Genetic Defects in Surfactant Protein A2 Are Associated with Pulmonary Fibrosis and Lung Cancer

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Idiopathic pulmonary fibrosis (IPF) is a lethal scarring lung disease that affects older adults. Heterozygous rare mutations in the genes encoding telomerase are found in ~15% of familial cases. We have used linkage to map another disease-causing gene in a large family with IPF and adenocarcinoma of the lung to a 15.7 Mb region on chromosome 10. We identified a rare missense mutation in a candidate gene, *SFTPA2,* within the interval encoding surfactant protein A2 (SP-A2). Another rare mutation in *SFTPA2* was identified in another family with IPF and lung cancer. Both mutations involve invariant residues in the highly conserved carbohydrate-recognition domain of the protein and are predicted to disrupt protein structure. Recombinant proteins carrying these mutations are retained in the endoplasmic reticulum and are not secreted. These data are consistent with *SFTPA2* germline mutations that interfere with protein trafficking and cause familial IPF and lung cancer.

Idiopathic pulmonary fibrosis (IPF, MIM 178500) is a scarring lung disease that presents in older adults with shortness of breath and cough. The mean length of time from disease diagnosis to death is only 3 years. IPF is distinguished by its progressive course, radiographic features, and pathologic evidence of patchy injury with foci of replicating fibroblasts at the interface of normal and scarred lung tissue.[1] Approximately 2% of subjects with IPF have a familial disease form characterized by an autosomal-dominant pattern of inheritance with incomplete penetrance.[2] In ~15% of these families, the disease is caused by mutations in the genes encoding the protein (*TERT,* MIM 187270) or RNA component (*TERC,* MIM 602322) of telomerase,[3,4] an enzyme that maintains the integrity of the chromosomal ends. An additional 25% of individuals who have either familial or sporadic pulmonary fibrosis but who do not have a mutation in *TERT* or *TERC* have evidence of telomere shortening of circulating leukocytes.[5] Although evidence of telomerase dysfunction is present in a large proportion of IPF cases, other genetic defects probably cause disease.

We have 59 kindreds who have familial pulmonary fibrosis and no identifiable mutations in the coding regions of *TERT* or *TERC*. To identify other genes that are defective in IPF, we performed a whole-genome linkage study in one of the largest of these families in our collection. The study was approved by the Institutional Review Board of the University of Texas Southwestern Medical Center at Dallas. Kindreds were described previously.[5] Written informed consent was obtained from all subjects. Genomic DNA was isolated from either circulating leukocytes or paraffin-embedded archived tissue.[4]

In family F27, multiple members have early-onset pulmonary fibrosis and lung cancer cosegregating in an autosomal-dominant pattern, suggesting that these two diseases share a common etiology. The proband of the family, subject IV:8 (Figure 1), is a 51-year-old white man who has ten relatives with pulmonary fibrosis; five of the ten died before age 50. Four individuals (III:8, III:12, IV:2, and IV:7) also had adenocarcinoma of the lung with features of bronchioloalveolar cell carcinoma (BAC). Three other related individuals had pulmonary adenocarcinoma or BAC in the absence of known fibrosis. Photomicrographs of hematoxylin and eosin-stained slides of resected lung tissue from affected family members are shown in Figure 2. Seven individuals, including the proband, were evaluated with pulmonary function testing and high-resolution CT scans of the chest; medical records or death certificates were obtained when possible. Additional clinical information is provided in Table S1.

We performed a whole genome linkage analysis of 29 family members by using the Illumina Linkage IVb SNP panel of >6,000 SNPs. Call rates varied from 97.2% to 98.5% for Autopure-purified DNA and from 56.4% to 73.9% for whole-genome-amplified DNA extracted from archived samples. Individuals with pulmonary fibrosis and/or lung cancer were classified as "affected," and all others were assigned an unknown affectation status. We used the software MERLIN[6] to screen the entire genome by using multipoint model-free linkage analysis,[7] and we then evaluated the regions with the highest signals by using a model-based method. Figure S1 in the Supplemental Data shows the analysis of family F27 with the highest peak on chromosome 10; the model-free LOD

