Experimental (continued)

The suspicions were allowed to propagate for 8 hours prior to sub-sampling. An analytical aliquot was then diluted twenty fold into 0.1% NaCl for introduction to the ICP-MS. Samples were delivered to the ICP-MS via syringe pump at a 10uL/min flow rate. A total consumption nebulizer and spray chamber were utilized to maximize transport efficiency. The nebulizer and spray chamber gas flows were optimized to ensure the cells stayed intact during sample transport to the plasma.

Results

• High nebulization efficiency achieved

• Multi-element scan successful for enriched and non-enriched yeast cultures (Mn, Ga, Zn, Sr, Fe)

• Noticeable difference between enriched and non-enriched strains for both enriched and metabolic elements

Future Work

The ability to analyze single cell suspensions by ICP-MS will complement other advanced analytical techniques, while also minimizing sample preparation time and providing a simplified analytical workflow.

Duchenne muscular dystrophy (DMD) tissue samples immunostained with RPE will be prepared utilizing collagenase to remove cells from the tissue. These samples will be analyzed for RPE single-cell analysis by ICP-MS.

Inorganic Mass Spectrometry Analysis of Single Cell Protein Quantification: A Road Map

Introduction

Duchenne muscular dystrophy (DMD), a genetic disorder characterized by the progressive degeneration of muscle, affects approximately 1 in 4,000 male births worldwide. DMD is caused by a variety of mutations in the gene encoding dystrophin, a critical structural component of muscle fibers. In this work, we will highlight the utilization of rare earth metal-tagged antibodies (M-Ab) to localize and quantify differentially expressed proteins of the Dystrophin-glycoprotein complex.

Objectives

• Develop novel methods for protein quantitation in biological and clinical research samples

• Exploit the low abundance of rare-earth metal elements (REE) in biological systems

• Combine cutting edge laser capture and laser ablation methods with ICP-MS to achieve highly multiplexed single cell protein quantification

Approach

We are developing a novel technique to protein quantification exploiting the inherently low concentration of rare earth elements (REE) in biological systems. By coupling REE-antibody immunolabeling with laser capture microdissection (LCM) of single cells or laser ablation of tissue sections, and measurement by ICP-MS, we are achieving multiplexed protein measurement in histological sections and single cells.

Results and Discussion

LCM-ICP-MS/MS multiplexing across individual cells

High dimensional mass cytometry of mouse quadriceps with multiplex REE protein and DNA quantification.

Experimental Design

Figure 1: Traditional immunofluorescence versus heavy metal immunostaining

Figure 2: Workflow for single cell protein detection by LCM-ICP-MS/MS

Table 1: REE Labeling Reagents

Table 2: ICP-QQQ data from single, REE-labeled muscle fibers.

Figure 3: Laser capture microdissection of a single REE-immunolabeled muscle cell.

Figure 4: WT mouse quadriceps stained for dystrophin (slide 1) – (Slide 9)

Figure 5: High dimensional mass cytometry of mouse quadriceps with multiplex REE protein and DNA quantification.

Figure 6: WT mouse quadriceps stained for dystrophin

Figure 7: Time Scan of Se signal on same scale for both selenium enriched and non-enriched yeast cultures.