BIOGRAPHICAL SKETCH

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NAME: **Yonghao Yu**

eRA COMMONS USER NAME (credential, e.g., agency login):

POSITION TITLE: **Associate Professor of Biochemistry**

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date	FIELD OF STUDY
Fudan University, Shanghai, China	B.S.	06/2001	Chemistry
University of California, Berkeley	Ph.D.	09/2006	Chemistry
Harvard Medical School, Boston, MA	Postdoctoral	12/2011	Cell Biology

A. Personal Statement

I have a long-standing interest (more than 18 years) in mass spectrometry-based **proteomics** (including sample preparation, instrumentation and computation methods).

My graduate work with Dr. Julie Leary was focused on developing mass spectrometry approaches to characterizing noncovalent protein-ligand interactions, and covalent protein post-translational modifications (PTMs). During my postdoctoral fellowship, I was co-mentored by a cell biologist (Dr. John Blenis) and a mass spectrometrist (Dr. Steven Gygi). There, we developed a series of novel mass spectrometric methods to study protein phosphorylation. We were particularly interested in an evolutionarily conserved Ser/Thr kinase called mTOR (mechanistic target of rapamycin), and were among the first to use quantitative phosphoproteomics to comprehensively characterize the downstream signaling networks of both mTORC1 and mTORC2.

My lab at UT Southwestern is extending these accomplishments, with long-term goals of developing cutting-edge mass spectrometry technologies, and applying them to systematically characterize protein posttranslational modification (PTM) events (e.g., phosphorylation and ADP-ribosylation) implicated in various pathophysiological processes, in particular metabolic syndrome and cancer.

Through these studies, we have gained extensive experience in extracting meaningful information from large-scale proteomic data for follow-up functional studies. The combination of quantitative proteomic analysis with classical biochemistry and molecular biology approaches put us in a unique position to identify aberrant protein modification patterns, decipher the mechanisms of their deregulation, establish the functional consequences of these molecular events, facilitate the development of relevant therapeutic strategies, and finally, identify proteomic signatures that may serve as diagnostic, prognostic or predictive biomarkers for neurodegenerative diseases.

1. Wang S, Han L, Han J, Li P, Ding Q, Zhang QJ, Liu ZP, Chen C*, **Yu Y***. Uncoupling of PARP1 trapping and inhibition using selective PARP1 degradation. ***Nature Chemical Biology***.15(12):1223-1231 (2019).

2. Zhang Y, Zhang Y and **Yu Y.***, "Global Phosphoproteomic analysis of Insulin/Akt/mTORC1/S6K signaling in Rat Hepatocytes", ***Journal of Proteome Research***, 16, 2825, (2017).

3. Xiang, S., Kato, M., Wu, L., Lin, Y., Ding, M., Zhang, Y., **Yu, Y.*** and McKnight, S.* (*, Co-corresponding authors) "The LC Domain of hnRNPA2 adopts similar conformations in hydrogel polymers, liquid-like droplets and nuclei", ***Cell***, 163, 829-839 (2015).

4. Zhang, Y., Wang, J., Ding, M. and **Yu, Y*.** "Site-Specific Characterization of the Asp- and Glu-ADP-ribosylated Proteome". ***Nature Methods***, 10(10):981-4 (2013). PMID: 23955771

B. Positions and Honors

Positions and Employment

2001-2006 Graduate Student Researcher, Department of Chemistry, University of California, Berkeley, Advisor, **Julie Leary**

2007-2011 Postdoctoral Research Fellow, Department of Cell Biology, Harvard Medical School, Boston Co-advisors, **Steven Gygi and John Blenis**

2012-2017 Assistant Professor, Department of Biochemistry, UT Southwestern Medical Center, Dallas, TX

2012- Member, Simmons Cancer Center, UT Southwestern Medical Center, Dallas, TX

2017- Associate Professor (with tenure), Department of Biochemistry, UT Southwestern Medical Center, Dallas, TX

Other Experience and Professional Memberships

2001- American Society for Mass Spectrometry (ASMS), Member

2013- American Association for Cancer Research (AACR), Member

2009- Ad hoc reviewer for scientific journals, including Molecular Cell, Analytical Chemistry, Journal of the American Society for Mass Spectrometry, Analytical Biochemistry, Rapid Communications in Mass Spectrometry, PNAS, Genes and Development, Nature, Nature Communications, etc.