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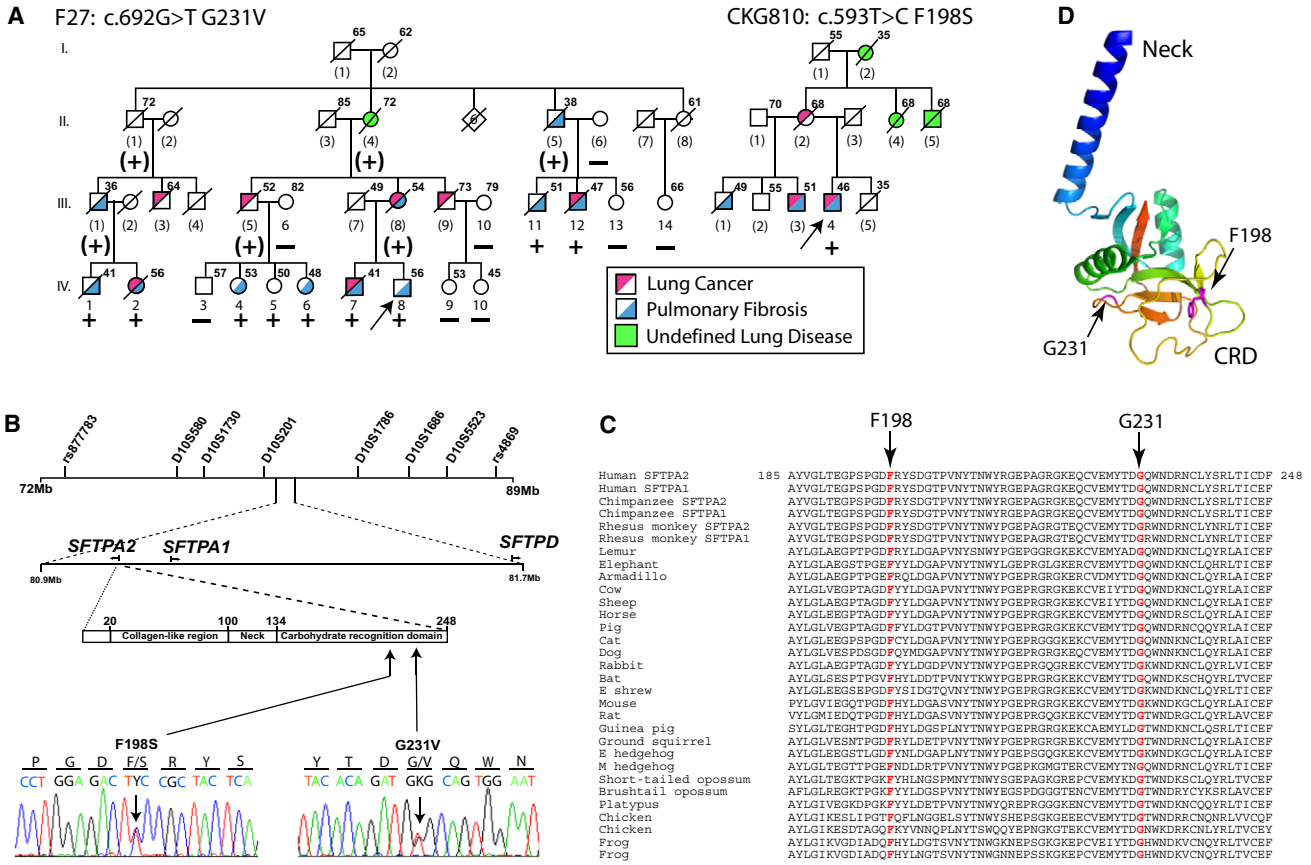

Figure 1. Mutations in *SFTPA2* Segregate with Familial Lung Cancer and Pulmonary Fibrosis

(A) Abridged pedigrees are shown. The arrow indicates the index case. Circles represent females; squares represent males. Symbols with a slash through them indicate deceased subjects. Individuals with pulmonary fibrosis, lung cancer, or undefined lung disease are indicated by blue, red, and green symbols, respectively. Numbers below each symbol correspond to the individuals described in Table S1; numbers in parentheses indicate individuals for whom no DNA sample was available. The presence or absence of a mutation in *SFTPA2* is indicated by plus or minus signs, respectively. When the mutation was inferred based upon the pattern of inheritance, the plus sign is placed in parentheses. The current age or the age at death is indicated to the upper right of each symbol. The *SFTPA2* mutations and predicted amino acid changes are listed above each family.

