Human Surfactant Protein B Expression in Humanized Transgenic Mice

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Human Surfactant Protein B Expression in Humanized Transgenic Mice

A Capstone Project Submitted in Partial Fulfillment of the Requirements of the Renée Crown University Honors Program at Syracuse University

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May 2014

Honors Capstone Project in Biology

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Abstract

Surfactant protein B (SP-B, gene name: sftpb) is essential for normal lung function. It reduces alveoli surface tension, thereby preventing the lung from collapse. A single nucleotide polymorphism (SP-B 1580 C/T) is associated with several lung diseases and altered N-linked glycosylation site at Asn^{129} of SP-B. This change, present in the human population, has been associated with negative effects on SP-B precursor (proSP-B) processing and function. In this study, hSP-B humanized transgenic mice were generated without mouse SP-B background. Four founding lines, showing only the hSP-B gene, were selected via PCR-based DNA analysis. Genomic sequencing of these mice revealed which allele variant (hSP-B-C/T) they carried. Western blot analysis of BALF samples revealed these hTG mice expressed hSP-B protein in levels significant for survival and comparable to that of a healthy human lung. Characterization of the two hSP-B allele variant hTG strains revealed significant differences in their relative alveolar size and total lipid concentration.
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Executive Summary

Surfactant protein B (SP-B, genus name: sftpb) is a vital component of a healthy and normally functioning lung. It coats the surface of alveoli and prevents them from collapsing by lowering surface tension. Alveoli, hollow sacs found in the lung, are the site of oxygen and carbon dioxide exchange in the body. Modification of the gene that codes for this protein can lead to its dysfunction and cause serious, even fatal, lung complications. This paper looks to analyze one such modification. SP-B gene encodes a protein that is 381 amino acids (building blocks of protein) in length. A single nucleotide change (from ‘T’ to ‘C’) in the DNA changes one of these amino-acids from a Threonine to an Isoleucine. This small change has a drastic effect on how the protein folds together and subsequently how it performs. The two forms (alleles) of this gene are labeled C-allele (SP-B-C), which contains the modification, and wild type (normal) T-allele variant (SP-B-T) which does not. This change, which has been associated with decreased lung function, is common in the human population and represents an important avenue of research.

To study the effect this genetic modification has on physiological function and structure, humanized transgenic mice (hTG) were created. hTG SP-B mice express human SP-B (hSP-B) but not mouse SP-B (mSP-B). To generate these mice, the human SP-B gene was inserted into mouse oocytes. These mice, which now contain both the mouse SP-B gene and human gene, were bred with ‘knockout’ mice (KO) that did not express either form of the gene. From the resulting offspring, mice that expressed only the human gene, and not the mouse SP-B gene, were selected for testing in this experiment. This selection was accomplished by taking tail samples and testing the DNA for mice that were human
SP-B conditional. This test confirmed that the human gene had successfully been inserted into the (now humanized transgenic) mice and was being expressed at levels sufficient for survival. Four ‘Founder’ strains had been selected and were given labels: 2004, 2007, 2012, and 2059. Next, the DNA from each of these four founder strains was sequenced to see if they contained the SP-B gene modification. Of the four strains, only one (2012) was determined to contain the C-allele while the rest (2004, 2007, 2012) contained the T-allele.

The goal of this paper was to characterize the hSP-B gene successfully established in these humanized transgenic mice and to compare the two allele variants (C/T) to samples taken from a healthy human. The following factors were tested: relative size of alveoli, SP-B expression levels, and total lipid concentration. Alveolar size was tested by taking lung samples from each of the four founder strains and viewing them under a microscope. Relative size was then determined by superimposing these images over a grid and counting the number of intersections (‘hits’) that each alveolus contained. 100 alveoli from each founder strain were tested and used to calculate average size. Results indicated that mice containing the C-allele (hSP-B-C) had significantly larger alveoli compared to those containing the T-allele (hSP-B-T). Protein expression levels and lipid concentration were determined by testing broncho-alveolar lavage fluid (BALF). BALF is obtained by injecting a sterilized solution into the lung and withdrawing it back (now containing lung material) for use in further testing. The level of hSP-B expression in the hTG mice was determined using western blot analysis. In this method, proteins are separated by their molecular weight. Since the molecular weight of human SP-B is known, western blot analysis allowed not only for confirmation that hSP-B was being expressed by the mice
but also to what extent. Results of this testing indicated that the level of hSP-B protein expression in the lung of these transgenic mice is comparable with that in the human lung. Lipid concentration was determined by testing the degree to which these samples could absorb UV light. These results indicated that the level of lipid concentration was significantly higher in the BALF taken from a C-allele mouse compared to that of the T-allele variant and also to that found in a healthy human. The T-allele showed comparable lipid concentration to that of the human sample.

