaluated this through an *in vitro* colony formation assay and an *in vivo* nude mouse assay. Mammalian expression vectors containing the two *p34* isoforms were prepared and transfected into the H1299 human lung cancer cell line. Ectopic expression of the A allele of *p34* significantly suppressed colony formation by 30% in comparison with the G allele–trasfected and vector alone–transfected cells (Fig. 3*A* and *B*). We next injected equal numbers of H1299 cells stably expressing the A or G allele of *p34* into nude mice to observe *in vivo* tumor-suppressing effects. Continuous monitoring of tumor sizes and weight showed significant retarded growth for the A allele–transfected cells compared with the G allele–transfected and vector-transfected cells (Fig. 3*C–F*). Protein expression of *p34* was confirmed in excised tumors (Fig. 3*D*). These data suggest that the A allele may function as a tumor suppressor in lung carcinogenesis, whereas the G allele acts as a loss-of-function allele.

p34 codon 106 SNP in human cancer cell lines

We sought to determine the prevalence of the p34 codon 106 SNP in other cancer cell lines. Genomic DNAs from the NCI-60 panel of cell lines, representing nine different cancers, were screened for the p34 A/G codon 106 SNP. We identified the less frequent G allele in multiple cancer cell lines (Table 1), including non–small cell lung cancer (one of nine), colon cancer (one of seven), melanoma (two of eight), renal cancer (one of eight), and both prostate cancer (two of two) cell lines. Interestingly, compared with its rare frequency in the general population (as discussed later), the homozygous G allelotype was seen more frequently in the cancer cell lines [i.e., in both prostate cancer cell lines (DU-145 and PC-3), a colon cancer cell line (COLO-205), and a melanoma cell line (SK-MEL-28)]. One might speculate that the tumor-suppressing A allele is lost due to LOH in these cell lines as was observed in 8 of the 11 sporadic lung tumors discussed previously (Fig. 1*B*).

Association of codon 106 A/G SNP with lung cancer risk

The above analyses suggest that the *p34* gene functions as a candidate tumor suppressor involved in the carcinogenesis of lung and potentially other organs. As previously mentioned, it is possible that, due to its role in sporadic tumors, *p34* may also be the 6q23-25 familial lung cancer susceptibility gene. The limited number of probands and controls sequenced for the p34 SNP in the initial analysis is not statistically sufficient to claim it as a candidate. Therefore, we conducted sequencing and association analyses of this

SNP on probands from various populations, including probands from GELCC lung cancer families (with three or more affected individuals), sporadic lung cancer patients, and various control populations. We screened lung cancer probands from 77 lung cancer families, which were initially genotyped by the CIDR for the initial linkage analysis (7). In addition, another 199 probands representing lung cancer families from other GELCC sources were also examined (Table 2). Overall, 12 of 77 (16%) probands of CIDR-typed lung cancer families and 20 of 199 (10%) probands of non-CIDR lung cancer families were found to have the A/G genotype. Among the 77 CIDR lung cancer families, 40 of them have shown positive LOD scores at 6q based on the initial 6q linkage study (7), of which 7 of those 40 (18%) carried the A/G SNP (Table 2). Of the remaining 37 families, which showed no significant linkage to 6q, 5 of those 37 (14%) had the A/G genotype (Table 2). Hence, there was no significant difference in the frequency of the G allele between 6q-linked and 6q-unlinked lung cancer families ($P = 0.87$). Linkage information for non-CIDR GELCC families was not available. Among sporadic lung cancer patients, 24 of 204 (12%) were found to have the A/G genotype.