2012-13 Ad hoc reviewer for Congressionally Directed Medical Research Programs (CDMRP/DoD), The Tuberous Sclerosis Complex Research Program

2012-17 Ad hoc reviewer for Tuberous Sclerosis Alliance, the Research Grants Program

2017 Ad hoc reviewer for NIH MIST (Molecular and Integrative Signal Transduction) study section

2017 Ad hoc reviewer for American Cancer Society Research Grant Program

2017- Member of the Executive Committee, Chinese American Society for Mass Spectrometry

2018 Ad hoc reviewer for NIH EBIT (Enabling Bioanalytical and Imaging Technologies) study section

2019-2025 Standing member of the NIH EBIT (Enabling Bioanalytical and Imaging Technologies) study section

Honors

1999 Chun-Tsung Scholar, Hui-Chun Chin and Tsung-Dao Lee Chinese Undergraduate Research Endowment

2007 Academic Excellence Award for Overseas Graduate Students, Ministry of Education, China

2008 Postdoctoral fellowship, The Tuberous Sclerosis Alliance.

2011 Virginia Murchison Linthicum Scholar in Medical Research, UT Southwestern Medical Center

2011 CPRIT Scholar in Cancer Research

2011 University of Texas STARs award

2015 American Cancer Society Research Scholar

2017 Young investigator award of the Chinese American Diabetes Association

2018 Special issue of "Future of Biochemistry", *Biochemistry*

C. Contributions to science.

To date we have produced 52 publications, with a total of 3632 citations and an h-index of 29 (google scholar).

See complete publication list

https://www.ncbi.nlm.nih.gov/myncbi/1F_avi54Qj1ky/bibliography/public/

The overarching theme of our science (and areas which we have contributed to) is summarized as follows:

1. Mass spectrometry technologies

We have been continuously developing and optimizing all the essential components of now a highly sophisticated proteomic pipeline. The efforts include those dedicated to better sample preparation procedures (e.g. for general proteomic analysis, and for the analysis of proteins/PTMs with unusual biochemical properties), improved liquid chromatography/MS hardware (e.g. data acquisition methods for bottom-up and top-down proteomics, on Orbitrap- and Fourier transform ion cyclotron resonance-based platforms, respectively) and novel data analysis algorithms (e.g. database search engine, MS1 calibration, PTM site-assignment, post-search processing, etc.).

(a) Zhao X, Huffman KE, Fujimoto J, Canales JR, Girard L, Nie G, Heymach JV, Wistuba II, Minna JD, **Yu Y***. "Quantitative Proteomics Analysis of Optimal Cutting Temperature (OCT) embedded core-needle biopsy of lung cancer", ***J. Am Soc. Mass Spectrom.***, 28, 2078, (2017).

(b) Hu R, Huffman KE, Chu M, Zhang Y, Minna JD, and **Yu Y***. "Quantitative Secretomic Analysis Identifies Extracellular Protein Factors that Modulate the Metastatic Phenotype of Non-Small Cell Lung Cancer." ***Journal of Proteome Research***, 15, 477, (2016).

(c) Xiang, S., Kato, M., Wu, L., Lin, Y., Ding, M., Zhang, Y., **Yu, Y.*** and McKnight, S.* **(*, Co-corresponding authors)** "The LC Domain of hnRNPA2 adopts similar conformations in hydrogel polymers, liquid-like droplets and nuclei", ***Cell***, 163, 829-839 (2015).

(d) Wang J, Zhang Y and **Yu Y*.**, "Crescendo: A Protein Sequence Database Search Engine for Tandem Mass Spectra", ***J. Am. Soc. Mass. Spectrom***., 26, 1077 (2015).