In summary, humanized transgenic mice were generated that carried human SP-B C-allele or T-allele. hSP-B expression levels were sufficient for survival and were comparable with that in the human lung. Characterization of these hSP-B two allele variants revealed significant differences in their relative alveolar size and total lipid concentration.

These differences should help drive the future research needed to ascertain the specific nature of the C-allele’s negative effect on the host’s ability to fight respiratory diseases. Since this mutation is common in the human population, the ability to identify patients containing this C-allele variant could lead to modified treatment and an increased success rate in fighting their respiratory disease.
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I wish to thank Dr. Guirong Wang for his all-encompassing mentorship over the past two years. His enduring belief in my work and in my future have meant more than he will ever know. I wish also to thank Dr. Osama Abdel Razek for his patience, expertise and friendship. His continued support of me and for this project were invaluable to its completion.
**Introduction**

Surfactant protein B (SP-B gene name: **sftpb**) is essential for effective pulmonary respiration and physiological function. Pulmonary surfactant, a lipid and protein complex synthesized by alveolar type II cells, lines alveoli at the gas-liquid interface and reduces surface tension, thereby preventing the lung from collapse. Surface tension arises as water molecules are more strongly attracted to one another than to air molecules causing an inwardly directed force that tends to reduce surface area [1]. During low lung volumes (expiration), surfactant is squeezed out of the surface of the alveoli and forms micelles. Upon inspiration, this film is spread over the surface of the alveoli reducing surface tension and maintaining the structural integrity of the lung. Surfactant-associated protein is made up of four primary functional components: surfactant protein A (SP-A), surfactant protein B (SP-B), surfactant protein C (SP-C), and surfactant protein D (SP-D). Surfactant proteins A and D are important in immunity and host defense while SP-B is a vital structural component of the surfactant. SP-B loss of function causes surfactant death and subsequently alveolar collapse and decreased lung function. Human SP-B (hSP-B) genetic variation has been associated with several fatal respiratory diseases, such as congenital alveolar proteinosis [2-4], and SP-B-deficient mice exhibit pulmonary dysfunction and die shortly after birth [5]. Two variants will be examined in this paper: T allele and C allele. A single nucleotide polymorphism (SN, rs1130866) SP-B 1580 C/T alters N-linked glycosylation of Asn\(^{129}\), which is present in the C allele but absent in the T allele [6]. This alteration in N-linked glycosylation pattern may influence SP-B
precursor processing and function. hSP-B precursor contains three Saposin-like protein (SAPLIP) domains, SP-B\textsuperscript{N}, SP-B\textsuperscript{M}, and SP-B\textsuperscript{C}. N-Linked glycosylation occurs in the SP-B\textsuperscript{N} domain. For this study, humanized transgenic mice were successfully generated that contain either the C or T allele of the human SP-B gene without mouse SP-B background. Transgenic mice showed comparable hSP-B expression with that in the human lung and also showed normal phenotypes as wild type (WT) mice. These results indicate that hSP-B is functional in the transgenic mice. The goal of this study was to characterize this humanized sftpb transgenic mouse line.

**Materials and Methods**

**Mice and animal husbandry**

Wild type mice used for this study were purchased from the Jackson Laboratory (Bar Harbor, ME). They were then maintained in the animal core facility of SUNY Upstate Medical University (SUNY UMU) throughout the duration of the study. hTG SP-B mice generated for this study were conditional hSP-B transgenic mice (contained the background of SP-B KO mice). All mice used for this study were handled by the protocols approved by the Institutional Animal Care and Use Committee of SUNY UMU.

