Identifying If a Denervated Muscle is No Longer Receptive to Reinnervation: The Use of Spatial Transcriptomics

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INTRODUCTION:

Peripheral nerve injuries (PNI) can produce prolonged muscle denervation and functional decline secondary to sensory and motor deficits. Although surgical repair of nerves or nerve transfer surgery is performed to create the possibility of reinnervation, recovery remains unpredictable as some patients regain strength months or years later, others do not. Our group has recently shown that identification of motor endplate morphometry from muscle biopsies could be used to forecast which muscles will successfully reinnervate, we sought to identify distinct biomarkers as positive predictors for reinnervation so that we can improve surgical decision-making. In this study, we demonstrate the value of high-resolution spatial transcriptomics to analyze human muscles so as to characterize distinct denervation-associated gene signatures as a foundation for future reinnervation biomarkers.

METHODS:

Under IRB approval, patients consented to intra-operative biopsy of upper extremity muscles. Samples of a healthy innervated trapezius (control) and denervated deltoid (as confirmed by pre-operative EMG after nerve injury) were collected from the same patient during a standard-of-care orthopaedic procedure. Biopsies were flash-frozen in liquid nitrogen-cooled isopentane and processed on the 10× Genomics Visium HD spatial transcriptomics platform using spatial kits funded by NIA R21AG078909 (M.H. and R.G.). Data were normalized, reduced in dimensionality by principal component analysis (PCA), and visualized using UMAP. Percent-of-spots genetic expression was calculated across each sample for neuromuscular junction genes (CHRNA1 and MUSK) as well as myogenic markers (PAX7, MYOG, MYOD1, and MYF5).

RESULTS:

The Visium HD workflow captured 18,085 expressed genes across 198,100 spots in the healthy trapezium and 327,715 spots in the denervated deltoid. Library sequencing quality was high for both samples, with valid barcodes of 90% in the healthy trapezius and 91.2% in the denervated deltoid. Over 98% of spatial transcriptomics sequencing reads aligned confidently to the human reference genome. Using an 8 μm binning resolution, the healthy trapezius averaged 99.6 UMIs and 71.9 genes per bin. The denervated deltoid averaged 173.2 UMIs and 125.8 genes per bin. Twenty-six genetic clusters were identified in the healthy trapezius sample, and twenty-five clusters in the denervated deltoid sample (Figure 1). In the healthy trapezius, CHRNA1 and MUSK were detected in 0.16 percent and 0.02 percent of spots respectively (Figures 2 and 3); PAX7 in 0.01 percent, MYF5 in 0.016 percent, MYOG in 0.80 percent, and MYOD1 in 2.66 percent of spots. In the denervated deltoid, CHRNA1 and MUSK appeared in 25.96 percent and 6.09 percent of spots (Figures 2 and 3); PAX7 in 0.26 percent, MYF5 in 0.036 percent, MYOG in 7.42 percent, and MYOD1 in 0.80 percent of spots.

DISCUSSION AND CONCLUSION:

Our workflow, from intra-operative biopsy and tissue preservation to implementation of Visium HD spatial transcriptomics, produced high-quality spatial data and demonstrates feasibility in the clinical setting. Spatial transcriptomics can be applied to standard surgical muscle biopsies and yields high-resolution mapping of genes. With this reliable workflow establish, we plan to analyze larger cohorts and matched muscle types to map biological states and explore their relevance to muscle reinnervation. Spatial transcriptomics may provide the groundwork for determining predictive biomarkers of reinnervation success, with the potential to inform surgical decisions and personalize nerve repair strategies.

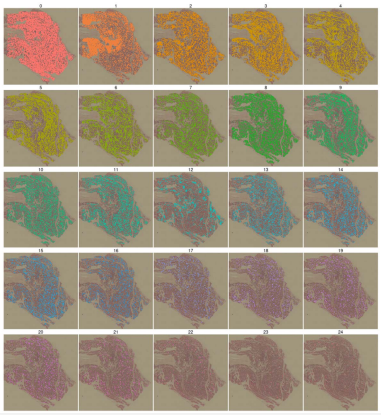

Figure 1 – Spatial Overlays of Each Unique Genetic Cluster of Denervated Deltoid Across the Tissue Sample

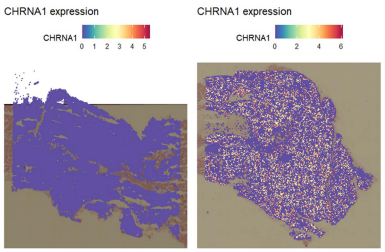

Figure 2 - CHRNA1 Expression Across Control (Left) and Denervated (Right) Samples

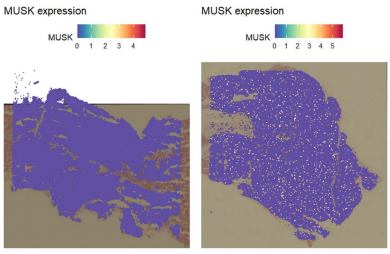

Figure 3 - MUSK Expression Across Control (Left) and Denervated (Right) Samples