UCLA David Geffen School of Medicine

Proteomics of the Pre-Operative Porcine Vocal Cord Via Tandem Mass Spectrometry

Anthony Chen[1], Yazeed Alhiyari, PhD[1], Kym Faull, PhD[2], Julian Whitelegge, PhD[2], Patrick Schlegel, PhD[1], and Jennifer Long, MD, PhD[1]

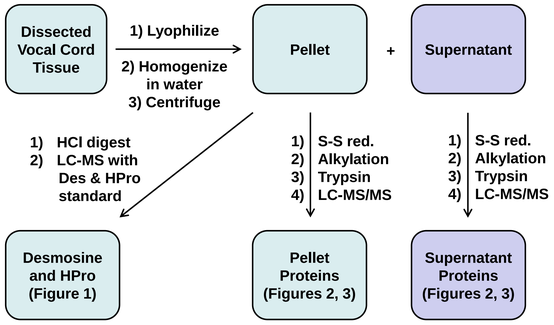
[1]Department of Head & Neck Surgery, David Geffen School of Medicine, University of California Los Angeles (UCLA)
[2]Pasarow Mass Spectrometry Laboratory, University of California, Los Angeles (UCLA)

Background

- Adipose-derived mesenchymal stem cell (ASC) implants are currently being studied developed as a potential therapy to restore normal voice following vocal cordectomy in patients with laryngeal cancer [1]. ASC implant studies are underway in the porcine model organism
- Proteomic study of the pig vocal cord allows for quantitation of protein composition pre- and post-cordectomy. Tandem MS/MS can be used to identify and quantitate up to thousands of proteins in a tissue sample simultaneously.

Methods

- Protocol and MS/MS performed by Alhiyari, Faull et al:



- Mascot tandem LC-MS/MS was used to identify proteins with associated emPAI [3] and Score data. Mol% of proteins were calculated following Mann et al [2].

Results

- In the right true vocal cord, the five most abundant proteins identified using MS/MS were **decorin** (50.6%), **vimentin** (9.7%), **fibrinogen** (8.5%), **histone H4** (1.2%), and **fructose-bisphosphate aldolase** (0.9%). Collagen and elastin should be highly abundant, but may not be detected due to low water solubility.
- Seven of the 15 most abundant proteins were shared between the left and right true vocal fold pellets.
- The true cord desmosine content ranged from 23-144 pmol/mg dry weight, and hydroxyproline content ranged from 23-26 nmol/mg dry weight.
- A minor amount of **Type VI collagen** (associated with basement membrane [4]) was identified in both left and right vocal fold pellets (0.2% and 0.3% respectively).

Results

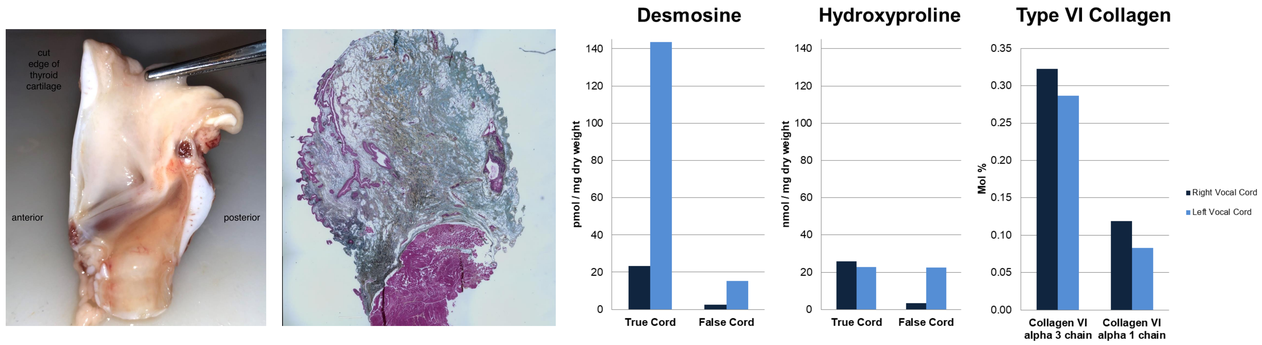


Figure 1) Left: Hemisection of pig larynx with true and false vocal cords. Center: Pentachrome stain of true vocal cord. Right: Desmosine (elastin amino acid) and hydroxyproline (collagen amino acid), collagen VI measured in pre-op vocal cords.