To determine the frequency of the p34 codon 106 SNP in a lung cancer–free population, we examined three groups of controls: (*a*) 94 healthy spouses from the GELCC lung cancer families, (*b*) 68 cancer-free persons studied by the Coriell Institute for Medical Research, and (*c*) 196 cancer-free young individuals (with an average age of 36.7 ± 5.4; data not shown) previously used in a nicotine addiction study. Overall, 43 of 358 (12%) control persons were identified with the A/G genotype. Hardy-Weinberg equilibrium remained at this SNP. χ^2 tests for comparisons between the two case populations (merged GELCC lung cancer families and sporadic lung cancer patients) and the merged control population did not show any significant association between the G allele and lung cancer susceptibility ($P = 0.80$ for familial lung cancer probands; $P = 0.93$ for sporadic lung cancer cases). In addition, there was no significant difference in the frequency of the G allele between the familial cases and sporadic cases (data not shown).

Discussion

Identification of the underlying familial lung cancer susceptibility gene at 6q23-25 will contribute significantly to human lung cancer prevention, diagnosis, and treatment. We hypothesized that, like many other known familial cancer susceptibility genes, this *6q* gene might also function as a tumor suppressor and show frequent LOH in sporadic lung cancers. Hence, one strategy was to look for genes in the 6q23-25 by analyzing LOH patterns in sporadic tumors. For example, human chromosomal region 17q21, which harbors the familial breast cancer susceptibility gene *BRCA1*, frequently shows LOH in tumors of sporadic and familial breast cancer patients (9, 10). Sporadic and familial colon cancer patients also frequently show LOH in chromosomal region 5q21, where the familial adenomatous polyposis susceptibility gene, *APC*, is located (11).

Several studies were previously conducted using cytogenetic techniques to detect large regions of allelic loss on 6q in non–small cell lung cancer (12–15). More recently, Picchio et al. (16) showed LOH in a region on 6q, which contains the *PARK2* (161.7–163.1) and *PACRG* (163.1–163.7) genes. They suggested that *PARK2* might be a tumor suppressor gene for lung cancer. Interestingly, numerous other tumor types have also shown allelic loss on human chromosome 6q and minimum regions of chromosomal deletion have been defined (7). In the present study, we observed frequent LOH events in sporadic lung tumors on chromosomal fragment 6q25.1 and determined a 2.2-Mb minimum region of LOH. To our knowledge, this study is the first to define such a small minimum region of deletion on 6q for lung cancer.

Through direct sequencing and SNP analysis of probands from lung cancer families with linkage to 6q23-25, we identified an A/G nonsynonymous polymorphism at codon 106 of the *p34* gene. In tumor samples that had this A/G SNP, frequent LOH events were observed at the *p34* locus (i.e., 12 of 14 in the present study). We further confirmed that the more prevalent A allele is lost in tumors with an A/G genotypes and which exhibit LOH (i.e., 8 of 11 sporadic tumors). Moreover, tumor suppressor activity of the *p34* A allele was shown in *in vitro* clonogenic and *in vivo* nude mouse assays. This might explain why the tumor suppressor A allele is commonly lost in the sporadic tumors we tested. Taken together, our data support *p34* as a candidate tumor suppressor gene in lung. We also used real-time PCR to determine the expression of p34 in normal versus sporadic lung tumor tissues but found no association between expression patterns and A/G SNP status or LOH (Supplementary Fig. S1).

Interestingly, we identified the *p34* codon 106 SNP in various types of human cancer cell lines (Table 1), which suggests that *p34* may also function in other types of cancers. Although its biological function remains unknown, *p34* seems to encode a zinc finger, C-x8-C-x5-C-x3-H type (ZNF-CCCH)–like protein. Proteins containing zinc finger domains of the C-x8-C-x5-C-x3-H motif have been shown to respond to extracellular antagonist and can also be transcription factors that regulate cell cycle or cell growth. For example, the human *ZFP36L1* (zinc finger protein 36, C3H type-like 1) gene encodes a zinc finger protein that regulates the response to growth factors (17). Of interest, the *p34* protein sequence also shows very high sequence identity in rhesus monkey, mouse, and rat and down to lower organisms, such as zebrafish and *Drosophila* (Fig. 4). Interestingly, the lysine corresponding to Lys^{106} in human is conserved in all these organisms, but *Drosophila*. Recently published data suggest that a highly homologous human gene, *ZC3H12A* (59% identity), is induced by monocyte chemoattractant-1, and expression of *ZC3H12A* could lead to cell death (18).