(B) A region of chromosome 10 cosegregates with pulmonary fibrosis and lung cancer. The genes that encode paralogs of both surfactant protein A and surfactant protein D lie within the linked region. The DNA sequence of a segment of *SFTPA2* exon 6 is shown for the heterozygous CKG810 (left) and the proband of family F27 (right). A single base substitution (arrow) changes the wild-type thymidine to cytosine and leads to the predicted substitution of phenylalanine to serine at codon 198 (left); substitution of the wild-type guanine to thymidine leads to the predicted substitution of glycine for valine at codon 231 (right). Both mutations affect the coding region of the carbohydrate-recognition domain of surfactant protein A2.

(C) The terminal portion of the amino acid sequence of the carbohydrate-recognition domain of surfactant A proteins is shown from diverse vertebrate species. This domain is highly conserved. Both the phenylalanine and glycine at positions 198 and 231, respectively, are conserved in all species shown. All sequences were obtained from NCBI; the comparison of the different proteins was based on a ClustalW-generated alignment.

(D) The ribbon diagram of the crystal structure of the monomeric carbohydrate recognition domain (CRD) and neck domains of rat surfactant protein A is shown; the positions of amino acids corresponding to codons 198 and 231 of the human sequence are indicated by arrows.

score is 3.22 (p value = 6.0×10^{-5}), and the model-based LOD score is 2.74 (p value $< 1.8 \times 10^{-3}$) as determined in a dominant genetic model with a penetrance of 0.95. All affected family members share an identical-by-descent 15.7 Mb region of chromosome 10 (bounded by markers rs877783 and rs4869, Figure S2). Additional polymorphic microsatellite markers within this region were genotyped, and haplotypes were determined by MERLIN.

Approximately 118 annotated genes, including the genes encoding surfactant proteins A and D, reside within the linked interval. These two genes were considered excellent candidates because they are both expressed at high levels in the lung and play key roles in maintaining the integrity of the air spaces (alveoli) of the lung. Surfactant A has two isoforms, SP-A1 and SP-A2, which are encoded by adjacent genes (*SFTPA1*, MIM 178630, and *SFTPA2*,