2. Site-specific characterization of the ADP-ribosylated proteome

Poly-ADP-ribosylation (PARylation) is a protein posttranslational modification (PTM) that was first discovered in 1963. It is catalyzed by a family of enzymes called Poly-ADP-ribose polymerases (PARPs). In particular, PARP1 is a nuclear enzyme that is involved in mediating DNA damage response (DDR). During genotoxic stress, PARP1 is recruited to nicked DNA and is rapidly activated, resulting in the synthesis of a large number of PARylated proteins and initiation of the DNA repair mechanisms. The critical role of PARP1 in DDR also provides the rationale for developing PARP1 inhibitors for the treatment of cancer (e.g. olaparib has recently been approved to treat *BRCA1/2*-mutated ovarian cancer).

Despite decades of research, we know surprisingly little about the mechanism by which PARP1 signals downstream. Specifically, very few genuine PARP1 substrates have been identified. In addition, site-localization of protein PARylation is also a daunting challenge, due to the labile and heterogeneous nature of this modification. Recently, we were able to develop the **first** large-scale mass spectrometric (MS) approach towards site-specific characterization of the Asp- and Glu-ADP-ribosylated proteome.

The development of this critical technology has greatly facilitated the functional characterization of this PTM. For example, we collaborated with Junjie Chen Lab (MD Anderson Cancer Center) and mapped the ADP-ribosylation sites on a critical tumor suppressor, PTEN. We found that these PARylation sites play an indispensable role in regulating PTEN stability, which then contributes to PTEN-dependent suppression of oncogenesis. More recently, we have collaborated with Lee Kraus lab (UT Southwestern medical center) and developed a robust analog-sensitive approach for PARPs, which allows the identification of the substrates of specific PARP family members. Using this approach, we mapped hundreds of protein targets and sites of ADP-ribosylation for PARPs 1, 2, and 3. Moreover, we found that PARP1 ADP-ribosylates and inhibits RNA-binding by NELF, a protein complex that regulates promoter-proximal pausing by RNA polymerase II (Pol II). Finally, we recently performed global characterization of the PARP1-dependent, Asp/Glu-ADP-ribosylated proteome in a panel of cell lines originating from benign breast epithelial cells, as well as common subtypes of breast cancer. Despite similar expression levels, we found that PARP1 is differentially activated in these cell lines under genotoxic conditions, which generates signaling outputs with substantial heterogeneity. By comparing protein abundances and ADP-ribosylation levels, we dissected cell-specific PARP1 targets that are driven by unique expression patterns versus cell-specific regulatory mechanisms of PARylation. Intriguingly, PARP1 modifies many proteins in a cell-specific manner, including those involved in transcriptional regulation, mRNA metabolism, and protein translation.

(a) Wang S, Han L, Han J, Li P, Ding Q, Zhang QJ, Liu ZP, Chen C*, **Yu Y***. Uncoupling of PARP1 trapping and inhibition using selective PARP1 degradation. ***Nature Chemical Biology***. **2019 Dec;**15(12):1223-1231.

(b) Zhen Y., Zhang Y., and **Yu, Y*.** "A cell line specific atlas of PARP-mediated Asp-/Glu-ADP ribosylation in breast cancer", ***Cell Reports***, 21, 2326 (2017)

(c) Gibson, B. A., Zhang, Y., Jiang, H., Hussey, K. H., Shrimp, J. H., Lin, H., Schwede, F., **Yu, Y.** and Kraus, W. L. "Chemical Genetic Discovery of PARP Targets Reveals a Role for PARP-1 in Transcription Elongation", ***Science***, 353, 45, (2016) ▪ Highlighted in Nature Molecular Cell Biology, 17, 397 (2016).