**Constructs for generation of TG SP-B and conditional TG SP-B mice**

To generate hSP-B mice, the cDNA of human *sftpb* had to first be cloned into a suitable vector and initiated by a human promoter. This was accomplished by cloning the cDNA into a basic 3.7-hSP-C/SV40 plasmid. A 5.4 kb DNA fragment of this vector was then
excised from the recombinant plasmids and microinjected into fertilized FVB/N oocytes from wild type mice.

**Generation of hTG SP-B Mice**

The generation of an hTG SP-B strain began by mating mice which carried both the mouse and human genes (hSP-B+/−, mSP-B+/+) with SP-B KO mice which expressed neither gene (hSP-B−/−, mSP-B−/−). This yielded hTG SP-B F1 mice (hSP-B+/-, mSP-B+/-). This strain was then mated with SP-B KO mice to block the mouse SP-B gene. KO mice (conditional transgenic) could not express mSP-B due to a neomycin resistant gene that was inserted into exon 4 of their SP-B gene. Finally, homozygous hTG hSP-B mice could be obtained by the self breeding of F2 mice from the same founder. hTG SP-B mice were therefore defined as being human SP-B positive and mouse SP-B negative (hSP-B +/+, mSP-B−/-)

**Genotype analysis of the hTG SP-B mice**

Genotyping was required to correctly identify the hTG SP-B mice. 0.5-1.0cm tail samples were obtained from new born pups after being weaned from their parents using a Qiagen DNeasy kit (Qiangen, Valencia, CA). The quality of the DNA was analyzed using Nano Drop. Two Polymerase Chain Reactions (PCR) amplifications were performed using the same DNA sample as the template: primer pair 1458/189 (which was used to screen hSP-B) and primer pair 75/76 (which was used to amplify mSP-B). Conditions for the running of the PCR were as follows: 95°C for 2 minutes, then 95°C for 40s, 58°C for 40s, and 68°C for 40s. This cycle was run 35 times with the final
extension step being performed for 7 minutes at 68°C. Products of this PCR were then examined utilizing 2% agarose gel electrophoresis and an EB stain solution. Only samples that showed the hSP-B band exclusively were labeled as human SP-B positive and mouse SP-B negative. To identify the polymorphism allele at site +1580, primer pair 1458/1401 was used to amplify the whole fragment of hSP-B gene from the positive DNA samples by High Fidelity PCR kit (Roche). The PCR for this sample was varied from the reaction prior. Initial denaturation was run for 2 minutes at 94°C followed by 10 cycles of the following conditions: 94°C 30s, 58°C 30s and 72°C 2 minutes. This was followed by two cycles of: 94°C 30s, 58°C 30s and 72°C 3 minutes. The final step was extension at 72°C for 7 minutes. The PCR products were sequenced by the Core Facility of SUNY Upstate Medical University.

**Western blot analysis of surfactant proteins from mouse BALF**

Western blot analysis was performed on the broncho-alveolar lavage fluid (BALF) of positive mice to identify expression levels of human SP-B protein in hTG SP-B mice. Samples were taken from mice (8-12 weeks) and lavaged with 0.5 mL of autoclaved PBS solution. Total protein concentration of the BALF was measured by BCA micro assay kit (Thermo). 10 µg of total protein was loaded on a 12% non-reducing SDS-PAGE gel. 10 µg of human BALF was also loaded onto the gel for use as a positive control. Electrophoresis was run on the samples at 60V for 30 minutes followed by 110V for 1 hour. The human SP-B protein was identified by anti-pig SP-B antibodies as well as two others, labeled as Antibody 1 and Antibody 2, which were created in the lab for use on this protein. Antibodies were used at 1:5000 and 1:10000 dilutions.
Histological and cellular analysis

To observe the histological structure and determination of relative alveolar size, lungs from positive mice (C and T allele) were fixed by 10% formalin solution at about 25 cm of water pressure, and then processed into paraffin blocks. Blocks were cut into pieces with thickness of roughly 5 µm. Sections were stained by hematoxylin and eosin kit while others were stained with ABC kit (Vector Laboratories, Burlingame, CA).