To determine the candidacy of *p34* as the 6q23-25 familial lung cancer gene, we conducted a SNP analysis using blood DNA from 14 GELCC familial lung cancer families and 17 spousal controls. However, such a limited number of samples may not be statistically sufficient to establish the *p34* gene candidacy. Therefore, we conducted a more extensive association analysis to include more 6q-linked familial lung cancer families, a larger sporadic lung cancer population, and different control groups. Based on our analysis, there were no significant differences in the allele frequencies and, thus, association for 6q-linked lung cancer families compared with 6q-unlinked lung cancer families (Table 2), suggesting that the codon 106 SNP does not account for 6q-linked lung cancer susceptibility. Furthermore, there was no significant difference in the allele frequency when familial and sporadic lung cancer patients were compared with control populations, and there was no significant difference in the allele frequency between familial and sporadic lung cancer patients either (Table 2). Taken together, our association analysis suggests that *p34* is likely not be the major 6q lung cancer susceptibility gene proposed by our previous study (7). It is likely that there are other tumor suppressors on 6q, perhaps also within our minimal region of deletion, which may function as the 6q-linked familial lung cancer susceptibility gene. Clearly, future studies on the 6q23-25 region are necessary for eventual identification of the gene.

Acknowledgments

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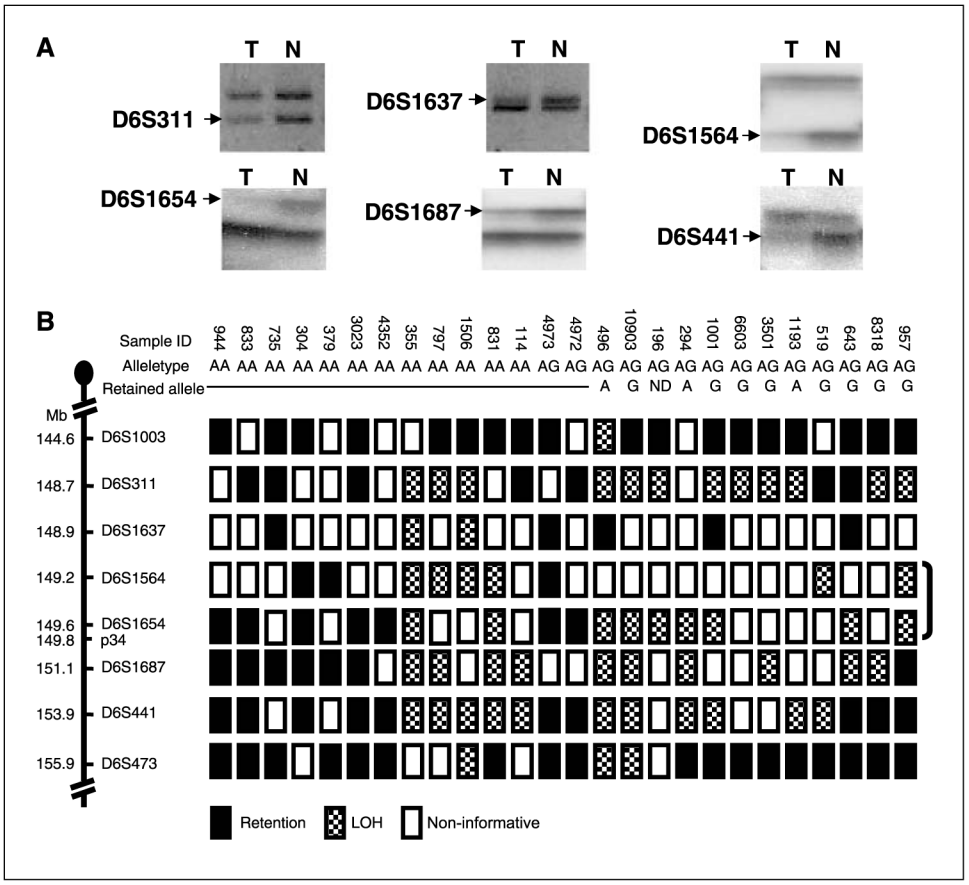

Figure 1.