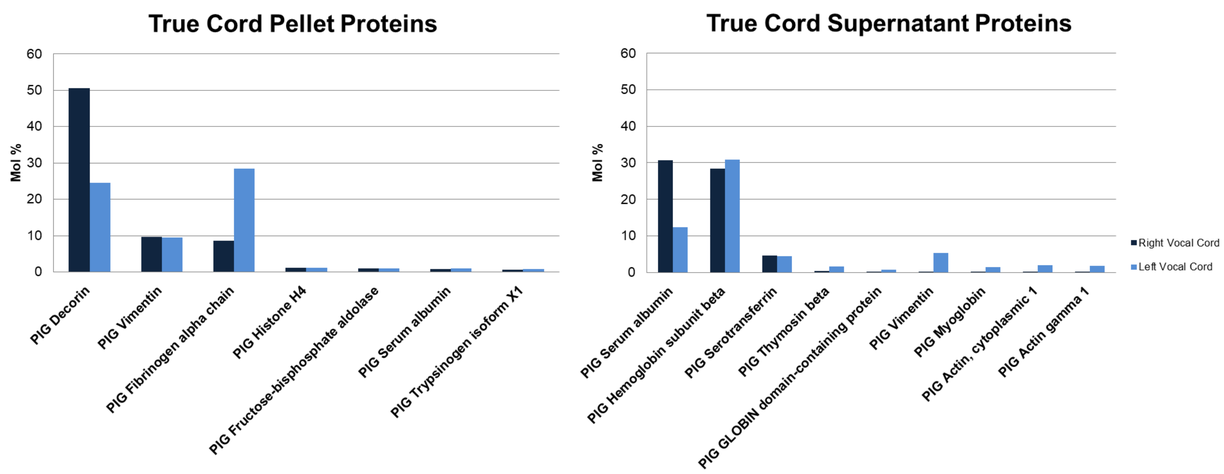


Figure 2) Proteins identified bilaterally within the right and left true vocal cord pellet and supernatant.