Determination of Relative Alveolar Size using Delesse Method

Samples prepared for histological and cellular analysis placed on microscope slides and viewed using a Nikon Eclipse TE2000-U microscope at 20x magnification. To conduct the Delesse Method of determining relative alveolar size, the images were superimposed on a grid. From this grid, the number of intersections (‘hits’) that each alveolus contained was counted. 100 alveoli were used for each founder strain and used to calculate average size.

Determination of Lipid Concentration from BALF

BALF samples from each founder variant (CT) and from a healthy human had to first be diluted to 1:10 adequate detection and determination of lipid concentration (mg/mL). For each sample, 15 µL of BALF was added to 135 µL of PBS buffer and the resulting solution was loaded into a Multiskan Ascent V1.24 to determine absorbance. To determine lipid concentration, absorbance of the samples were compared to the known absorbance of albumin at a preset concentration. Lipid concentration of each variant
strain of hTG mice (hSP-B-C/T) were compared to one another and also to that of the sample from a healthy human.

* All experiments were repeated at least three times. Western blot bands were quantified by *Quantity One* (version 4.6.1). Statistical analysis was performed by *Sigma Stat* version 3.5 software. Significant difference in statistics among groups was considered when p < 0.05 by t-test or ANOVA

**Results**

1. **Generation of SP-B-T and SP-B-C mice and transgene transmission in the offspring**

The generation of human SP-B transgenic mice began by cloning the cDNA of human *sftpb* into a basic 3.7-hSP-C/SV40 vector and driven by the human *sftpb* promoter. A 5.4 kb DNA fragment was then excised from the recombinant plasmid and microinjected into fertilized FVB/N oocytes from wild type mice. Four founder lines of hTG SP-B mice were generated and identified by PCR amplification of DNA fragment of SP-B: 2004, 2007, 2012, and 2059. Endogenous mouse SP-B background was eliminated by the use of conditional human SP-B transgenic mice, while expression of the mouse SP-B gene was shut down by the insertion of a Neomycin resistant gene into SP-B exon 4. These mice were named as KO (hSP-B -/mSP-B -). Next, an F₁ generation of hTG mice (hSP-B +/mSP-B +) was generated by the mating of these conditional human SP-B transgenic mice with hSP-B positive transgenic founders. Positive mice in the F₁ generation were
then bred with KO mice to generate a line of F₂ positive mice (hSP-B+/mSP-B-).

Summary of this breeding process can be seen below in Figure 1:

**Figure 1: hSP-B Transgenic Mouse Model**

Generation of hSP-B hTG mice began by the cross breeding of a conditional transgenic mouse (containing neither gene) with a WT mouse which had hSP-B gene cloned into its genome. The resulting F1 generation was crossbred once again with KO mice to form an F2 generation. Mice with only the hSP-B gene were selected from this group and cross bred to generate hSP-B mouse line (F3).
Genotype analysis was then conducted via PCR to amplify fragments of both the mSP-B and hSP-B. hSP-B was amplified with primer pair 1458/1401 locating on the SP-C promoter and the 3’ end of SP-B cDNA respectively. Amplification of this gene yields a PCR product of 577bp. Conversely, mSP-B was amplified with primers 75 and 76 which locate the 3’ end of intron 3 and the 5’ end of intron 4 respectively. Amplification of this gene leaves a product that is 279 bp. Humanized SP-B mice showing only the hSP-B band (577 bp) were selected out of this F2 generation and used for this study (Figure 2).

