Video Article

Protein Transfection of Mouse Lung

Patrick Geraghty[1], Robert Foronjy[1]

[1]Department of Medicine, St. Luke's Roosevelt Medical Center

Correspondence to: Robert Foronjy at rforonjy@chpnet.org

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Abstract

Increasing protein expression enables researchers to better understand the functional role of that protein in regulating key biological processes[1]. In the lung, this has been achieved typically through genetic approaches that utilize transgenic mice[2,3] or viral or non-viral vectors that elevate protein levels via increased gene expression[4]. Transgenic mice are costly and time-consuming to generate and the random insertion of a transgene or chronic gene expression can alter normal lung development and thus limit the utility of the model[5]. While conditional transgenics avert problems associated with chronic gene expression[6], the reverse tetracycline-controlled transactivator (rtTA) mice, which are used to generate conditional expression, develop spontaneous air space enlargement[7]. As with transgenics, the use of viral and non-viral vectors is expensive[8] and can provoke dose-dependent inflammatory responses that confound results[9] and hinder expression[10]. Moreover, the efficacy of repeated doses are limited by enhanced immune responses to the vector[11,12]. Researchers are developing adeno-associated viral (AAV) vectors that provoke less inflammation and have longer expression within the lung[13].

Using β-galactosidase, we present a method for rapidly and effectively increasing protein expression within the lung using a direct protein transfection technique. This protocol mixes a fixed amount of purified protein with 20 µl of a lipid-based transfection reagent (Pro-Ject, Pierce Bio) to allow penetration into the lung tissue itself. The liposomal protein mixture is then injected into the lungs of the mice via the trachea using a microsprayer (Penn Century, Philadelphia, PA). The microsprayer generates a fine plume of liquid aerosol throughout the lungs. Using the technique we have demonstrated uniform deposition of the injected protein throughout the airways and the alveoli of mice[14]. The lipid transfection technique allows the use of a small amount of protein to achieve effect. This limits the inflammatory response that otherwise would be provoked by high protein administration. Indeed, using this technique we published that we were able to significantly increase PP2A activity in the lung without affecting lung lavage cellularity[15]. Lung lavage cellularity taken 24 hr after challenge was comparable to controls (27±4 control vs. 31±5 albumin transfected; N=6 per group). Moreover, it increases protein levels without inducing lung developmental changes or architectural changes that can occur in transgenic models. However, the need for repeated administrations may make this technique less favorable for studies examining the effects of long-term increases in protein expression. This would be particularly true for proteins with short half-lives.

Video Link

The video component of this article can be found at http://www.jove.com/video/50080/

Protocol

1. Preparation of Protein Transfection Reagent

1. Dissolve the Pro-Ject Reagent by adding 250 µl of methanol or chloroform to the tube containing the dry film.
2. Vortex for 10-20 sec at top speed.
3. Pipette 20 µl of Pro-Ject reagent into separate microcentrifuge tubes.
4. Evaporate the solvent by placing the microcentrifuge tubes containing the Pro-Ject Reagent under a laminar flow hood for a minimum of 6 hr at room temperature. It must be completely dry. Alternatively, dry the Pro-Ject reagent using a vacuum desiccator.
5. Store the tubes at -20 °C. They are good for one year at this temperature.

2. Adding Protein to Pro-Ject Reagent

1. Pipette 50 µl of PBS, containing 2.0 µg of the protein of interest, into a tube containing 20 µl of dried Pro-Ject transfection reagent.
2. After adding the protein, mix the solution by vortexing at low speed for 3-5 sec followed by incubating for 30 min at room temperature.

3. Intratracheal Administration of Protein

1. Anesthetize mice by intraperitoneal injection of ketamine/xylazine (100 mg/kg and 10 mg/kg).

2. After sedation is achieved, mice are suspended from their upper incisors using a specially designed platform (**Figure 1**).
3. Using forceps and a paper clip as a laryngoscope, the oropharynx is opened so that the catheter can be introduced (**Figure 2**). The vocal cords are visualized by placing a light source against the trachea of the mouse.
4. The protein/ProJect reagent mixture (50 µl) is injected through the trachea using a Penn Century Microsprayer.

Representative Results

To demonstrate the effectiveness of our technique we used a microsprayer (Penn Century) to inject the trachea of mice with 2 µg of mouse albumin (Sigma) dissolved in 50 µl of PBS that contained 20 µl of Pro-Ject transfection reagent. The albumin treated mice were compared with mice that were treated in an identical manner with 2 µg of beta-galactosidase protein (Pierce Bio). After twenty-four hours, the mice were euthanized and the lungs were processed for histological analysis. Immunohistochemistry for beta-galactosidase was conducted on formalin fixed lung tissue sections from the albumin and beta-galactosidase treated mice. Even 24 hr after protein transfection, we detected intense staining within the airways (large arrows) of the beta-galactosidase treated mice but not the albumin treated mice (**Figure 3A** and **3B**).

Figure 1. To position the mice for the intratracheal injection, a wood platform was constructed that has a ramp at a 45 ° angle from its base[16]. The mice are suspended from their incisors by a metal wire that is attached to two metal hooks at the top of the ramp. The tongue is gently repositioned with forceps and paper clip laryngoscope is used to open the airway.