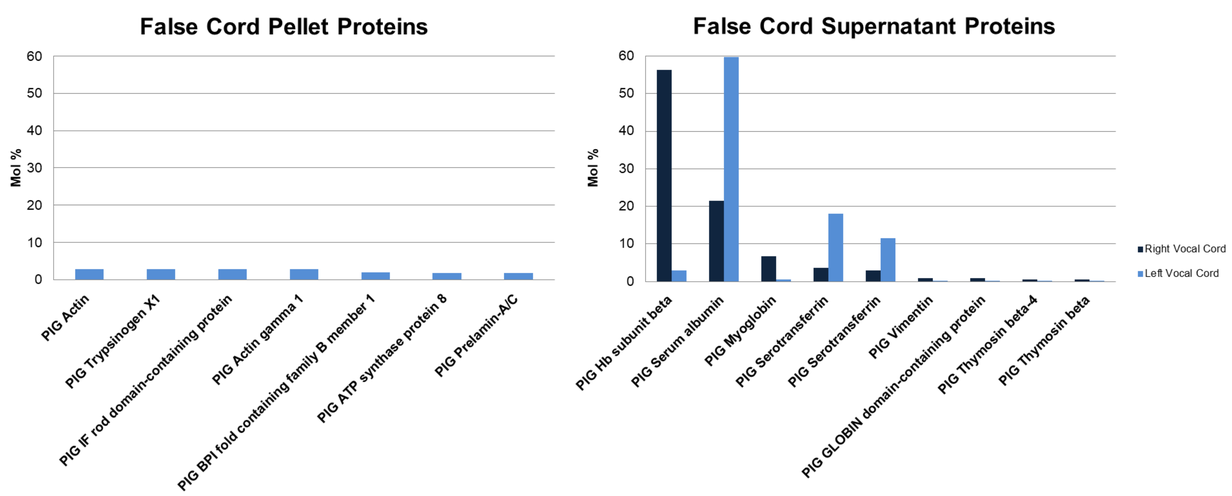


Figure 3) Proteins identified bilaterally within the left and right false vocal cord pellet (left cord only) and supernatant.

Discussion

- Desmosine and hydroxyproline are amino acids found in elastin and collagen respectively, and can be used to quantitate post-operative changes in the lamina propria [6].
- Decorin is a proteoglycan found in the extracellular matrix and binds to type 1 collagen to facilitate assembly of the ECM [5]. Vimentin is a major intermediate filament protein, which is found in fibroblasts, melanocytes, and endothelial cells [7].
- Differences between left and right vocal cord pellets could possibly be due to the area of resection (Figure 1). Histology of the vocal cord reveals variation in tissue distribution along both AP and superficial/deep axes.

Conclusion

- The most abundant proteins in the pig vocal cords were identified using tandem mass spectrometry for the first time, in addition to desmosine and hydroxyproline content as markers for elastin and collagen.
- A reasonable level of congruence in protein composition was observed between 2 sides of the same vocal cord. Future studies to evaluate variability in vocal cord protein abundance between pigs and to monitor changes post-cordectomy are underway.

References

1. Long, Jennifer L. "Repairing the Vibratory Vocal Fold." The Laryngoscope 128, no. 1 (January 2018): 153–59. https://doi.org/10.1002/lary.26801.
2. Rappsilber, Juri, Ursula Ryder, Angus I. Lamond, and Matthias Mann. "Large-Scale Proteomic Analysis of the Human Spliceosome." *Genome Research* 12, no. 8 (August 2002): 1231–45. https://doi.org/10.1101/gr.473902.
3. Ishihama, Yasushi, Yoshiya Oda, Tsuyoshi Tabata, Toshitaka Sato, Takeshi Nagasu, Juri Rappsilber, and Matthias Mann. "Exponentially Modified Protein Abundance Index (EmPAI) for Estimation of Absolute Protein Amount in Proteomics by the Number of Sequenced Peptides per Protein * S." *Molecular & Cellular Proteomics* 4, no. 9 (September 1, 2005): 1265–72. https://doi.org/10.1074/mcp.M500061-MCP200.
4. Sun, S., and M. A. Karsdal. "Chapter 6 - Type VI Collagen." In Biochemistry of Collagens, Laminins and Elastin, edited by Morten A. Karsdal, 49–55. Academic Press, 2016. https://doi.org/10.1016/B978-0-12-809847-9.00006-4.
5. Zhang, Wen, Yan Ge, Qian Cheng, Qi Zhang, Lin Fang, and Junnian Zheng. "Decorin Is a Pivotal Effector in the Extracellular Matrix and Tumour Microenvironment." *Oncotarget* 9, no. 4 (January 3, 2018): 5480–91. https://doi.org/10.18632/oncotarget.23869.
6. Hahn, Mariah S., James B. Kobler, Barry C. Starcher, Steven M. Zeitels, and Robert Langer. "Quantitative and Comparative Studies of the Vocal Fold Extracellular Matrix I: Elastic Fibers and Hyaluronic Acid." Annals of Otology, Rhinology & Laryngology 115, no. 2 (February 1, 2006): 156–64. https://doi.org/10.1177/000348940611500213.
7. "Vimentin - an Overview | ScienceDirect Topics." Accessed July 11, 2021. https://www.sciencedirect.com/topics/medicine-and-dentistry/vimentin.