Biomolecular Analysis Facility (BAF)
An Omics Resource

Nicholas E. Sherman, PhD, Director

OVERVIEW

Services:

Proteomics/Metabolomics: Mass Analysis (MALDI-TOF, LC-ESI, High Resolution), Simple Identification (gels, solutions), Complex Mixture (GeLC-MS/MS, tissues, biomarker), Post-translational Modifications/De Novo (site specification, cloning), and Quantification (relative by tags, absolute by MRM), Small Molecule Quantification.

Park, Jeong-Jin <jp2ht@eservices.virginia.edu>

DNA Sciences: DNA Genome Analysis/Library Preparation (single read, paired-end, DNA/RNA, small RNA), Digital PCR, RT-PCR (SNP, miRNA, gene expression), DNA Fragment Analysis.

Bao, Yongde <yb8d@eservices.virginia.edu>

Shared Instrumentation: HPLC/FPLC Separations, Molecular Interactions, Circular Dichroism, Plate Reading

Shannon, John D. <jds1c@eservices.virginia.edu>

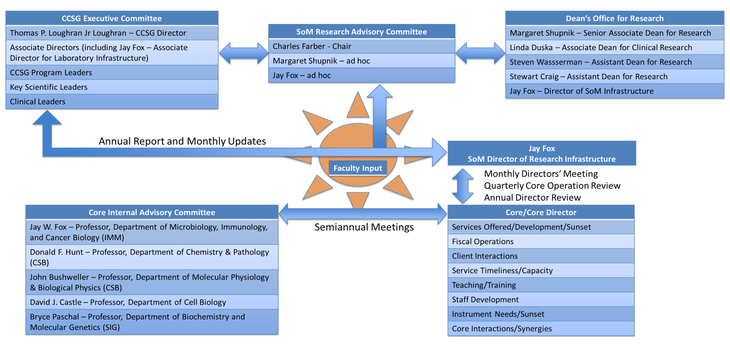
Personnel:

- Director: Nicholas E. Sherman, Ph.D.
- Group Leader, DNA Sciences: Yongde Bao, Ph.D.
- Group Leader, Proteomics/Metabolomics: JJ Park, Ph.D.
- Group Leader, Shared Instruments: John Shannon, Ph.D.
- Data Analyst: Brian Capaldo, Ph.D.
- Lab Specialist: Alyson Prorock, M.S.

INSTRUMENTATION

- fortéBIO Octet Red
- Bio-Rad QX200 Digital PCR
- ABI 7900HT DNA Detection System
- Illumina MiSeq
- Illumina NextSeq500 **(New)**
- Sage Pippin and Agilent TapeStation 4200 **(New)**
- Thermo Velos Orbitrap
- Thermo TSQ Quantum
- Bruker MicroFlex MALDI-TOF

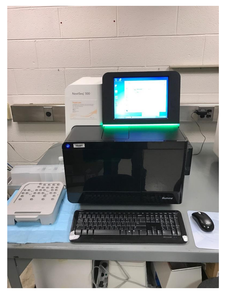
PLANNING AND OVERSIGHT



VALUE ADDED

The BAF enhances the work/productivity of PIs by:

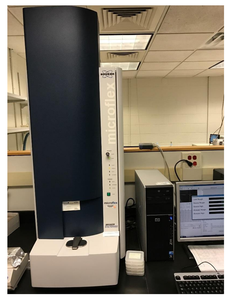
- Providing a centralized setting for services and instrumentation in 'omics': genomics, transcriptomics, proteomics, lipidomics, metabolomics, and molecular interaction. BAF provides expertise in experimental design, cost-effective data collection, data analysis, and post-experiment processing for manuscripts and grants.
- Giving investigators access locally to a wide variety of services that can determine qualitative and quantitative changes at the cellular level. The consolidated organization of the facility allows for researchers to perform many of these experiments on the same or related samples.
- Being a local repository of "omics" knowledge and instrumentation and offering a strategic and economical advantage for UVA.



NextSeq500 NGS

MiSeq NGS

Molecular Interactions



QA/QC

Identification, PTM

Quantitation

FUTURE PLANS

New initiatives – NextSeq500 and Molecular Interactions:

- Octet Red determining binding constants and interactions
- NextSeq500 installed Jan. 2017
- First NGS experiments underway

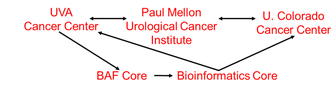
Upgrade the instruments supporting heavily utilized services:

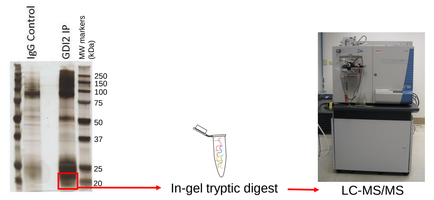
- BAF will submit two grants to fund replacement of instruments that are 7 years old.
  - NIH S10 Shared Instrument Grant and UVA SIF to replace Orbitrap Velos with an Orbitrap Fusion Lumos.

BAF-SUPPORTED RESEARCH HIGHLIGHTS

Identification of RhoC as a Target of RhoGDI2 for Prevention of Lung Colonization of Bladder Cancer (Proteomics)

David L. Brautigan, Ph.D.