LOH at chromosomal 6q25.1 in sporadic lung tumors. *A,* LOH determination by PCR. PCR products from the indicated microsatellite markers were amplified from tumor (*T*) tissue and corresponding normal (*N*) lung tissue. Products were resolved on either 3% MetaPhor agarose (D6S311 and D6S1637) or 6% polyacrylamide gel for ^{32}P-labeled products (D6S1564, D6S1654, D6S1687, and D6S441). *Arrows,* allele loss. *B,* determination of minimum LOH region. ■, retention; ◘, LOH; □, noninformative; ND, not determined. The minimum LOH region is a 2.2-Mb chromosomal region encompassed by marker *D6S1637* (148.9 Mb) and *D6S1687* (151.1 Mb).

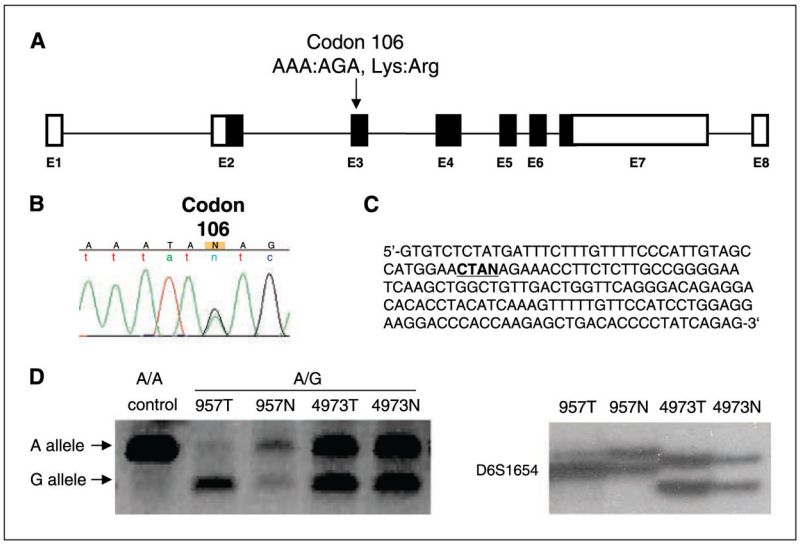

Figure 2.

p34 codon 106 A/G nonsynonymous SNP. *A,* exon organization of *p34* gene. The largest transcript consists of six coding exons (*solid boxes*) and two nontranslated exons (*open boxes*), which encode a 321-amino acid protein (NCBI accession no. *XM_291154*). *B,* sequencing chromatogram of p34 codon 106 A/G SNP. The nonsynonymous A/G SNP is at the second nucleotide of codon 106, which alters lysine (AAA) to arginine (AGA). *C,* p34 A/G allele loss of RFLP amplicon. Sequence of the p34 A/G containing amplicon. The *Bfa*I restriction site is in *bold font. N,* "A" or "G" nucleotide. There is a 43-bp difference between cut product (CTAG, G allele) and uncut product (CTAA, A allele). *D,* RFLP assay to determine p34 allele loss. Following PCR amplification and purification, amplified products from the G allele were cut by *Bfa*I restriction enzyme, whereas products from the A allele were not cut. Samples 957 and 4973 are heterozygous A/G at codon 106. *Right,* LOH was previously identified in sample 957 but not in sample 4973. *Bfa*I restriction enzyme digestion indicates that the lost allele in sample 957 is allele A, whereas no allele is lost in sample 4973. The control sample is homozygous A/A genotype and is not cut by *Bfa*I enzyme.