(d) Zhang, Y., Wang, J., Ding, M. and **Yu, Y*.** "Site-Specific Characterization of the Asp- and Glu-ADP-ribosylated Proteome". ***Nature Methods***, 10(10):981-4 (2013). ▪ **Cited 200 times**

3. Site-specific characterization of the phospho-proteome

We have been continuously involved in the development of novel mass spectrometric technologies in the field of phosphoproteomics. These unbiased approaches have been deployed to systematically interrogate the phosphoproteome regulated by several key kinases, including those in the PI3K/Akt/mTOR (also see contribution #4), ERK/MAPK, and chemokine signaling pathways. For example, we created a multiplexed, mass-spectrometry-based in vitro kinase assay, using stable isotope-labeled peptides as the internal standard. We applied this unique kinome-activity profiling strategy in a variety of cellular settings, to report the change in intrinsic kinase activities upon mitogen stimulation, cell cycle and pharmacological inhibition of pathways.

(a) Wang Z, Ma J, Miyoshi C, Li Y, Sato M, Ogawa Y, Lou T, Ma C, Gao X, Lee C, Fujiyama T, Yang X, Zhou S, Hotta-Hirashima N, Klewe-Nebenius D, Ikkyu A, Kakizaki M, Kanno S, Cao L, Takahashi S, Peng J, **Yu Y**, Funato H, Yanagisawa M, Liu Q, "Quantitative phosphoproteomic analysis of the molecular substrates of sleep need", ***Nature***, 558, 435-439 (2018).

(b) Zhang Y, Zhang Y and **Yu Y.***, "Global Phosphoproteomic analysis of Insulin/Akt/mTORC1/S6K signaling in Rat Hepatocytes", ***Journal of Proteome Research***, 16, 2825, (2017).

(c) **Yu, Y.,** Anjum, R., Kubota, K., Rush, J., Villen, J. & Gygi, S. P. "A site-specific, multiplexed kinase activity assay using stable-isotope dilution and high-resolution mass spectrometry" ***Proc. Natl. Acad. Sci. U. S. A.,*** **106** (28), 11606-11611 (2009). ▪ Highlighted in Nature Methods 6, 631 (2009) and Faculty of 1000 Biology: http://f1000biology.com/article/id/1162909/evaluation

(d) Kubota, K., Anjum, R., **Yu, Y.,** Kunz, R. C., Andersen, J. N., Kraus, M., Keilhack, H., Nagashima, K., Krauss, S., Paweletz, C., Hendrickson, R. C., Feldman, A. S., Wu, C. L., Rush, J., Villen, J. & Gygi, S. P. "Sensitive multiplexed analysis of kinase activities and activity-based kinase identification." ***Nature Biotechnology***, ***27***, 933-940 (2009). ▪ Highlighted in **Nature Biotechnology** (2009) 27: 912

4. Downstream signaling networks of mTOR

The evolutionarily conserved kinase, mTOR, plays a critical role in regulating cell growth, proliferation, migration and survival. Numerous upstream genetic alterations converge on mTOR, leading to its hyperactivation in a broad spectrum of human cancers. However, the clinical evaluation of the mTORC1 inhibitor, rapamycin, in treating cancers has yielded mostly disappointing results, which is due in part to rapidly developed resistance to the drug. To improve on rapamycin therapies, understanding how resistance occurs, identifying new therapeutic targets for future drug development, and revealing new biomarkers to improve on current assays used to monitor disease progression, we took a quantitative mass spectrometric approach towards defining the signaling landscape downstream of both mTORC1 and mTORC2 (including the proteome, phosphoproteome and secretome).

We identified hundreds of potential mTORC1 downstream effector proteins. Detailed biochemical characterization of one such hit, Grb10, showed that it is a direct mTORC1 substrate. Grb10 is phosphorylated by mTORC1, which results in its stabilization and accumulation. Grb10 then functions as a pseudosubstrate that competitively inhibits the activity of insulin receptor and IGF1 receptor. Rapamycin treatment abrogates the expression of Grb10, leading to the activation of insulin/IGF1 signaling. The activation of these pathways provides an alternative mechanism that fuels cell survival and proliferation, under these mTORC1-suppressed conditions. Grb10 expression is frequently down-regulated in various cancers, and loss of Grb10 and loss of the well-established tumor suppressor phosphatase PTEN appear to be mutually exclusive events, suggesting that Grb10 might be a tumor suppressor regulated by mTORC1.

