Prof. Mohamed L. Salem's visit to Mass Spectrometry Proteomic Core Facility Medical Univ. of South Carolina, USA on November 13, 2014

Lauren E. Ball, PhD; Assistant Professor

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Department of Pharmacology

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Charleston, SC, USA

http://academicdepartments.musc.edu/cohr/projects/ball.htm

http://academicdepartments.musc.edu/pharmacology/mass_spectrometry/

November

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A brief summary on the visit

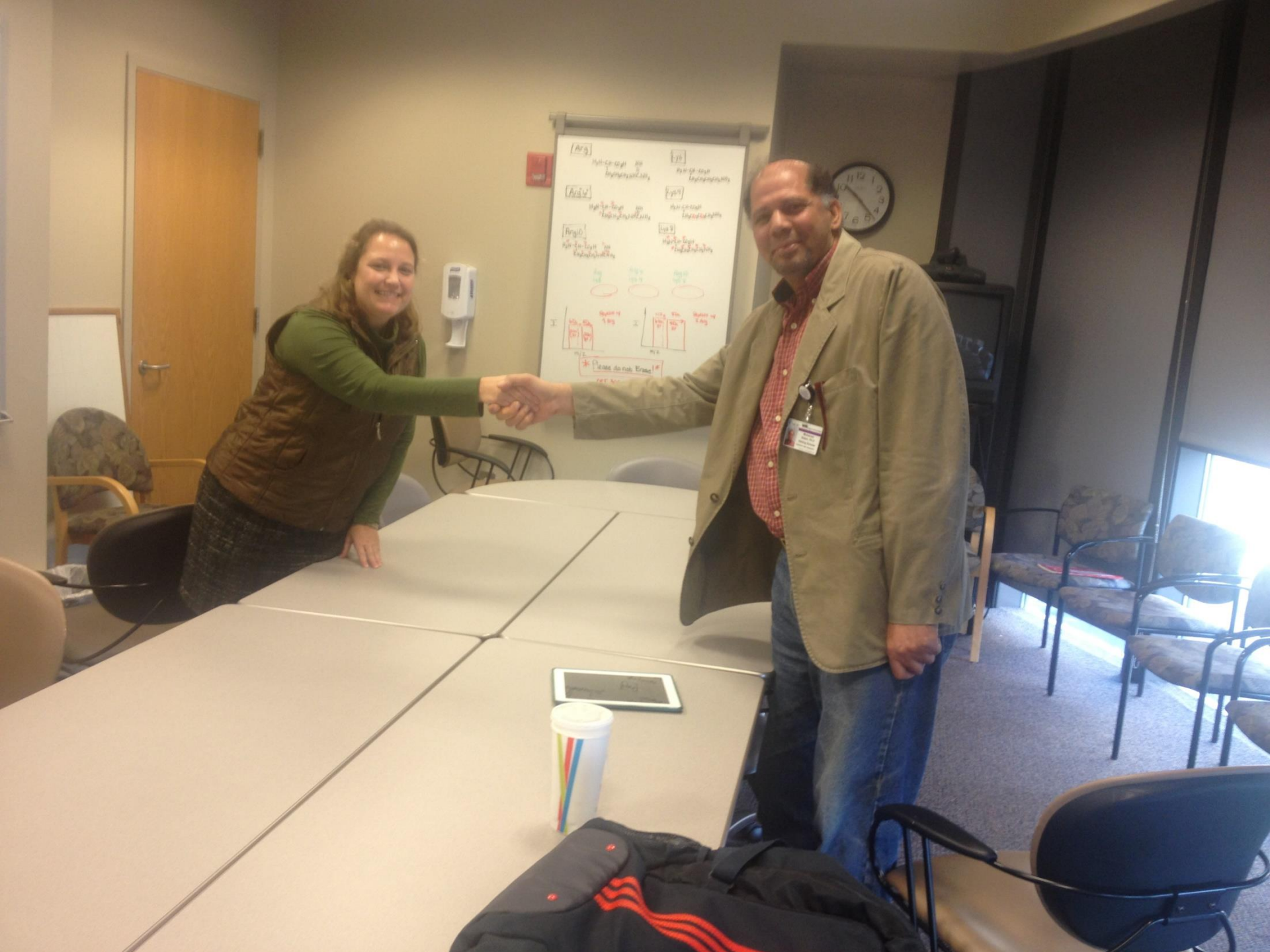
On November 12, Prof. Mohamed L. Salem, currently a visiting Prof. at Department of Microbiology and Immunology, College of Medicine, Medical University of South Carolina, USA visited the Mass Spec Proteomic core facility at Children's Research Institute, Medical Univ. of South Carolina, USA. Dr. Lauren E. Ball, the facility Director welcomed him and presented a brief presentation on the facility and her work on bone.