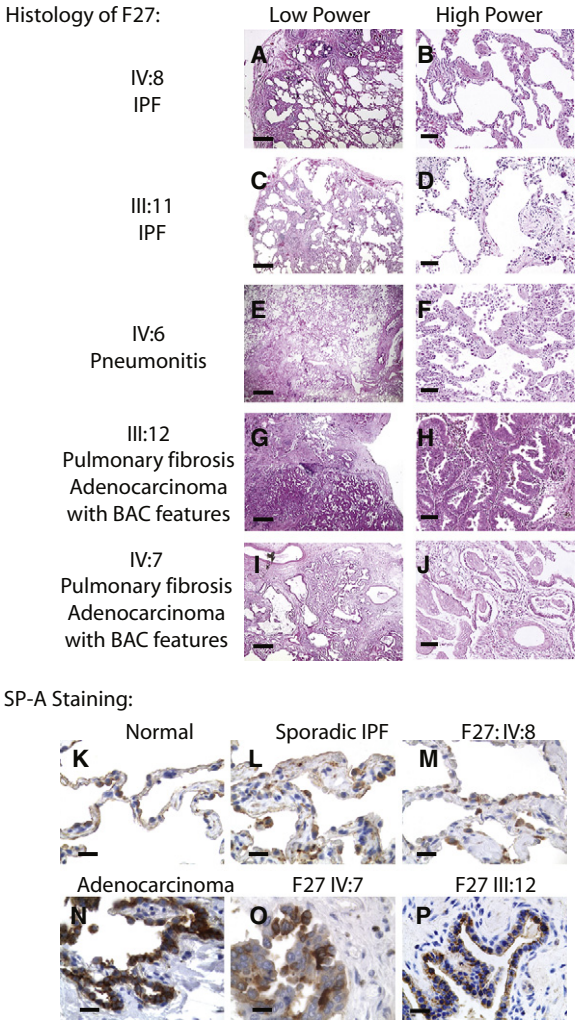

Figure 2. Histology and Immunohistochemical Staining of SP-A in Lung Specimens from Affected Individuals in Family F27
The hemotoxylin and eosin-stained slides (A–J) show different pathologic subtypes of pulmonary fibrosis with and without adenocarcinoma with features of bronchioloalveolar cell carcinoma (BAC). Specimens from the proband IV:8 (A, B) and III:11 (C, D) demonstrate the pathologic findings of usual interstitial pneumonia, consistent with IPF. The specimen shown from IV:6 (E and F), a 29-year-old female diagnosed with hypersensitivity pneumonitis resulting from bird exposures, shows organizing pneumonitis with minimal fibrosis; her disease remitted with prednisone treatment and avoidance of birds. The specimen from III:12 (G and H) shows pulmonary fibrosis and adenocarcinoma with features of BAC. An autopsy sample from IV:7 (I and J) shows extensive severe interstitial fibrosis with cystic remodeling and marked epithelial proliferation. In some areas the epithelial cells show severe cytologic atypical and papillary and acinar growth patterns, consistent with adenocarcinoma with features of BAC confined to the lungs. Photographs were obtained as previously described.[4] Slides from a normal subject (K), a sporadic patient with IPF without any mutations in *SFTPA2* (L), a patient with adenocarcinoma with BAC features and no *SFTPA2* genomic mutations (N) and F27 family members IV:8 (M), IV:7 (O), and III:12 (P) were stained for immunochemical localization of SP-A. Type II cells lining the alveoli and neoplastic adenocarcinoma cells demonstrate SP-A staining (indicated by the brown color). Slides were deparaffinized with xylene for 10 min two times and rehydrated by immersion in serial ethanol baths: 100% twice, 95% once, 75% once, 50% once, and then three washes in PBS for 5 min each. Antigen retrieval was performed with 1× Citra buffer (Biogenex, San Ramon, CA) for 10 min at 100°C. Endogenous peroxidase activity was quenched by incubation with 0.6% H_2O_2 in methanol for 30 min at room temperature. Immunohistochemical staining was performed with the Vectastain Elite ABC kit (Vector Laboratories) according to the manufacturer's protocol with anti-SP-A antibody (1:2000 dilution with overnight incubation at 4°C), biotinylated GAR (1:500 with a 1 hr room-temperature incubation) and the chromagen DAB (Dako). Slides were counterstained with Mayer's Hematoxylin (Dako, Carpinteria, CA) and mounted with VectaMount AQ (Vector Laboratories). Scale bars represent 1 mm and 100 μm for the low-power (A, C, E, G, and I) and high-power (B, D, F, H, and J–P) magnifications, respectively.

MIM 178642) that are >98% and >90% identical in their coding and noncoding regions, respectively. Only four amino acids differ between the two human proteins. We selectively amplified the coding exons and consensus splicing sequences of *SFTPA1* and *SFTPA2* for the proband of family F27 by using gene-specific oligonucleotides. Sequencing was then performed as described;[4] all PCR conditions and primers are listed in Table S2. A transversion mutation (GGG → GTG) was identified in codon 231 of one *SFTPA2* allele; this c.692G → T G231V mutation is predicted to change a highly conserved glycine residue to a valine (Figure 1B). All individuals in kindred F27 with pulmonary fibrosis and/or lung cancer were heterozygous for this mutation (Figure 1A).

SFTPA1 and *SFTPA2* were then sequenced in the other 58 probands in our collection of families who have pulmonary fibrosis and no mutations in *TERT* or *TERC*. A heterozygous transversion mutation (TTC → TCC) in codon 198 of *SFTPA2* was found in a 45-year-old white individual with IPF and lung cancer. This c.593T → C F198S mutation substitutes a serine residue for a highly conserved phenylalanine. Although this individual had multiple family members with pulmonary fibrosis and lung cancer, these individuals were not available for study.

We used Taqman allelic discrimination assays[9] to test for both *SFTPA2* mutations in a large (n = 3557) multiethnic population-based probability sample of Dallas County; this sample included 1048 whites.[8] The oligonucleotides used for the assays are listed in Table S3. No other subjects were found with either of the sequence variants. We also sequenced the coding exons and consensus splicing sequences of the gene encoding SP-D in the proband of family F27 and in 32 other probands. No rare variants were found. All the DNA sequence variants we identified in the genes encoding SP-A1, SP-A2, and SP-D are provided in Table S4.

Pulmonary surfactant protein A (SP-A) is the major protein of pulmonary surfactant, a mixture of phospholipids and proteins synthesized by the type II alveolar epithelial cells. Surfactant stabilizes the gas-exchanging surface of the alveoli and plays a role in pulmonary host

defense by binding, aggregating and opsonizing various microorganisms.[10,11] SP-A belongs to a structurally homologous family of innate-immune defense proteins that are known as collectins and contain a collagen-like N-terminal region that is involved in trimerization of the protein and a C-terminal carbohydrate recognition (CRD) lectin domain.[12,13] It assembles into 18-subunit oligomers that are composed of six disulfide-linked trimers assembled in a "flower bouquet" pattern. Mice that lack SP-A are susceptible to pulmonary infections from multiple organisms and lack tubular myelin, the ordered lattice-like array of intersecting membranes and proteins in the alveolar space.[14]

