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Hyperpolarized ^{3}He diffusion MRI and histology of secreted frizzled related protein-1 (SFRP1) deficient lungs in a Murine model

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ABSTRACT

Secreted frizzled related protein-1 (SFRP1) plays a key role in many diverse processes, including embryogenesis, tissue repair, bone formation, and tumor genesis. Previous studies have shown the effects of the SFRP1 gene on lung development using the SFRP1 knockout mouse model via histological and physiological studies. In this study, the feasibility of ADC (acquired via HP ^{3}He) to detect altered lung structure in the SFRP1 knockout (SFRP1$^{-/-}$) mice was investigated, and compared to analysis by histology. This study consisted of two groups, the wild-type (WT) mice and the knockout (KO) mice with $n = 6$ mice for each group. ^{3}He ADC MRI and histology were performed on all of the animals. The global L_m values of WT and KO mice were 35.0 ± 0.8 μm and 38.4 ± 3.8 μm, respectively, which translated to an increase of 9.58% in the L_m of KO mice. The mean global ADCs for the WT and KO mice were 0.12 ± 0.01 cm^2/s and 0.13 ± 0.01 cm^2/s, respectively, which equated to a relative increase of 8.0% in the KO mice compared to the WT mice. In the sub-analysis of the anterior, medial and posterior lung regions, L_m increased by 10.50%, 6.66% and 11.84% in the KO mice, respectively, whereas the differences in ADC between the two groups in the anterior, medial, and posterior regions were 7.3%, 8.3%, and 4.6%, respectively. These results suggest that HP MRI measurements can be used as a suitable substitute for histology to obtain valuable information about lung geometry non-invasively. This technique is also advantageous as regional measurements can be performed, which can identify lung destruction more precisely. Most importantly, this approach extends far beyond the specific pathology analyzed in this study, as it can be applied to many other pathological conditions in the lung tissue, as well to many other embryonic studies.

1. Introduction

Secreted frizzled related protein-1 (SFRP1) is a member of a family of secreted proteins that contain a cysteine-rich domain (CRD) analogous to the one found in the WNT binding region of the frizzled family of transmembrane receptors. It has been shown that SFRP1 acts as an antagonist to the WNT pathway by competing with the frizzled receptor for binding sites on WNT proteins. The WNT signaling pathway and its associated proteins play a key role in many diverse processes, including embryogenesis, tissue repair, bone formation, and tumor genesis.

Elucidating the mechanism of the SFRP family on the WNT pathway is an ongoing process. Previous work has suggested that SFRP1 acts as a tumor suppressor protein in various types of cancers, including breast cancer, colorectal tumors and pleural mesothelioma [1–3]. More recently, SFRP1 was demonstrated to play an important role in lung morphogenesis during embryogenesis. In the normal lung, SFRP1 is tightly expressed during early stages of development concomitant with the expression of WNT5A and WNT10B, resulting in healthy lung parenchyma and normal alveoli size. Knockout mouse studies demonstrate that blocking the expression of SFRP1 results in abnormally dilated alveoli with structural changes similar to what is seen in emphysematous tissue of adult lung parenchyma [4].

Although SFRP1$^{-/-}$ knockout mice exhibit increased alveoli size similar to that observed in emphysematous tissue, there are several important differences between the two conditions. Firstly, although both conditions exhibit abnormally large alveoli, in emphysema SFRP1 expression is increased, contrary to what is observed in the knockout. Secondly, while both emphysema and loss of SFRP1 affect the lung fairly heterogeneously, the change in alveoli size due to SFRP1 deficiency is more heterogeneous and is greater in the periphery of the lung. Lastly, unlike emphysema, loss of SFRP1 does not result in an

Abbreviations: SFRP1, secreted frizzled receptor protein-1; HP, HYPERPOLARIZED; L_m, mean linear intercept; WT, wild-type; KO, knockout.

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ongoing destructive process, since the alveoli do not continue to enlarge over time. Consequently, the compliance of the lungs is not altered in the lungs of SFRP1$^{-/-}$ mice after development [4].

Previous studies have primarily relied on histology to identify structural changes in animal models, both during development and adulthood. Histology is widely used to obtain anatomical measurements of both large-scale changes, such as alveoli size, as well as smaller scale changes, such as those that occur in the lung epithelium and parenchyma. However, using histology to obtain these data requires the animal to be sacrificed in the process. This approach becomes especially problematic if one is studying a developmental animal model longitudinally, or developmental abnormalities clinically. Hyperpolarized (HP) ^{3}He MRI provides an alternative to histology; it does not require animal sacrifice, and can provide both structural and functional data on the lungs.

Hyperpolarized gases have been used previously to study structural and functional changes in lung tissue in human and animal species. Human pathologies studied include several disorders, such as, emphysema, asthma and COPD [5–7]. Animal models studied include rat and mouse models of elastase-induced emphysema [8,9]. The simplest method to probe lung structure utilizes the apparent diffusion coefficient (ADC) of inhaled HP ^{3}He, acquired by using diffusion-weighted magnetic resonance imaging (DW-MRI). An increase in alveolar size correlates with elevated ^{3}He ADC values. This increase is caused by the diminished restriction of gas diffusion in larger airspaces due to the disease process [10]. Similarly, HP gas MRI can also provide functional information such as regional ventilation and regional alveolar partial pressure maps of lungs [11,12].