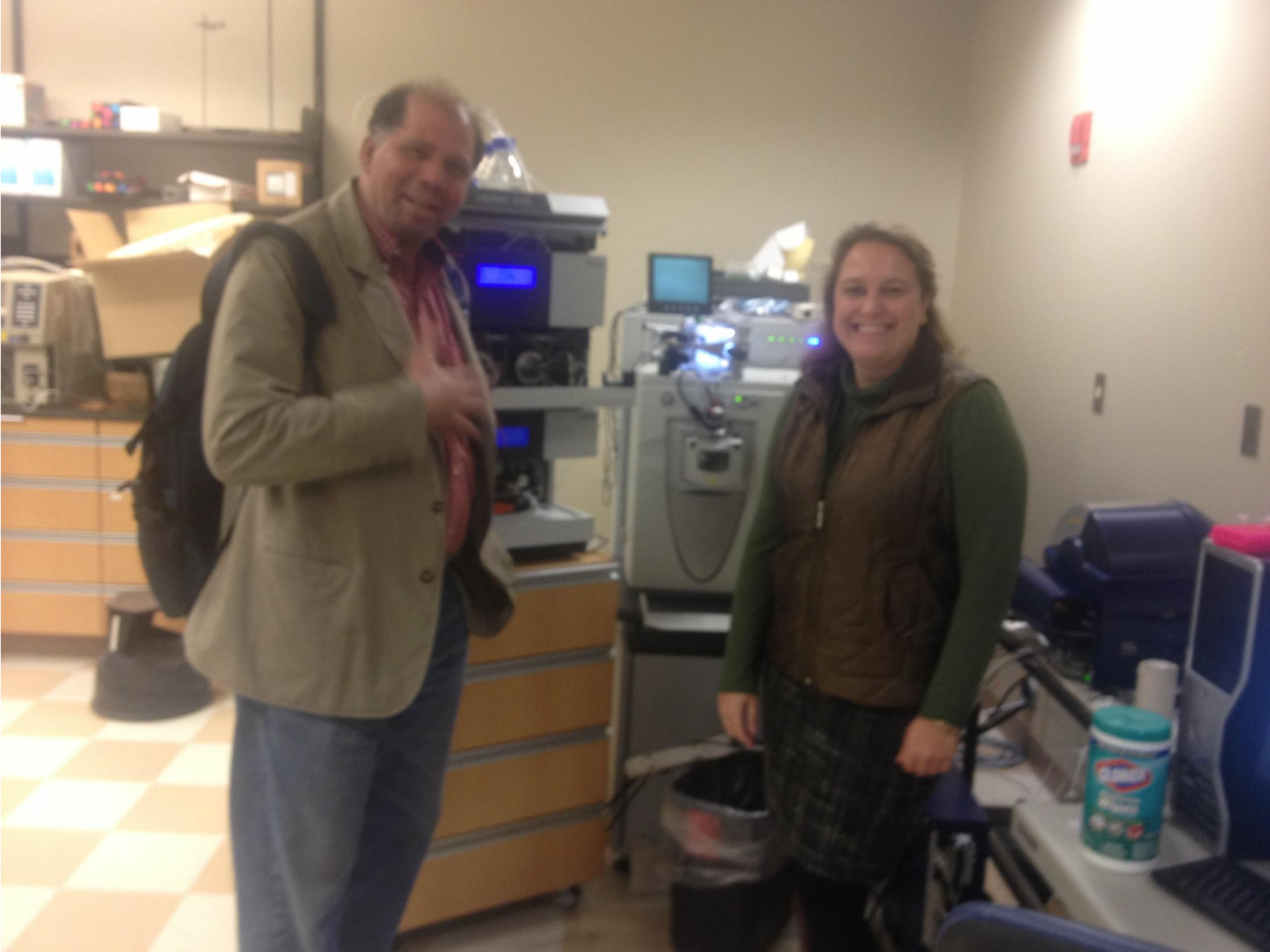
Prof. Salem also gave a brief presentation on Tanta University and Center of Excellence in Cancer Research and the proteomic array available at the center and expressed collaborative activities. Then Prof. Salem had a tour at the 10-million $ facility with the guidance of Dr. **Lauren and Dr. Hesham ElShewy, Assistant Prof. of Pharmacology and a collaborator.**