Both of the mutations we identified in SP-A2 involve amino acids in the CRD, which is highly conserved among all surfactant orthologs from *Xenopus* to humans (Figure 1C). Both mutations are predicted to disrupt the tertiary structure of the CRD domain (Figure 1D). The bulky aromatic ring of phenylalanine at position 198 is located in the hydrophobic core of the protein, and substitution of a serine at this position would be predicted to destabilize the protein on the basis of the crystal structure of the rat protein.[15] The glycine at position 231 lies adjacent to an alpha helix, and the substitution of a larger residue such as a valine at this position would be predicted to result in disruptive steric clashes.

Immunodetectable SP-A was detected in the type II alveolar cells via a rabbit polyclonal antibody raised against purified human SP-A (a kind gift from C. R. Mendelson); this antibody does not distinguish between SP-A1 and SP-A2. The staining of type II cells in the affected individual IV:8 in family F27 was similar to the staining of normal lung or lung tissue from a patient with sporadic IPF and no mutations in *SFTPA2*. In both individuals IV:7 and III:12, the adenocarcinoma cells lining the air spaces demonstrate expression of SP-A (Figure 2), which is characteristic of this type of lung cancer.[16]

To determine what effect the sequence variations identified in SP-A2 in this study have on the synthesis and secretion of the protein, we expressed the wild-type and the variant proteins in A549 cells, an immortalized human alveolar epithelial cell line derived from a lung adenocarcinoma. High-fidelity DNA polymerases were used for cloning, and all subclones were confirmed by sequence analysis. Full-length human *SFTPA1* cDNA was PCR amplified from clone LIFESEQ90096303 (Open Biosystems, Waltham, MA) and subcloned into pcDNA3 (Invitrogen, Carlsbad, CA) downstream of the CMV promoter. We used two partial IMAGE cDNA clones, 5184888 and 841707 (Invitrogen), to construct a full-length *SFTPA2* cDNA. Site-directed mutagenesis (QuickChange, Stratagene) was utilized so that the DNA sequence of wild-type SP-A1 and SP-A2 clones exactly matched NM_005411.3 and NM_006926.2, respectively. In a parallel set of experiments, we used expression constructs containing an in-frame 14 amino acid V5-tag immediately after the glutamic acid at position 21 by primer extension mutagenesis and zipper PCR.

A549 cells were transfected with the expression plasmids as follows: 350,000 cells were plated on 35 mm wells in 2 ml complete medium (Ham's F12 with 10% FBS and 1% P/S) and transfected on day 1 with 1–2 μg DNA and with 3 μl FuGENE HD Transfection Reagent (Roche, Basel, Switzerland) per μg DNA according to the manufacturer's protocol. The cells were refed with complete medium on day 2. Seventy-two hours after transfection, we examined the cell lysate and medium by immunoblotting to assay for the presence of SP-A. One milliliter of cultured medium was removed from each well and centrifuged at 16,000 × *g* for 10 min at 4°C. The cells were washed once with 2 ml ice-cold PBS, harvested in 300 μl of RIPA lysis buffer (150 mM NaCl, 50 mM Tris-HCl [pH 8], 1 mM EDTA [pH 8], 1% NP-40, 0.1% SDS, and 0.1% deoxycholate with 1 tablet of protease cocktail [Roche] per 10 ml buffer), sonicated for 10 s., and centrifuged at 16,000 × g at 4°C for 10 min. The protein concentration of the medium and cellular lysates was determined with the BCA Assay (Pierce, Rockford, IL).

Protein aliquots were eletrophoresed on 10% SDS-PAGE Bio-Rad minigels and transferred to nitrocellulose Protran membranes (Whatman, Dassel, Germany). The blots were incubated for 1 hr at room temperature in blocking buffer (5% dried milk in TBST [150 mM NaCl, 10 mM Tris (pH 8), 0.1% Tween-20]), incubated with primary antibody overnight at a dilution of 1:10,000 (anti-SP-A or anti-V5 mAb R960-25 from Invitrogen) in blocking buffer, washed four times in TBST for 5 min each, incubated with secondary antibody (Southern Biotech; Birmington, AL) at 1:20,000 in blocking buffer for 1 hr at room temperature, washed four times in TBST for 5 min each, and developed with SuperSignal West Pico Chemiluminescent substrate (Pierce) according to the manufacturer's protocol.