![PCR gel image](image)

**Figure 2** Genotype of hSP-B Transgenic Mice. The transgenic mice were genotyped by amplification of both human SP-B and mouse SP-B fragment by PCR with genomic DNA extracted from mouse tails (8-12 weeks). hSP-B yields a PCR product of 577bp. mSP-B, which was also amplified, yields wild type mSP-B product of 279bp. The SP-B fragment in conditional transgenic mice (named as KO mice in this study) cannot be amplified because a neomycin resistance gene (1.8kb) was inserted into exon 4 of their SP-B gene. The mice expressing hSP-B only (lane 1,2,4-6,8) were defined as F2. Mice expressing both hSP-B and mSP-B (lane 7) were defined as F1. The mice expressing mSP-B only (lane 9,10) were defined as WT while the mice expressing neither hSP-B nor mSP-B (lane 3) were defined as KO. The recombinant plasmid was used as positive control and the KO mouse genomic DNA was used as negative control.
Genetic sequencing of these mice (labeled F3) was conducted to detect the presence of a single-nucleotide polymorphism (SNP) in SP-B gene exon 4 position 1580 (Figure 3A). The T/C allele variation changes amino acid 131, causing a change from threonine to isoleucine (Thr\textsuperscript{131}-Ile). This change eliminates an N-linked glycosylation site, Asn\textsuperscript{129}-Gln-Thr\textsuperscript{131}, present in the C allele (hSP-B-C) but not the wild type T allele (hSP-B-T). It has been reported that the C allele is associated with increased risk of acute respiratory distress syndrome (ARDS) and respiratory failure in adults [7-8]. Samples were sent from each founder strain to the University’s Core facility for sequencing analysis. The data reported that three of the 4 founders (2004, 2007, 2059) carried the T allele while founder 2012 carried the lone C allele (Figure 3B).

**Figure 3A**

![Image of genetic sequencing results]

**Figure 3B**

<table>
<thead>
<tr>
<th>Founder</th>
<th>Allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004</td>
<td>T</td>
</tr>
<tr>
<td>2007</td>
<td>T</td>
</tr>
<tr>
<td>2012</td>
<td>C</td>
</tr>
<tr>
<td>2059</td>
<td>T</td>
</tr>
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</table>

**Figure 3  Genotype of hTG Mouse Founder Strains**

Genetic sequencing performed to detect T/C allele variation of hTG mice strains. A SNP in exon 4 position 1580 of SP-B gene (Figure 3A) causes a change from threonine to isoleucine (Thr\textsuperscript{131}-Ile). Determination of allele variation was conducted for each of the four Founder strains, and is shown in Figure 3B. Only the 2012 strain was C allele while the other three strains were T allele.
2. **Comparison of alveolar size in hSP-B-C and hSP-B-T transgenic mice.**

To compare the relative size of alveoli between humanized transgenic mice, lung tissue was harvested from each of the four founder strain (8-12 weeks). Samples were fixed by 10% formalin solution at about 25 cm of water pressure and then processed into paraffin blocks. From these blocks, 5 μm thick sections were cut, placed on microscope slides, and stained by hematoxylin and eosin staining kits. Relative size of alveoli was estimated using the Delesse Method in which microscopic images of alveoli cells were superimposed on a grid. From this grid, the number of line intersections (hits) within each alveoli was counted and averaged for each founder strain thus estimating relative differences in total size the alveoli themselves. All images were taken when viewed at 20x magnification on a Nikon Eclipse TE2000-U microscope and overlain on the same grid. For each founder, 50 alveoli from two separate mice were sampled and used for calculation. The results (Figure 4) indicate a sizable difference in the relative size of alveoli between hSP-B-C and hSP-B-T transgenic mice.

![Figure 4. Relative Size of Alveoli in hSP Mice using the Delesse Method](image-url)

*Figure 4. Relative Size of Alveoli in hSP Mice using the Delesse Method.* To determine relative alveolar size between hTG mice variants (hSP-B-C/+) microscope slides were prepared from the lung tissue of each founder strain (previously identified by allele variation type). The 2012 founder strain (C-allele) recorded a significantly larger average alveolar size compared to all other T-allele strains (2004, 2007, 2059).
3. **Western Blot Analysis**

SP-B gene codes a 381 amino acid long precursor (pro-SP-B) that has a molecular mass of 42 kDa. This proSP-B undergoes extensive post-translational processing (See Figure 5) to yield mature SP-B (region comprising 201-279) which has a molecular mass of 8 kDa and is found in broncho-alveolar lavage material.