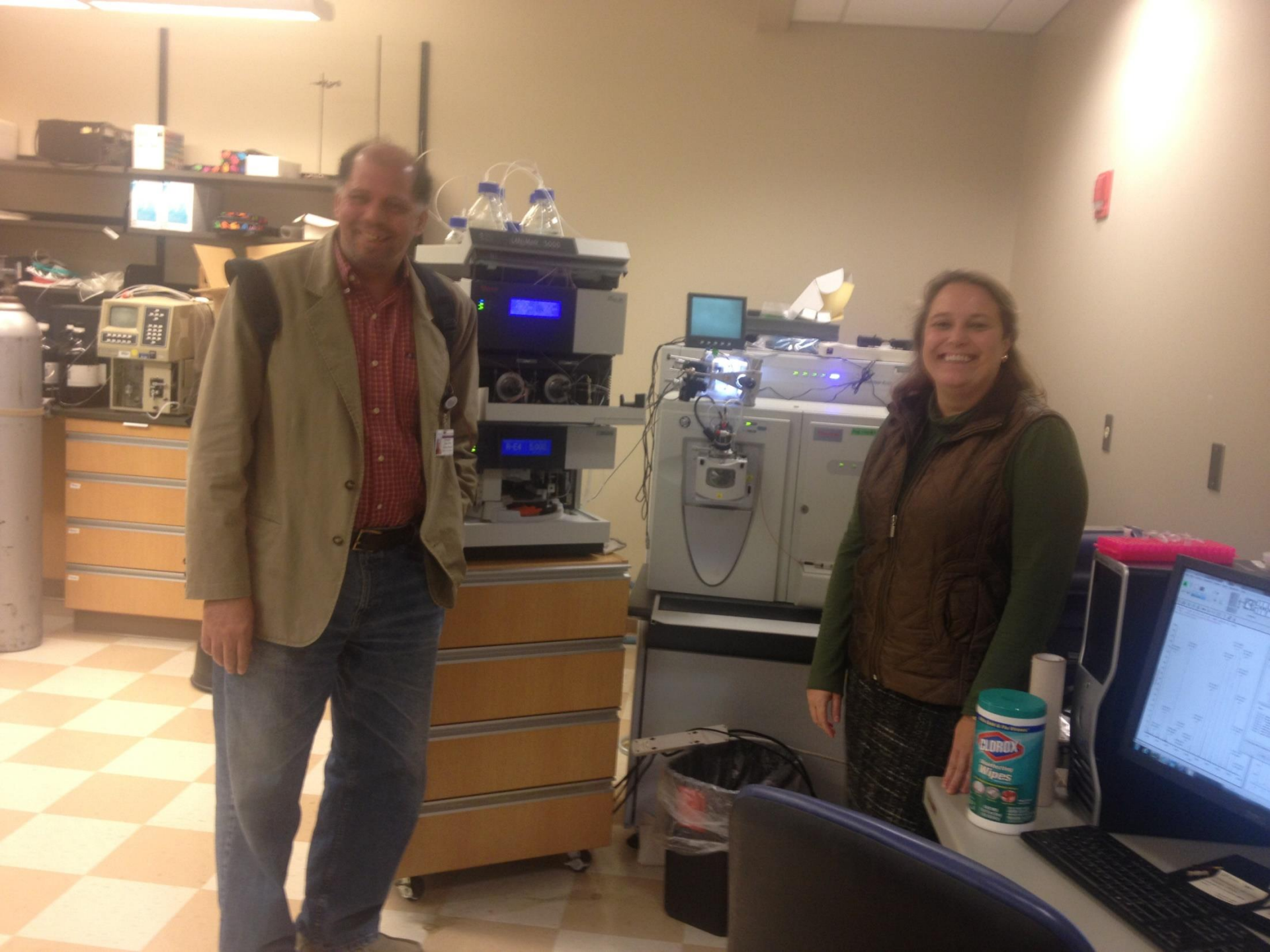
Dr. Lauren expressed her willingness to assess any efforts at Tanta to establish a similar facility as well as to run samples at a discounted rate for who is interested in phosphoproteomic mass spec analysis.

Mass Spectrometry Core Facility/MUSC

- Protein analysis: in-gel or in-solution protease digestion, chromatographic separation and tandem mass spectrometric analysis of the resulting peptides, and interpretation of MS/MS data using Sequest , Mascot, Protein Pilot, MaxQuant, and other search algorithms.
- Develop customized applications for: the isolation, detection and characterization of posttranslationally modified peptides (e.g. phosphorylation, glycosylation, oxidation, glutathionylation, and O-GlcNAc modification).
- Orbitrap Elite Mass Spectrometer couple quantitative approaches (SILAC, iTRAQ®, ICAT®, TMT®) to modification-specific experiments (*eg.,*phosphoproteomics, redox proteomics).