Other alternatives to histological measurements for studying structural and functional changes in the lung include CT and PET. However, both of these methods suffer from their own shortcomings: the former requires ionizing radiation, and the latter requires a radionuclide. In contrast, HP ^{3}He MRI is a safe, non-invasive, non-ionizing technique that only requires the inhalation of an inert gas. As such, HP ^{3}He MRI is potentially a very useful tool for longitudinal studies as well.

Previous studies have shown the effects of the SFRP1 gene on lung development using the SFRP1 knockout mouse model via histological and physiological studies [4]. In the present study, the feasibility of ADC (acquired via HP ^{3}He) to detect altered lung structure in the SFRP1 knockout (SFRP1$^{-/-}$) mice was investigated, and compared to analysis by histology.

2. Methods

2.1. Mouse model

This study was performed in accordance with protocols approved by the respective Institutional Animal Care and Use Committees of the University of Pennsylvania and Columbia University. The mice were divided into a control ($n = 6$) and a knockout (SFRP1$^{-/-}$) group ($n = 5$). The SFRP1$^{-/-}$ mouse model was generated using the protocol by Bodine et al. [13]. In brief, exon 1 of the SFRP1 gene was deleted because it encodes the majority of the functional protein. This exon was replaced with a LacZ/MC1-Neo gene to ensure that the SFRP1 promoter activity could be monitored with β-galactosidase expression in SFRP1$^{-/-}$ mice. The control mice (SFRP1$^{+/+}$) carried the wild-type (+/+) allele; the knockout (SFRP1$^{-/-}$) mice lacked this allele and contained the LacZ gene.

2.2. Mechanical ventilation

The mice were sedated with 100 mg/kg intraperitoneal ketamine and 10 mg/kg xylazine. This dosage was repeated every 60–90 min or if any movement was detected. They were then tracheotomized with a 1.5 mm endotracheal tube for intubation with a custom-designed, MRI-compatible, small animal ventilator equipped with real time monitoring of peak inspiration pressure (PIP) with an accuracy of 100 μL. A gas mixture of HP ^{3}He and oxygen in a 4:1 ratio was used for imaging. The heart rate and oxygen saturation were constantly monitored using a veterinary pulse-oximeter (Vet/Ox, Heska, Loveland, CO) with an optical probe attached to the hind foot. The temperature was monitored using a rectal probe and was maintained at 37 °C by a flow of warm air through the bore of the magnet.

2.3. Helium hyperpolarization

The helium gas used for imaging was hyperpolarized using spin exchange collisions with optically pumped rubidium (Rb) atoms, as described [14] using a commercial prototype polarizer (IGI.9600.He, GE Healthcare, Durham, NC). The gas was polarized to approximately 30% over a period of 14 hours prior to use. During the imaging session, the HP ^{3}He was stored in a 400 mL Tedlar bag (Jensen Inert Products, Coral Springs, FA) that was placed inside the magnet's bore. The T_1 relaxation time constant of this gas was in the order of 30–40 min inside the bag. The ^{3}He was mixed with O_2 in a 4:1 ratio and delivered to the subjects at 100 BMP with $I/E = 1{:}2$ and $V_T = 1.2$ mL/100 g body mass.

2.4. MR imaging and parameters

Imaging was performed in a 50-cm horizontal bore, 4.7 T MRI scanner (Varian, Palo Alto, CA) equipped with 3.8 cm, 25 G/cm gradients and an 8-leg bird-cage chest coil (Stark Contrast, Erlangen, Germany). The coil was tuned to the ^{3}He resonance frequency of 152.95 MHz. The animal was placed in the supine position in the coil and inserted into the bore of the magnet. Diffusion imaging was performed using a double acquisition diffusion-weighted gradient echo pulse sequence [15]. In the double acquisition method a single pair of gradients with inverted amplitudes is used to obtain two sets of images. The following acquisition parameters were used: b-values = 0.00 and 2.18 s/cm^2, field of view = 3x3 cm^2, slice thickness = 4 mm, flip angle (α) = ~8°, matrix size = 64 × 64 pixels, TR = 5.6 ms, and TE = 3.2 ms. Three coronal slices were acquired: anterior, middle (plane through which the trachea passes), and the posterior lung. The RF pulse width was calibrated to estimate the flip angle by acquiring a series of consecutive images during a 2-second single breath hold. Subsequently, series of 10 pre-wash breaths were administrated before the 11th breath hold of 3 seconds, during which the diffusion-weighted images were acquired.

2.5. Histology

Each animal was euthanized following the image acquisition using intravenous, high-concentration potassium cyanide. The drug was administered through the tail vein instead of the more commonly used intracardiac route to avoid damaging the lung with the needle. After euthanasia, the trachea and lungs were removed and filled with a 10% formalin solution at a pressure of 25 cm H_2O for 24 hours. The lungs were then paraffin embedded before being sectioned and stained with hematoxylin and eosin (H&E) [16]. A total of 10 5 μm-thick axial tissue slices (5-μm gap between slices) were cut for histology.

2.6. Data analysis

2.6.1. Histology analysis

The histology tissue slices were used to generate morphometric measurements, particularly the mean linear intercepts (L_m) for each subject. L_m is a measurement corresponding to the volume-to-surface-