![Western Blot Analysis Diagram](image-url)

**Figure 5  Partial SP-B Processing**

Human SP-B gene encodes a 381 amino-acid precursor. Proposed N-linked glycosylation sites are located at residues 311 (for both C/T allele variants) and 129 (C allele variant only). The signal peptide is comprised of the first 23 residues of the N-terminal sequence. Mature SP-B is the region comprising residues 201-279. Following removal of the signal peptide, proSP-B is cleaved at residue 185. The C-terminal fragment is approximately 25 kDa (still containing the N-linked glycosylation). The N-terminal fragment is approximately 17 kDa in the T-allele variant and does not contain any N-linked glycosylation. The same fragment in the C-allele variant is approximately 21 kDa and contains the N-linked glycosylation at residue 131.
Processing of proSP-B includes cleavage of signal peptides, glycosylation of C-terminus, and cleavage of N and C terminal propeptides [9]. Pepsinogen C is an aspartyl protease that has been identified as an important player in this post-translational modification by cleaving proSP-B at Met$^{302}$ and Ser$^{197}$ [10]. Western Blot analysis of BAL fluid was conducted to determine the extent of hSP-B gene expression in each of the four strains of hTG mice. BAL fluid and lung tissue were prepared from each founder and compared to the BAL fluid from a healthy adult which was used as a positive control. Human SP-B gene was identified by use of 3 antibodies: Alpha-pig antibody, Antibody 1, and Antibody 2. Alpha-pig is a commercially purchased identification antibody that detects mature SP-B. Non-reducing gel for was used for these samples as it allowed for better detection. As a result, mature SP-B presented as a dimer and showed a band at 16 kDa instead of its actual molecular mass of 8 kDa. Antibodies 1 and 2 are used to detect proSP-B and fragments of partially processed SP-B (42, 28 kDa). Our results indicate that human SP-B was successfully integrated and expressed in hTG mice at levels sufficient for survival (see figure 6-8). Bands of known molecular mass, corresponding to regions in proSP-B and mature SP-B, were seen in both human BAL fluid samples as well as in each hTG mouse strain. Quantification of hSP-B expression was then conducted using Quantity One software (version 4.6.1). Results of this analysis indicate that hSP-B was expressed in both variants (C/T) of hTG mice in comparable levels to that found in human BAL fluid (see Figure 9).
**Figures 6-8 Western Blot Analysis**
hSP-B expression in hTG mice was confirmed through western blot testing of BALF samples taken from each of the four Founder strains (2004, 2007, 2012, 2059). 20µg of total protein from F2 mice of 4 different founders was subjected to 12% SDS-PAGE under non-reducing condition. A human BALF was used as a positive control Gels were subjected to one of four different antibodies used for identification. Figures 6 shows both sham and infected (pneumonia) mouse BALF detected with AB1 which identifies proSP-B (42 kDa). Figure 7 shows the same samples when detected by AB2 which also identifies portions of proSP-B (28 kDa). Figure 8 shows the dimer (a 16kD band) of mature hSP-B after being detected with Alpha-pig antibody These results indicate all four of founders can express sufficient hSP-B protein for survival.
Figure 9: Human SP-B Expression In hTG Mice
Quantification of the hSP-B expression was performed using Quantity One software. Results indicate that hSP-B was expressed in the four Founder hTG mice in comparable levels to that of the human sample (positive control). Western blots identified with the three antibodies used during this experiment (AB1, AB2, Alpha-pig) were quantified. Both of bands of interest pertaining to AB2 detection (28,42 kDa) were able to be quantified.

4. Lipid Concentration
Total lipid concentration was determined from BAL fluid samples from both variant strains of hTG mice (hSP-B-C/T) and compared to that of BAL fluid taken from a healthy human. Samples were diluted to 1:10 by PBS and run through a Multiskan Ascent V1.24. Samples were compared to albumin which served as the calibration blank. Results (Figure 11) showed that the T variant (2007) showed comparable lipid concentration levels (0.0708 ± 0.00203 mg/ml) to that of the human sample (0.054
mg/ml) while the C variant (2012) showed significantly higher lipid concentration (0.13825 ± 0.00256 mg/ml).