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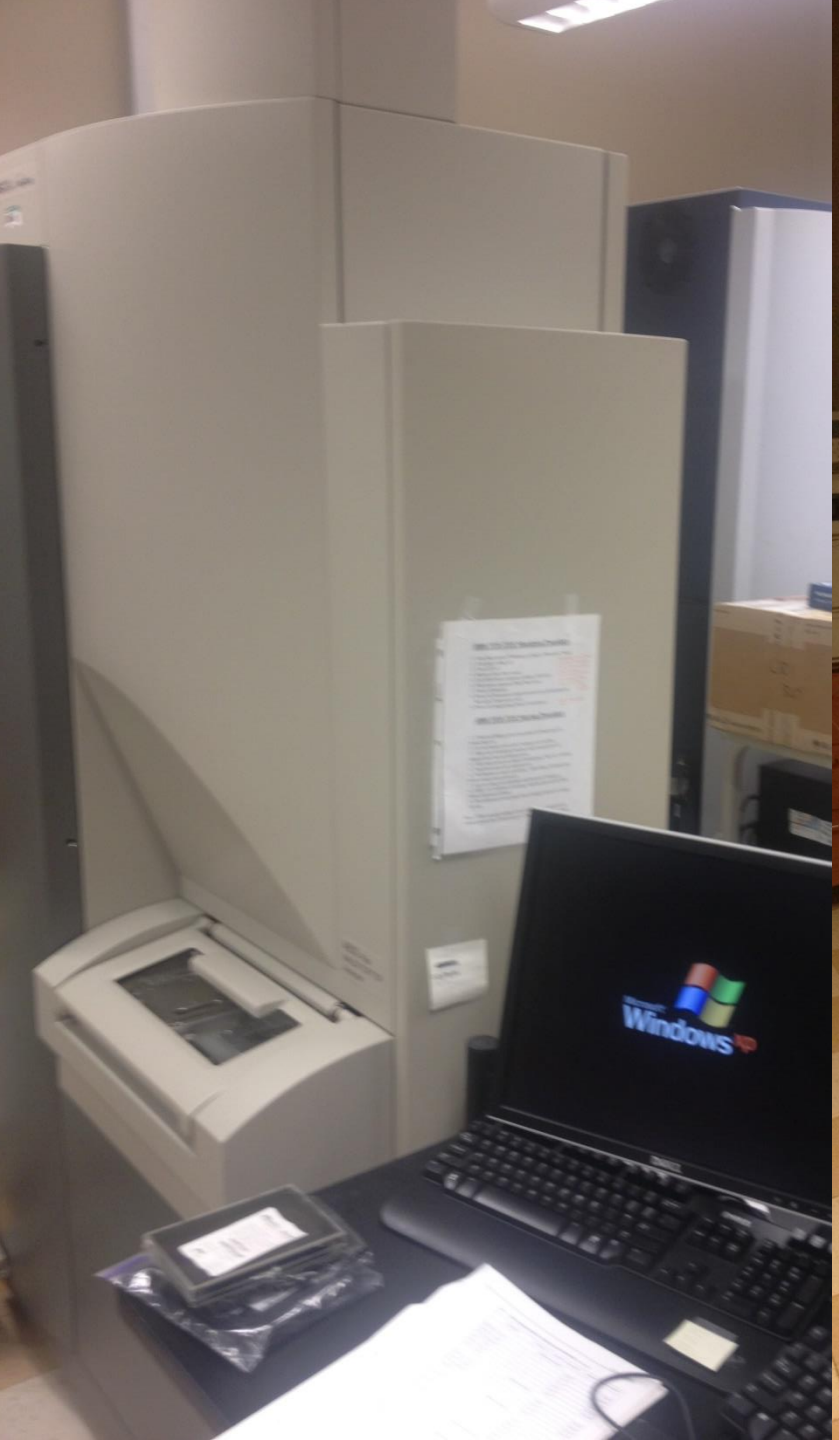
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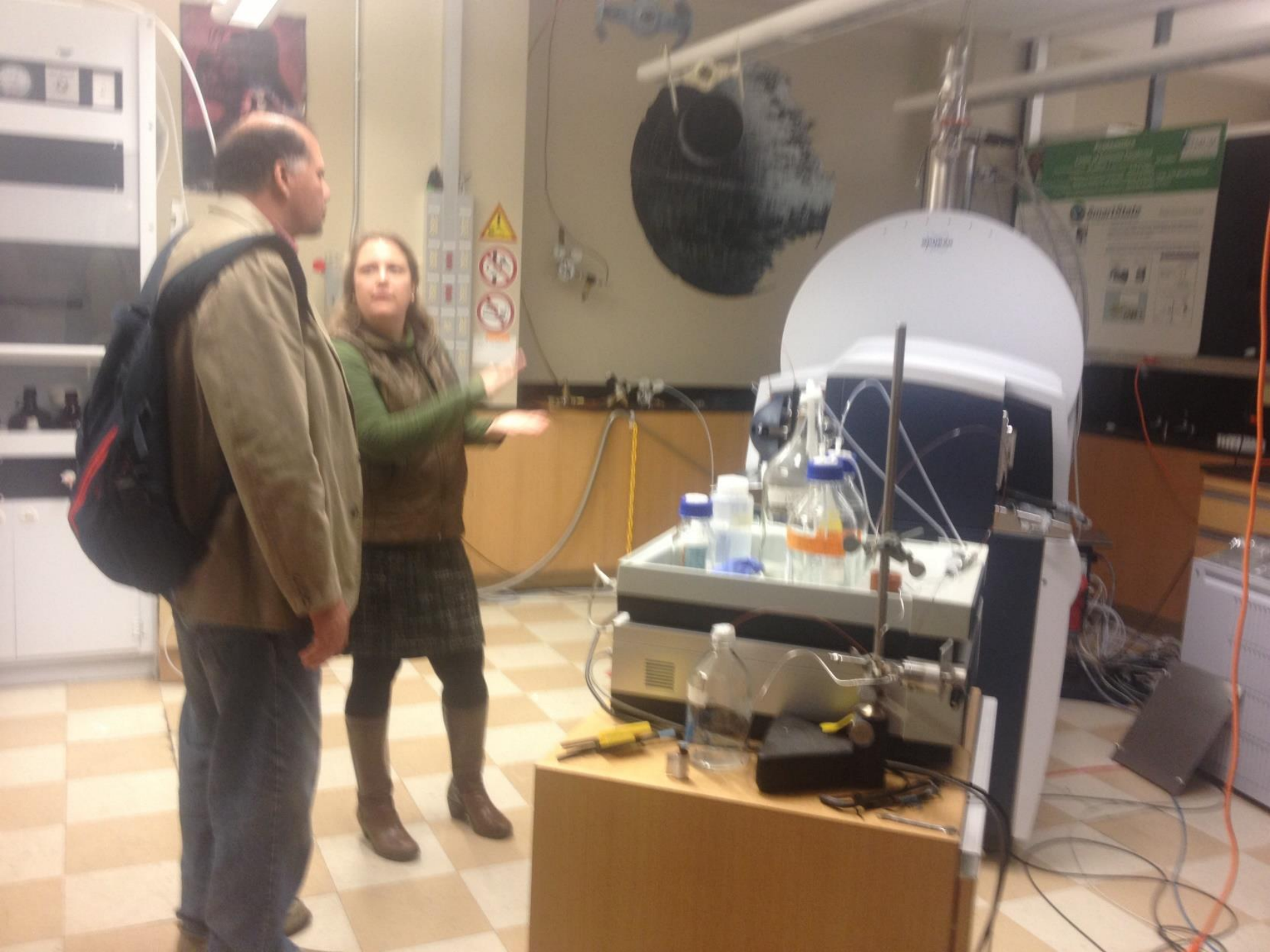