In cells expressing either wild-type SP-A or one of the SP-A variants with an allele frequency >5%, immunoreactive protein was present in both the cell lysate and the medium (Figure 3A). In contrast, the SP-A2 G231V and F198S variants were poorly expressed in the cells, and no protein was detected in the culture medium (Figure 3A). When a V5-specific antibody was used in a parallel set of experiments to analyze cells expressing the V5-tagged constructs, the protein was readily detected in the cell lysates but not in the medium. Another rare variant identified in this study, SP-A2 L12W, was expressed and secreted in amounts comparable to the wild-type protein. This variant failed to cosegregate with pulmonary fibrosis in the family in which the mutation was identified.

We also examined all the other rare and common SP-A1 variants that were identified in this study. All of these forms of SP-A1 had an expression pattern that was similar to the wild-type protein (Figure S3).

To determine whether the wild-type SP-A1 or SP-A2 interacted with the G231V and F198S SP-A2 variants, we coexpressed an in-frame Myc-tagged wild-type SP-A1 or SP-A2 (three copies of EQKLISEEDLN engineered after the signal peptide as described above) with the V5-tagged SP-A2 variants and immunoprecipitated the cellular lysates

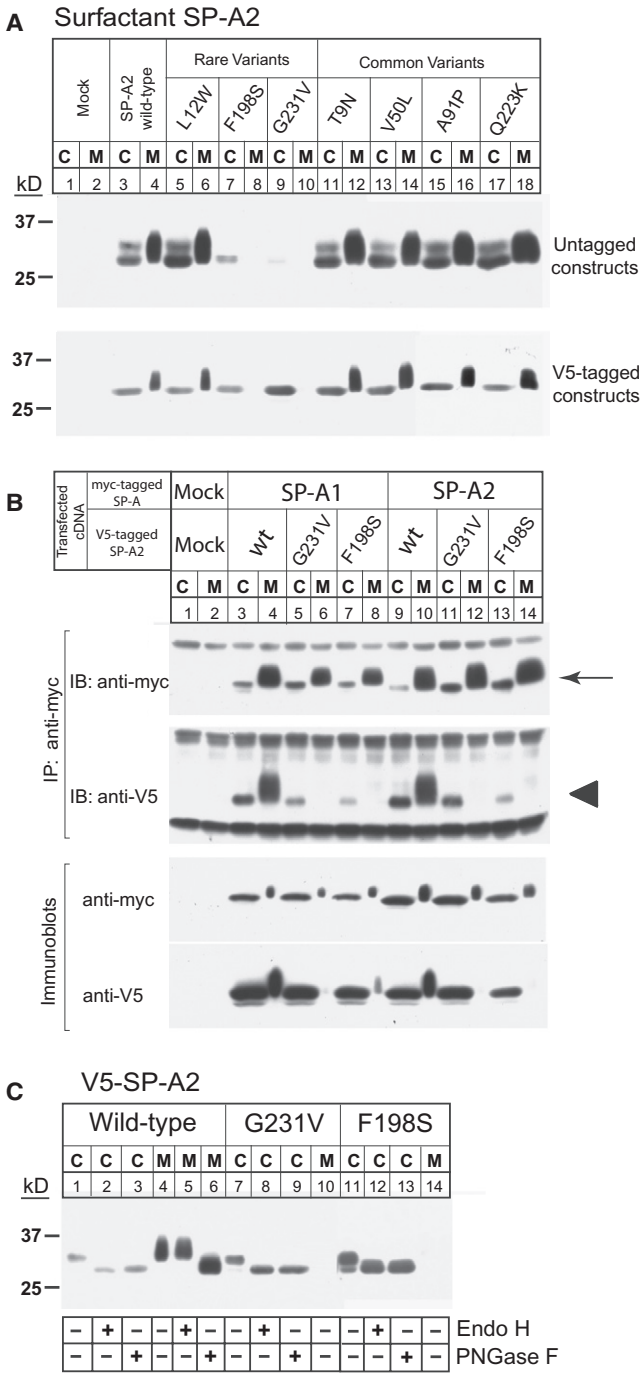

Figure 3. The Effects of the G231V and F198S *SFTPA2* Mutations on Protein Expression, Secretion, Interactions, and Endoglycosidase Sensitivity in Transiently Transfected A549 Cells