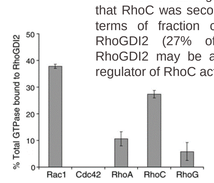




To determine binding partners for RhoGDI2 in bladder cells, endogenous RhoGDI2 was immunoprecipitated from RT4 cells. Proteins were separated by SDS-PAGE and silver stained, and a band 15 to 23 kDa was excised from the gel and analyzed by MS

Mass spectrometry results of proteins co-immunoprecipitating with RhoGDI2

Protein	Accesson#	MW	Xcorr
Ras-related C3 botulinum toxin substrate 2 (Rac2)	IPI00010270	21 kDa	14.63
Isoform A of Ras-related C3 botulinum toxin substrate 1 (Rac1)	IPI00010271	21 kDa	13.01
Rho-related GTP-binding protein RhoC	IPI00027434	22 kDa	9.76
Isoform 2 of cell division contorl protein 42 homolog (Cdc42)	IPI00016786	21 kDa	9.76
Transforming protein RhoA	IPI00478231	22 kDa	4.88
Isoform 1 of cell division control protein 42 homolog	IPI00007189	21 kDa	4.88
Rho-related GTP-binding protein RhoG	IPI00017342	21 kDa	4.88
Rho-related GTP-binding protein RhoB	IPI00000041	22 kDa	3.25



Fluorescent staining of Western blots showed that RhoC was second among the GTPases in terms of fraction coimmunoprecipitated with RhoGDI2 (27% of total) suggesting that RhoGDI2 may be an unappreciated negative regulator of RhoC activation in bladder cells.

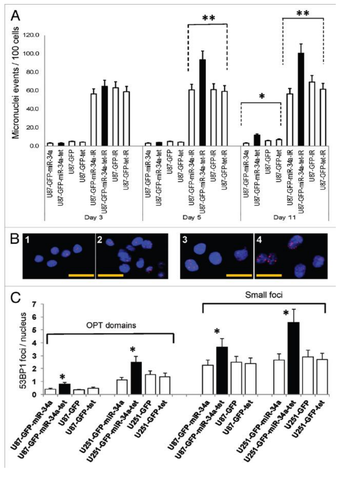
- Further analysis showed knockdown of RhoGDI2 increased RhoC activation in response to serum stimulation. Overexpression of RhoGDI2 decreased RhoC activation.
- RhoC promoted bladder cancer cell growth and invasion. RhoC knockdown increased cell doubling time, decreased invasion through Matrigel, and decreased colony formation in soft agar.
- RhoC knockdown reduced *in vivo* lung colonization by bladder cancer cells in immunocompromised mice.
- Unbiased transcriptome analysis revealed a set of genes regulated by RhoGDI2 overexpression and RhoC knockdown in bladder cancer cells.

***Funding* –CA910935, 5R01CA143971-05**

***Publications* – *"RhoC is an unexpected target of RhoGDI2 in Prevention of Lung Colonization of Bladder Cancer"* Griner et al. Mol Cancer Res 2015;13:483-492**
http://www.coloradocancerblogs.org/study-pinpoints-rhogdi2-suppresses-bladder-cancer-metastasis/

microRNA-34a Promotes DNA Damage and Mitotic Catastrophe (DNA Sciences)

James Larner, M.D. and Roger Abounader, M.D., Ph.D.



- miR-34a, a potent tumor suppressor, influences a large set of p53-regulated genes and contributes to p53-mediated apoptosis.
- Using tet-inducible miR-34a-expressing human p53 wild-type and R273H p53 mutant GBM cell lines, we found that miR-34a influences the broad spectrum of 53BP1-mediated DNA damage response.
- It escalates both post-irradiation and endogenous DNA damage, abrogates radiation-induced G2/M arrest and increases the number of irradiated cells undergoing mitotic catastrophe. Also, miR-34a downregulates 53BP1 and inhibits its recruitment to the sites of DNA double-strand breaks.
- These properties of miR-34a can potentially be exploited for DNA damage-effecting therapies of malignancies.

❖ DNA Section staff worked very closely with the investigators from the conception of the project in providing consultation in the experimental design and protocol selection for optimization of extraction of microRNA from cells and tissues and in gene expression of mRNA and microRNAs. For Taqman quantitative real time PCR, the core staff provided assay design and validation.

❖ The figure shows that miR-34a increases DNA damage. Black bars indicate miR-34a-over- expressing cells; stars * indicate statistical significance of $P < 0.05$; X-ray irradiation dose, 4 G.

(**A**) The frequency of spontaneous Mn events (small extra-nuclear chromatin-containing bodies) was higher in U251- than in U87-derived cell lines, showing elevated genomic instability of U251 cells. X-ray irradiation of U251 cells produced even higher numbers of Mn, thus making it difficult to discriminate between IR-induced chromosomal breaks and nuclear fragmentation (characteristic of mitotic catastrophe).
(**B**) miR-34a increases the number of spontaneous 53Bp1 foci. (1 and 2), U87-GFp-miR-34a non-induced and induced cells respectively; (3 and 4), U251-GFp-miR-34a non-induced and induced cells. Scale bars, 25 μm.
(**C**) Average numbers of spontaneous 53Bp1 foci per nucleus. Stars show a statistically significant difference ($P < 0.05$).

***Funding* – 1R01CA192669-01 (PI: Larner) and 5R01CA134843-05 (PI: Abounader)**

***Publications* – Kofman, et. al., Cell Cycle. 2013. 12(22):3500-3511.**