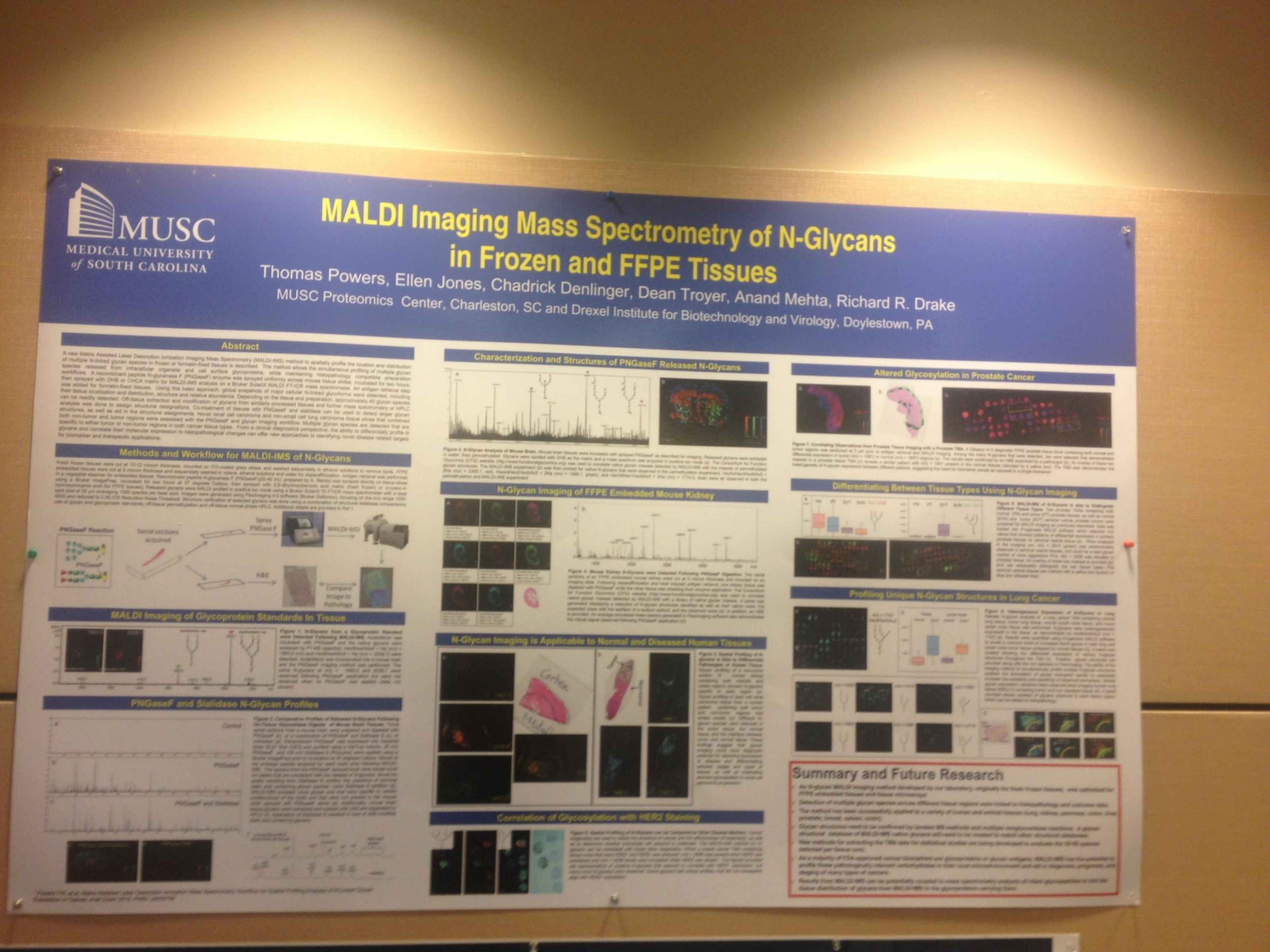
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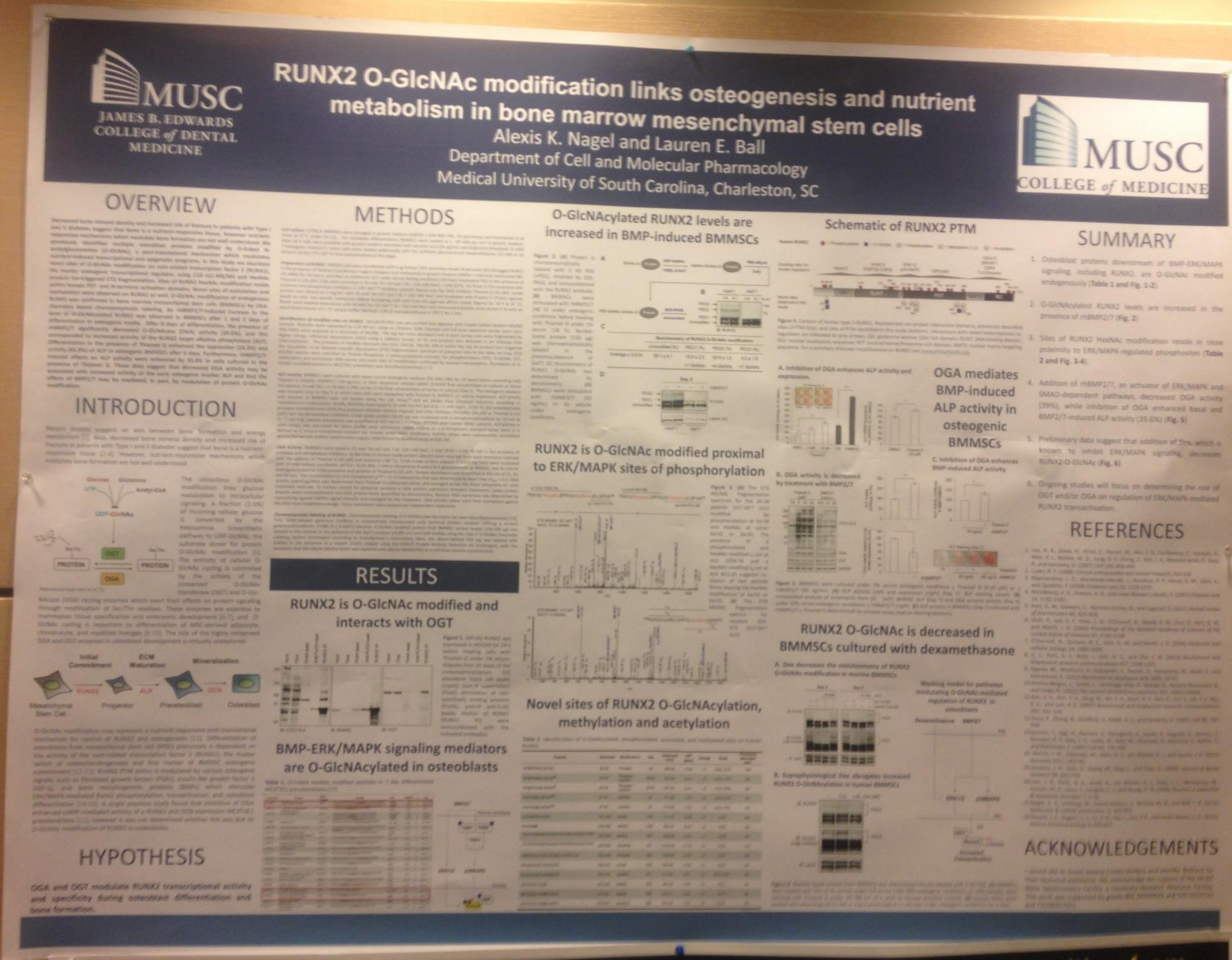
MALDI Imaging Mass Spectrometry of N-Glycans
in Frozen and FFPE Tissues
Thomas Powers, Ellen Jones, Chadrick Denlinger, Dean Troyer, Anand Mehta, Richard R. Drake
MUSC Proteomics Center, Charleston, SC and Drexel Institute for Biotechnology and Virology, Doylestown, PA
Abstract
Methods and Workflow for MALDI-IMS of N-Glycans
MALDI Imaging of Glycoprotein Standards in Tissue
PNGaseF and Sialidase N-Glycan Profiles
Characterization and Structures of PNGaseF Released N-Glycans
N-Glycan Imaging of FFPE Embedded Mouse Kidney
N-Glycan Imaging is Applicable to Normal and Diseased Human Tissues
Correlation of Glycosylation with HER2 Staining
Altered Glycosylation in Prostate Cancer
Differentiating Between Tissue Types Using N-Glycan Imaging
Profiling Unique N-Glycan Structures in Lung Cancer
Summary and Future Research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JAMES B. EDWARDS COLLEGE of DENTAL MEDICINE
RUNX2 O-GlcNAc modification links osteogenesis and nutrient metabolism in bone marrow mesenchymal stem cells
Alexis K. Nagel and Lauren E. Ball
Department of Cell and Molecular Pharmacology
Medical University of South Carolina, Charleston, SC
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OVERVIEW
INTRODUCTION
METHODS
RESULTS
RUNX2 is O-GlcNAc modified and interacts with OGT