(A) A549 cells were transiently transfected with vector plasmid (Mock), the wild-type SP-A2 expression construct and various untagged SP-A2 constructs (upper panels) or V5-tagged constructs (lower panels). Rare variants are those that were found at a frequency of <5%. Aliquots of cellular lysates (C, 40 μg) and medium (M, 80 μg) were analyzed via immunoblotting with either a rabbit polyclonal that was raised against purified human SP-A (upper panel) or a mouse monoclonal antibody that recognizes the V5 epitope (lower panel). Blots were exposed for 1–20 min or 1–20 s for the anti-SP-A and anti-V5 antibodies, respectively.

by using a monoclonal antibody directed against the c-Myc epitope. 48 hr after transfection, A549 cells were washed with ice-cold PBS, scraped on ice in 0.5 ml of freshly prepared lysis buffer (100 mM NaCl, 50 mM HEPES [pH 7.4], 1.5 mM $MgCl_2$, and 0.5% NP-40 with 1 tablet of protease cocktail (Roche) per 10 ml buffer), and centrifuged 16,000 × g at 4°C for 15 min. The supernatants were transferred to a new tube and incubated with 2 μg of 9E10 mAb (Santa Cruz Biotechnology) directed against the c-Myc epitope and 200 μg (20 μl) of a protein A-agarose slurry (Calbiochem, San Diego, CA) for 16 hr at 4°C. Protein A-bound antibodies and proteins were separated from the supernatant by centrifugation (800 × g, 15 s) and washed twice for 10 min in 800 ul of the lysis buffer at 4°C. The agarose pellet was resuspended in 2× Laemli buffer and boiled 5 min prior to SDS-PAGE. Immunoblot analysis of reserved aliquots of cellular lysate and medium was performed with anti-Myc (1:1000 dilution of 9E10) or anti-V5 antibodies as described above.

Immunoprecipitation of Myc-tagged wild-type SP-A1 resulted in the coprecipitation of wild-type SP-A2 from both the cell lysate and the medium (Figure 3B). In contrast to these results, no V5-tagged SP-A2 G231V or F198S coimmuniprecipitated with wild-type SP-A1 in the medium, although they were detected in the cellular lysate. Similar results were obtained when the Myc-tagged wild-type SP-A2 was used. Thus, the variants were expressed and interacted with both wild-type SP-A1 and SP-A2 in the cells but were not secreted into the medium.

To determine whether the mutant forms of SP-A2 (G231V and F198S) were retained in the endoplasmic reticulum (ER), we subjected aliquots of cellular lysates (25 μg) or medium (80 μg) to treatment with either Endoglycosidase H or PNGase F (New England Biolabs, Ipswich, MA) for 1 hr at 37°C in the provided buffers. These endoglycosidases differ in their specificities according to the degree of maturation of the N-linked sugar chain attached to SP-A2. Removal of the N-linked sugar results in an ~8 kDa reduction in the apparent molecular mass of the protein.[17]

(B) A549 cells transfected with empty vector (Mock), plasmid expressing the Myc-tagged SP-A1 or SP-A2, and the V5-tagged wild-type or variant SP-A2. Forty-eight hours after transfection the cell lysates (C) and medium (M) were split into two aliquots. The Myc-tagged SP-A proteins were immunoprecipitated from the cellular lysates and media, subjected to SDS-PAGE, transferred to nitrocellulose membrane, and immunoblotted with antibodies directed against the Myc or V5 epitopes (upper panels). The arrow and arrowhead indicate the position of the Myc-tagged SP-A and V5-tagged SP-A2, respectively. Aliquots of cell lysates (C) and medium (M) (40 μg) were subjected to SDS-PAGE, transferred to nitrocellulose membranes, and immunobloted with antibodies directed against the Myc or V5 epitopes (lower panels).

(C) A549 cells were transiently transfected with V5-tagged SP-A2 wild-type, G231V, or F198S constructs. Aliquots of lysates (C) or media (M) were treated with the indicated endoglycosidases and subjected to immunoblot analysis. Untreated samples were treated in parallel tubes without the addition of enzyme.

Analysis of the wild-type and mutant proteins in the cell lysates revealed a similar reduction in molecular mass after treatment with PNGase F, which removes all N-linked sugars, and Endo H, which removes high-mannose sugars that have not yet undergone complete maturation (Figure 3C). Thus, all the protein in the cells is located in the endoplasmic reticulum. In the medium, a reduction in the molecular mass of the wild-type SP-A2 was seen after treatment with PNGase F but not Endo H because the sugars have fully matured coincidently with secretion. Immunofluorescence of the V5-tagged recombinant proteins demonstrates colocalization of both the wild-type SP-A2 and SP-A2 G231V and F198S variants with calnexin, an ER resident protein (data not shown).