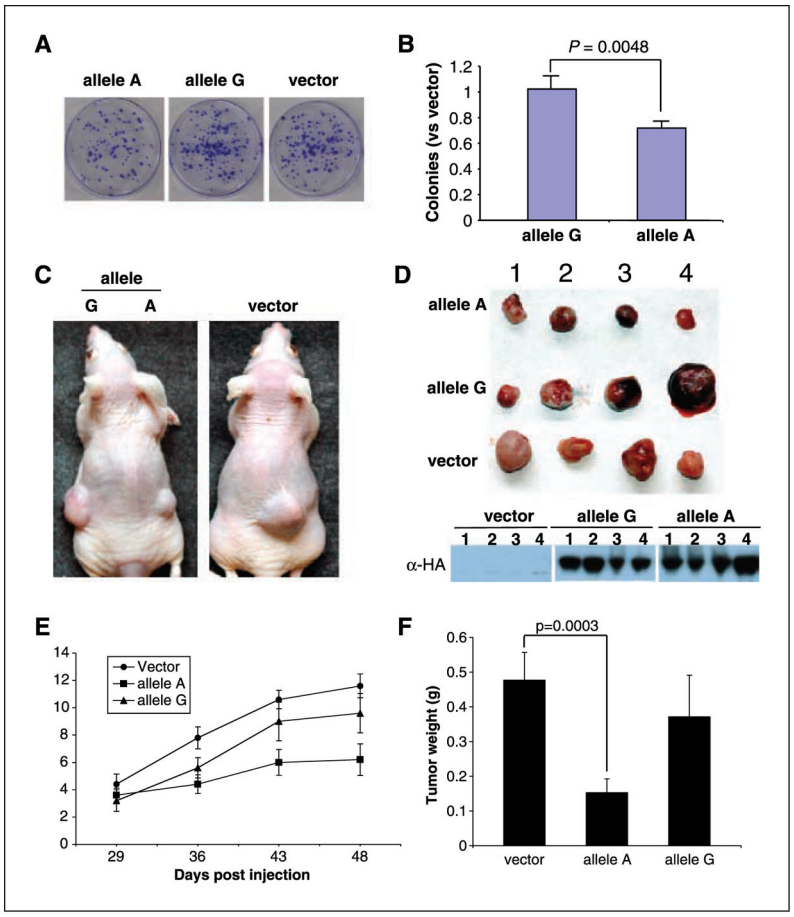

Figure 3.

p34 A allele exhibits tumor suppressor function *in vitro* and *in vivo. A* and *B, in vitro* clonogenic assay. H1299 cells were seeded at 1.5×10^6 per 10-cm dish and transfected with 4 μg of p34 A allele, p34 G allele, or empty vector with LipofectAMINE. The transfected cells were cultured in the presence of 0.6 mg/mL G418 for 2 weeks. *A,* cells that survived selection were fixed with 10% formalin and stained with 0.125% crystal violet. *B,* numbers of colonies (≥1 mm) were counted from triplicate dishes. *C* and *D, in vivo* nude mouse tumor assay. Pools of H1299 cells stably expressing the *p34* A or G alleles were collected after G418 selection. Cells (3 million) were injected s.c. into the flank of the nude mouse. Five animals per expression construct were injected. The health of the animals was monitored, and the sizes of tumors were measured weekly for 6 to 8 weeks. *D,* tumors were excised from the mice, weighed, and then homogenized. Equal amounts (10 μg) of cell lysate were subjected to α-HA Western blot. *E,* allelic differences in tumor diameter. Tumor diameter was monitored from day 29 to day 48. Five mice were used for each time point. *Bars,* SE. *F,* allelic differences in tumor weight. Tumors were excised on day 48 and weighed. Allele A suppressed tumor weight compared with vector-injected mice ($P = 0.0003$). Allele G did not suppress tumor weight compared with vector-injected mice ($P = 0.22$).