BMP-ERK/MAPK signaling mediators are O-GlcNAcylated in osteoblasts
O-GlcNAcylated RUNX2 levels are increased in BMP-induced BMMSCs
RUNX2 is O-GlcNAc modified proximal to ERK/MAPK sites of phosphorylation
Novel sites of RUNX2 O-GlcNAcylation, methylation and acetylation
Schematic of RUNX2 PTM
OGA mediates BMP-induced ALP activity in osteogenic BMMSCs
RUNX2 O-GlcNAc is decreased in BMMSCs cultured with dexamethasone
HYPOTHESIS
OGA and OGT modulate RUNX2 transcriptional activity and specificity during osteoblast differentiation and bone formation.
SUMMARY
1. Osteoblast proteins downstream of BMP-ERK/MAPK signaling, including RUNX2, are O-GlcNAc modified endogenously (Table 1 and Fig. 1-2).
2. O-GlcNAcylated RUNX2 levels are increased in the presence of rhBMP2/7 (Fig. 2).
3. Sites of RUNX2 HexNAc modification reside in close proximity to ERK/MAPK-regulated phosphosites (Table 2 and Fig. 3-4).
4. Addition of rhBMP2/7, an activator of ERK/MAPK and SMAD-dependent pathways, decreased OGA activity (39%), while inhibition of OGA enhanced basal and BMP2/7-induced ALP activity (35.6%) (Fig. 5).
5. Preliminary data suggest that addition of Dex, which is known to inhibit ERK/MAPK signaling, decreases RUNX2 O-GlcNAc (Fig. 6).
6. Ongoing studies will focus on determining the role of OGT and/or OGA on regulation of ERK/MAPK-mediated RUNX2 transactivation.
REFERENCES
ACKNOWLEDGEMENTS