![Graph showing lipid concentration](image)

**Figure 10:** Lipid Concentration in BALF samples of hTG

Lipid concentration was determined for each of the two allele variants (hSP-B-C/T). Samples were diluted with PBS and run through a Multiskan Ascent V1.24 with comparison to albumin (calibration blank). The T-allele variant (2007) showed comparable lipid concentration to that of human BALF while the C-allele (2012) recorded a noticeably larger average concentration.

**Discussion**

Surfactant Protein B (SP-B), a lipid protein complex, plays a critical role in the lung’s physiological function and structural integrity by reducing alveolar surface tension. A single polymorphism SP-B (1580 C/T) causes the change of amino acid 131 from threonine to isoleucine (Thr^{131}-Ile). This change alters N-linked glycosylation which may
in turn alter SP-B precursor (proSP-B) post-translational processing and function. This glycosylation, which occurs at Asn\(^{129}\), is present in the C-allele variant (hSP-B-C) but is absent in the T-allele variant (hSP-B-T). It has been reported that the C-allele is associated with increased risk of ARDS and other respiratory conditions in adults.

Generation of an hTG mouse line, containing hSP-B without background mSP-B, began by the cloning of human sftpb cDNA into a suitable vector and initiated by a human promoter. A DNA fragment of the recombinant plasmid was microinjected into fertilized WT mouse oocytes. These mice, now containing both the mouse and human SP-B gene were bred with conditional transgenic mice (KO) to remove the mSP-B background (Figure 1). SP-B fragment in the KO mice could not be expressed due to a neomycin resistance gene that was inserted into their SP-B gene. Mouse tail samples were collected from these transgenic mice and genotyped along with positive and negative controls by amplification of both human SP-B and mouse SP-B fragments via PCR (Figure 2). Mice that showed the hSP-B gene without mSP-B background (hSP-B+/mSP-B-) were selected and used in this study. Of this group, four Founder strains were selected (2004, 2007, 2012, 2059) and subjected to genetic sequencing. This sequencing was performed to identify the C/T allele variant type of each Founder strain. Only the 2012 strain showed the C-allele while the other three strains exhibited the T-allele (Figure 3). After identifying the gene in the four Founder strains, attention shifted to characterization of hSP-B in these hTG mice. Comparison of alveolar size was conducted via the Delesse Method. Results (Figure 4) indicated that the 2012 C-allele variants have significantly larger alveoli (26.98±1.227 hits/alveoli) than any of the T-allele variant strains. Western Blot Analysis was performed to identify expression of the hSP-B in the BALF of hTG
Founder strains. SP-B gene codes a 381 amino-acid precursor that undergoes extensive post-translational modification (Figure 5). Three antibodies (AB1, AB2, Alpha-pig) were used for detection of both pro-SP-B and mature SP-B fragments. Results show that hSP-B was expressed in all of the founder strains at levels sufficient for survival (Figures 6-9). To compare SP-B expression levels with those found in humans, quantification of the western blot bands was performed. Scans of the western blot gels were subjected to analysis by Quantity One software. Results of the quantification (Figure 10) indicate that hSP-B was expressed in the four Founder hTG mice in comparable levels to that of the human sample (positive control). BALF samples from each variant type (C/T) were then subjected to lipid concentration testing and compared BALF from a healthy human. Albumin was used as the calibration blank. Results indicate that the 2012 C-allele variant exhibited significantly higher lipid concentration than either the T-allele variant or human sample. The T-allele variant and the human sample exhibited similar concentration levels (Figure 11).

**Conclusion**

Humanized *sftpb* transgenic founder lines carrying the human SP-B C allele or T allele were generated and showed normal phenotypes as WT mice. The level of hSP-B protein expression in the lungs of these transgenic mice is comparable with that in the human lung indicating hSP-B is functional within these founder lines. Characterization of the two hSP-B allele hTG strains revealed significant differences in their relative alveolar size and total lipid concentration. These differences should help drive the future research needed to ascertain the specific nature of the C-allele’s negative effect on the host’s
ability to fight respiratory diseases. Since this mutation is common in the human population, the ability to identify patients containing this C-allele variant could lead to modified treatment and an increased success rate in fighting their respiratory disease.
Works Cited