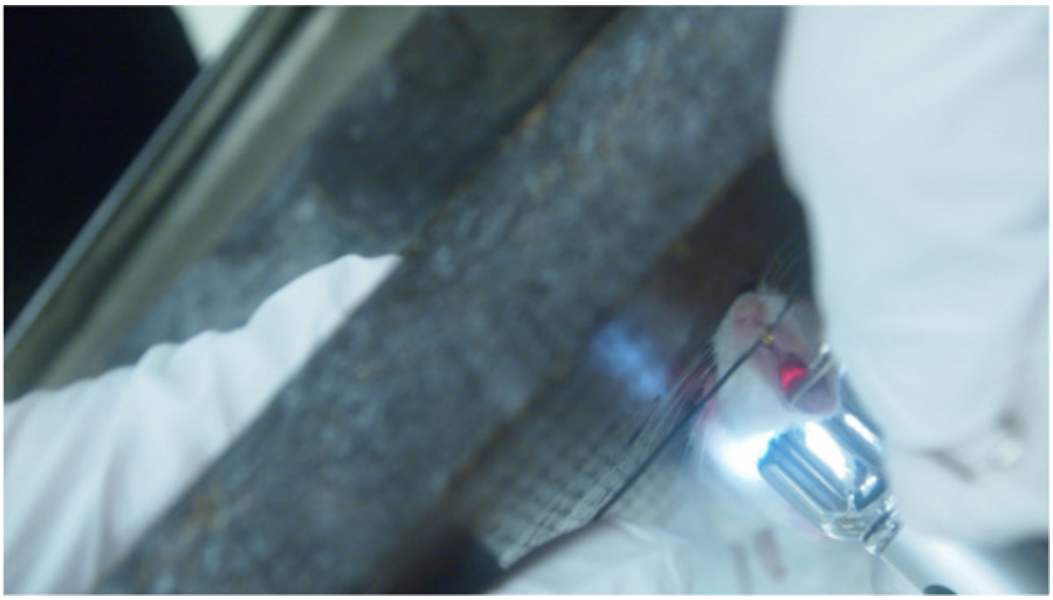

Figure 2. After placing a light source on the neck, the larynx can be visualized for intratracheal injection[16].

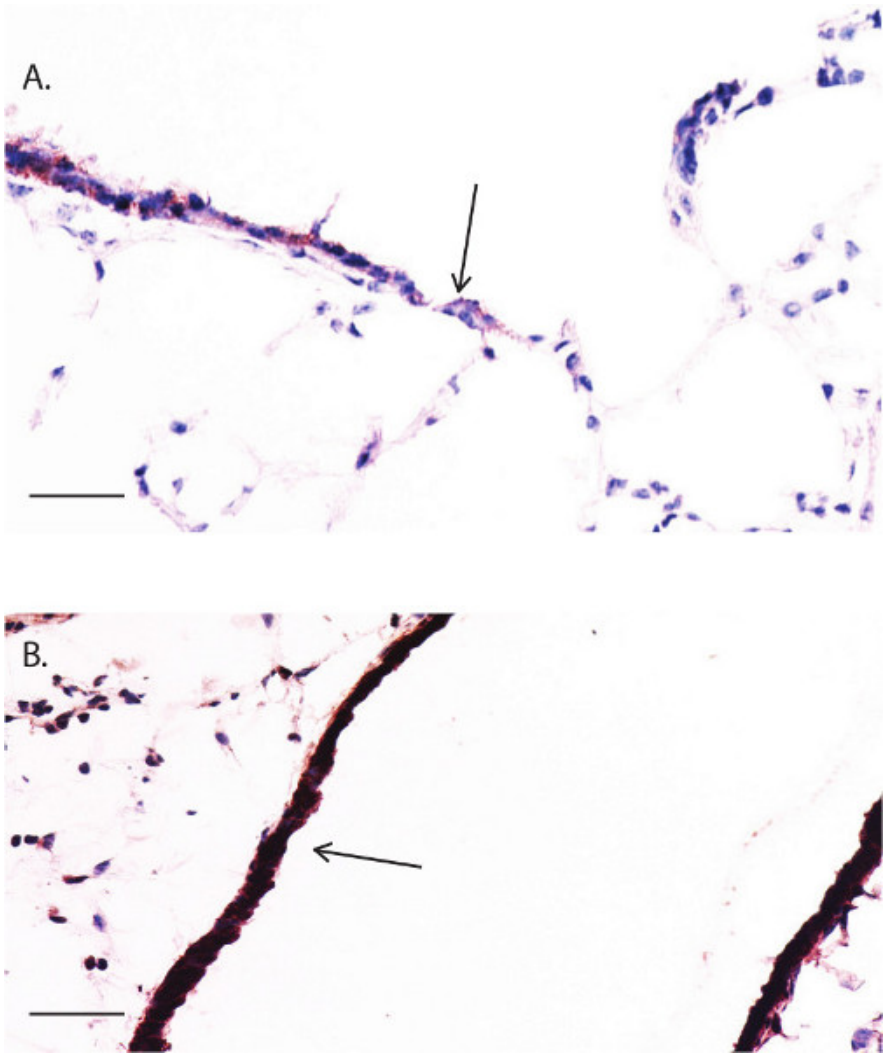

Figure 3. β-galactosidase immunostains were conducted on 4-µm lung sections from mice injected intratracheally with 2 µg of albumin (A) or β-galactosidase (B). Arrows indicate β-galactosidase staining within airways. Images are at 40X magnification. Scale bar=100 µm.

Discussion

The advantage of this technique over other methods is that it produces increases in protein levels and activity within the lung tissue itself. Moreover, it penetrates to the most distal regions of the lung as opposed to staying just within the airways. We have measured increased protein activity of our injectate even after lavaging the airways with saline[15]. Tissue activity analyses indicate that the protein is entering cells and does not just remain in the air spaces of the mouse. This was further confirmed by immunohistochemistry demonstrating diffuse staining of our injected protein within the alveoli and airways of the mouse (**Figure 3**). Importantly, this protocol does not provoke lung inflammation or protease expression. Thus, any changes that do occur can be ascribed to the effects of the administered protein. The lack of inflammatory response allows for the use of repeated injections to examine effects over a longer period of time.

Disclosures

The authors have no conflicts of interest to disclose.

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