Defining the Molecular Tumor Margin Regionss of Clear Cell Renal Cell Carcinoma Tissues by MALDI-MS Imaging of Lipid and Glycan Species

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Richard R. Drake, Thomas Powers, E. Ellen Jones, Anand S. Mehta, Raymond S; Lance, Dean A. Troyer

Department of Cell and Molecular Pharmacology, Hollings Cancer Center and MUSC Proteomics Center, Charleston SC;
Drexel Institute for Biotechnology and Virology Research, Doylestown, PA; Eastern Virginia Medical School and Urology of Virginia, Norfolk, VA

Abstract

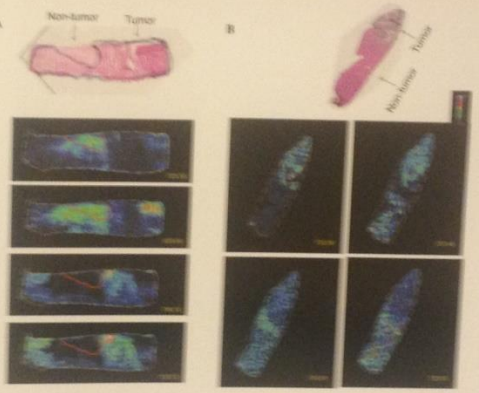
Background: The frequent tumor recurrence associated with clear cell renal cell carcinoma (ccRCC) suggests that there are underlying molecular processes present in the remaining tissue following nephrectomy that are not identified through conventional histopathological techniques. At the molecular level, transcript, metabolomic and protein expression patterns have indicated a striking Warburg Effect profile in ccRCC tissues, with major affects on sugar and lipid metabolism. Our group has been applying MALDI mass spectrometry imaging approaches to uniquely profile lipids and glycans associated with disease progression directly in frozen tissue slides.