These findings are consistent with a causal relationship between mutations in *SFTPA2* and both IPF and lung cancer. *SFTPA2* is located within the linkage interval for the disease in a large family with autosomal-dominant pulmonary fibrosis and adenocarcinoma. An independent mutation in the same gene was identified in another family with an identical phenotype. With two exceptions, all carriers who were older than age 45 had pulmonary fibrosis in this family, and several family members also had adenocarcinoma with BAC features. Neither of these two mutations was present in any of more than 1000 ethnically matched subjects. Both mutations involve highly conserved residues and disrupt cellular trafficking of the protein, probably by interfering with its proper folding.

Subjects with IPF have a 7- to 14-fold greater risk of developing lung cancer than those without IPF, and this risk is not completely accounted for by smoking.[18] Hyperplasia of type II alveolar cells is frequently seen with IPF, but case reports of individuals or families with coincident IPF and BAC are rare.[19,20] Development of adenocarcinoma is believed to involve the multistep progression from atypical adenomatous epithelial hyperplasia (preneoplastic lesion) to BAC and invasive adenocarcinomas.[21] The finding that people who belong to the same family (F27) and carry a germline *SFTPA2* mutation can have either isolated IPF or IPF with atypical bronchiolar epithelial proliferation, BAC, or adenocarcinoma supports this paradigm that progenitor cells expressing SP-A are relevant to the pathogenesis of alveolar-type lung cancer. No other family in our cohort had multiple individuals with coincident pulmonary fibrosis and either BAC or adenocarcinoma with bronchioloalveolar features. The finding of both IPF and BAC in the same individuals, especially in individuals younger than 50 years of age, may be a phenotype specifically associated with *SFTPA2* mutations.

Mutations in the collectin mannose-binding protein, which shares partial structural similarity with SP-A2, are associated with recurrent pediatric infections.[22] We failed to find any evidence that the family members with mutations in SP-A2 have an increased frequency of respiratory infections. One of the mutation carriers in family F27, IV:6, was diagnosed at the age of 29 years with hypersensitivity pneumonitis. She was successfully treated with prednisone and avoidance of birds, the presumed inciting factor leading to her lung disease. At a molecular level, transcriptional downregulation of the gene encoding human SP-A2 is known to be more responsive to the inhibitory effects of glucocorticoids than SP-A1.[23] The clinical and pathologic subtype of pulmonary fibrosis for other mutation carriers in family F27 differ from that of individual IV:6. It is unknown whether any of the other mutation carriers might experience a therapeutic benefit from steroids.

Currently we can only speculate as to how the mutations we identified in *SFTPA2* cause IPF. The accumulation of misfolded SP-A2 variants may trigger the unfolded protein response and induce ER stress in the secretory alveolar type II cells, as is seen for mutations in the gene that encodes surfactant protein C (*SFTPC*).[24,25] Mutations in this gene can cause interstitial lung disease, including IPF, in pediatric patients and, rarely, adults.[26,27] Markers of elevated ER stress and alveolar epithelial apoptosis have been demonstrated in sporadic cases of IPF[28], and it is possible that ER stress of type II alveolar cells is a common feature of IPF. Because alveolar type II cells can trans-differentiate into alveolar type I cells to repopulate the alveolar epithelium after injury[29] and express telomerase[30], a parsimonious hypothesis for the pathogenesis of familial IPF, resulting from mutations in genes encoding either surfactant proteins or telomerase, involves alveolar type II cell dysfunction and an impaired regenerative capacity of the lung. In addition, the mutations may cause a pathologic decrease in the secretion of SP-A into the alveolar space. Surfactant A has known anti-inflammatory properties[31]: an alteration in the balance of immunomodulatory regulators in the alveolar space may lead to increased fibrosis and lung cancer. If this were the case, then specific replacement of SP-A or SP-A2 may offer a potential therapeutic strategy for the treatment of this lethal disease.

Supplemental Data

Three figures and four tables are available with this paper online at http://www.ajhg.org/.

Acknowledgments

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Web Resources

The URLs for data presented herein are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/omim/
National Center for Biotechnology Information (NCBI), http://www.ncbi.nlm.nih.gov/
ClustalW, www.ebi.ac.uk/clustalw
All SNPs have been submitted to the dbSNP database, http://www.ncbi.nlm.nih.gov/SNP/

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