Although not required for the Grb10 paper, we decided that in addition to the mTORC1 results, we would also made all of the mTORC2 targets available to the public, due to **our philosophy** that this kind of data should be shared with the scientific community to promote more rapid discovery in general. Indeed, the dissemination of this critical resource has greatly facilitated many follow-up studies within the mTOR field.

More recently, we found that cells with hyperactive mTORC1 secrete a protein that potently inhibits the function of IGF-1. Using a large-scale, unbiased quantitative proteomic platform, we comprehensively characterized the rapamycin-sensitive secretome in mouse embryonic fibroblasts, and identified IGFBP5 as a secreted, mTORC1 downstream effector protein. IGFBP5 is a direct transcriptional target of HIF1, which itself is

a known mTORC1 target. IGFBP5 is a potent inhibitor of both the signaling and functional outputs of IGF-1. Once secreted, IGFBP5 cooperates with intracellular branches of the feedback mechanisms to block the activation of IGF-1 signaling. Finally, IGFBP5 is a potential tumor suppressor, and the proliferation of IGFBP5-mutated cancer cells is selectively blocked by IGF-1R inhibitors.

(a) Lee G, Zheng Y, Cho S, Jang C, England C, Dempsey J, **Yu Y**, Liu X, He L, Cavaliere P, Chavez A, Zhang E, Isik M, Couvillon A, Dephoure N, Blackwell T, Yu J, Rabinowitz J, Cantley L, Blenis J, "Post-transcriptional regulation of de novo lipogenesis by mTORC1-S6K1-SRPK2 signaling"**,** ***Cell*****,** 171, 1545, (2017).

(b) Zhang Y, Zhang Y and **Yu Y.***, "Global Phosphoproteomic analysis of Insulin/Akt/mTORC1/S6K signaling in Rat Hepatocytes", ***Journal of Proteome Research***, 16, 2825, (2017).

(c) Ding M, Bruick R, and **Yu Y***. "Secreted IGFBP5 mediates mTORC1-dependent feedback inhibition of IGF-1 signaling", ***Nature Cell Biology,*** 18, 319, (2016).

(d) **Yu, Y.,** Yoon, S., Poulogiannis, G., Yang, Q., Ma, M., Villen, J., Kubica, N., Hoffman, G., Cantley, L. C., Gygi, S. P. & Blenis, J. "Phosphoproteomic Analysis Identifies Grb10 as an mTORC1 Substrate That Negatively Regulates Insulin Signaling" ***Science*****, 332**(6035), 1322-1326 (2011). (PMCID: PMC3195509)
- **Cited 700 times,** Highlighted in Science, 332, 1270 (2011) and in Faculty 1000: Sheikh S: 2011. F1000.com/11376956; Chernoff J: 2011. F1000.com/11376956; Fruman D: 2011. F1000.com/11376956

D. Research Support

Ongoing Research Support

R01GM114160 (PI Yonghao Yu) 09/01/2015 – 8/31/2020
NIH/NIGMS
Molecular and biochemical basis of mTORC1-mediated feedback loops
The goal of this project is to elucidate the molecular mechanisms by which mTORC1 inhibits its upstream receptor tyrosine kinases.

R01GM122932 (PI Yonghao Yu) 09/30/2017 – 5/31/2021
NIH/NIGMS
Mass Spectrometric Approaches to Protein ADP-ribosylation
The goal of this project is to develop novel mass spectrometric methods to study protein ADP-ribosylation

Completed Research Support (past three years)

R1103 (PI Yonghao Yu) 01/02/2012 – 12/31/2015
CPRIT/Junior faculty recruitment award
The major goal of this project is to establish a quantitative proteomic platform (including both software and hardware components) for the functional characterization of the cancer proteome.

I-1800 (PI Yonghao Yu) 06/2012-05/2015
Welch Foundation
Large-scale isolation and identification of Poly-ADP-Ribosylated proteins
The primary goal is to develop and optimize an analytical chemistry method for isolation of Poly-ADP-ribosylated proteins.