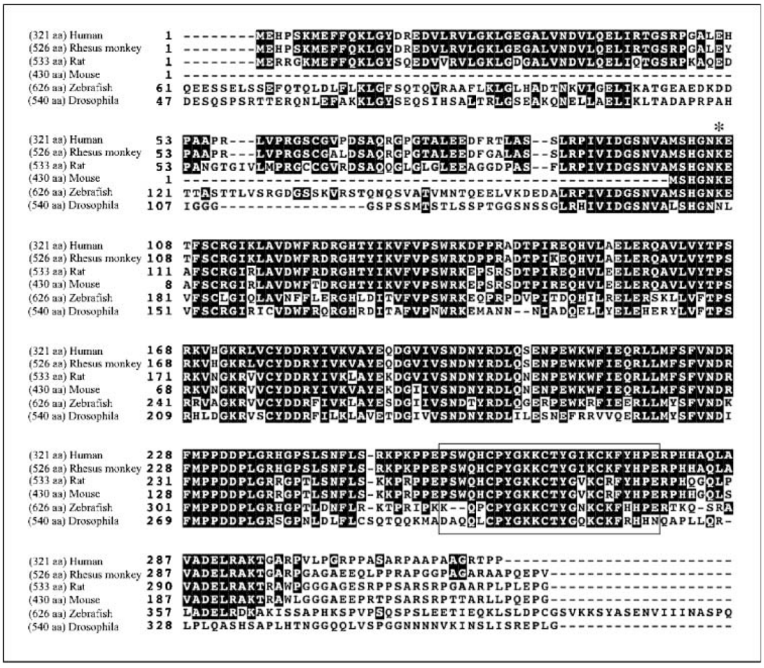

Figure 4.

Amino acid similarity between human *p34* and homologues from other organisms. Identical amino acids are highlighted in *black* (i.e., highlighted if three or more of the sequences exhibit identical residues), whereas similarities are highlighted in *gray*. The conserved ZNF-CCCH domain is *boxed*. *, codon 106 SNP in human p34. NCBI accession no.: XP_291154 for human, XP_001087043 for rhesus monkey, XP_001068699 for rat, NP_766373 for mouse, XP_691566 for zebrafish, and AAF55738 for *Drosophila melanogaster*.

Table 1

p34 codon 106 SNP in cancer cell lines

Cell line cancer type	Cell lines with SNP	Cell line	SNP genotype
Non–small cell lung	1/9	EKVX	A/G
Renal	1/8	RXF-393	A/G
Prostate	2/2	DU-145	G
		PC-3	G
Melanoma	2/8	LOX-IMVI	A/G
		SK-MEL-28	G
Colon	1/7	COLO-205	G
Leukemia	0/6		
Central nervous system	0/6		
Ovarian	0/6		
Breast	0/8		

Table 2

Association of *p34* codon 106 SNP with lung cancer risk

	No.	Genotype			Frequency		*P*
		A/A	A/G	G/G	Allele A	Allele G	
GELCC lung cancer families*							
Genotyped by CIDR							
6q linked	40	33	7	0	0.91	0.09	0.87†
6q unlinked	37	32	5	0	0.93	0.07	—
Other GELCC families	199	177	20	2			
Total	276	242	32	2	0.94	0.07	0.80‡
Sporadic lung cancer	204	180	24	0	0.95	0.06	0.94§
Unaffected control populations							
GELCC spouses	94	85	9	0			
Coriell	68	62	6	0			
Nicotine addicted	196	168	28	0			
Total	359	316	43	0	0.94	0.06	—

*Families defined by three or more family members with lung cancer.

†Comparison between 6q-linked and 6q-unlinked lung cancer families.

‡Comparison between total GELCC lung cancer families and total control population.

§Comparison between sporadic lung cancer patients and total control population.

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