Methods: Frozen ccRCC tissues with tumor, non-tumor adjacent and tumor margin regions were selected by a pathologist. Lipid profiles from fresh-frozen tissue slides coated in DHB matrix were obtained on a dual source Bruker Solarix 7tt FT/ICR mass spectrometer. Glycans were imaged in a similar fashion in ethanol-washed tissues using on-tissue protein N-glycanase F digestion to release surface N-glycans. Detected lipid and glycan ion intensities were converted to a color pixel scale for creating an image of individual peaks, linked directly to the histopathology of the tissue.

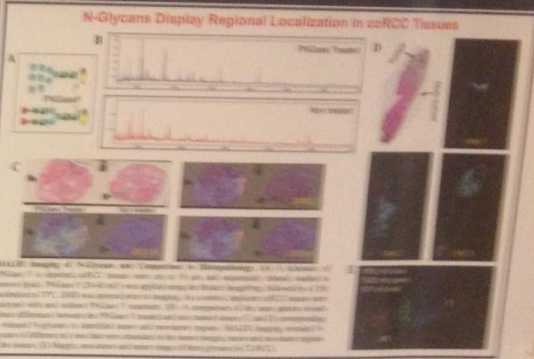
Results: Five groups of lipid and N-glycan species were identified following MALDI tissue imaging, those present in: the immediate margin area of non-tumor tissue adjacent to tumor; only in non-tumor regions; only in tumor regions; primarily in tumor regions but extended beyond the margin, and present throughout the tissue. Specific lipid and glycan species associated with margin and tumor regions are being correlated with disease progression and pathology data.

Conclusions: Analysis of the periphery of the tumor tissue and the normal parenchyma or capsule regions at the biomolecular level may better define the metastatic potential of the tumor as compared to analysis of the central tumor region. This approach has the potential to not only improve prognostic assessment and treatment choices, but also to inform on the underlying biology of ccRCC metastasis and new rational targets for therapeutic intervention.

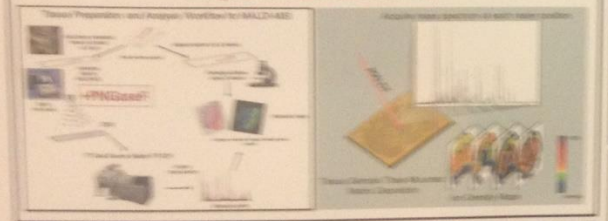
Comparison of Localized Lipid Distribution and Histological Analysis



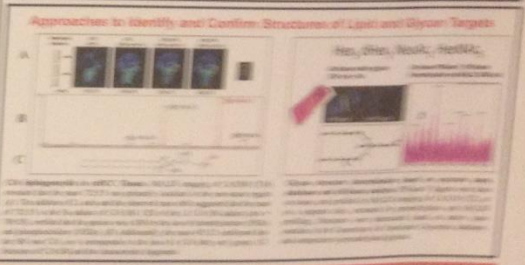
N-Glycans Display Regional Localization in ccRCC Tissues



Analysis Workflow for MALDI-MSI

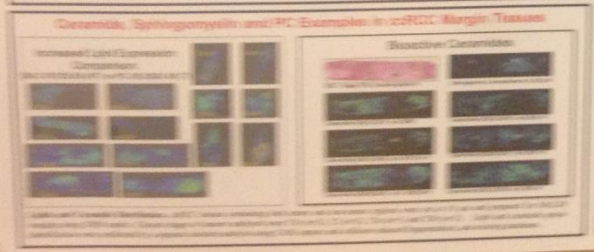


Approaches to Identify and Confirm Structures of Lipid and Glycan Targets

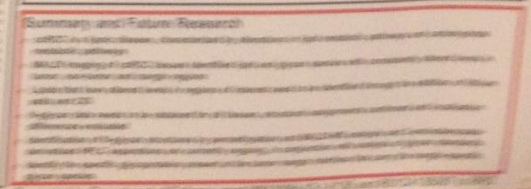


Reproducibility of Differential Lipid Detection in Tumor and Non-Tumor Regions

Ceramide, Sphingomyelin and PC Examples in ccRCC Margin Tissues



Summary and Future Research

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Children's Hospital
CHILDREN'S HOSPITAL
VISITOR ELEVATORS
Rutledge Tower Connector - Take
Elevator to Second Floor


If someone interested to get more information or reach **Dr. Lauren E. Ball**, please contact me at: cecr@unv.tanta.edu.eg

Thank you

Prof. Mohamed L. Salem