COMMENTARY

Proteomics illuminates fat as key tissue in aging

Jonathan Z. Long[a,b,1]

Proteomics, defined broadly as the large-scale study of genes at the level of proteins, has entirely revolutionized our understanding of the chemical composition and organization of biological systems. Over the past two decades, mass spectrometry-based proteomics has emerged as the dominant technique owing to its quantitative measurements in even highly complex biological samples (1, 2). However, nearly all proteomics experiments balance a critical trade-off between breadth of proteome coverage and sensitivity in detection (3, 4). In PNAS, Yu et al. (5) present an alternative proteomics workflow termed "Tomahto," which enables discovery-mode proteomics experiments with the speed and sensitivity afforded by targeted approaches. By applying Tomahto to a diverse panel of young versus old murine tissues, Yu et al. uncover white fat as a unique tissue that is especially sensitive to age-associated changes.

Accelerating Proteomic Discoveries

Proteomics workflows are typically classified into untargeted and targeted approaches. Untargeted approaches maximize proteome coverage and are typically used for hypothesis-generating experiments but lack the sensitivity required for detecting low-abundance proteins (3, 4). Targeted proteomic strategies exhibit dramatically improved sensitivity by measuring only a small number of peptides in a given experiment, but at the cost of proteome coverage (6). More recently, strategies that combine both targeted and untargeted approaches have emerged. These approaches include "peptide multiplexing" where hundreds of peptides are measured in a targeted manner in a single experiment (7) and "sample multiplexing" in which multiple biological samples are isobarically labeled and pooled together to increase throughput (8). When used together, combined sample and peptide multiplexing enables the targeted measurement of hundreds of proteins from multiple biological samples simultaneously and with high sensitivity (9). However, in practice these two-dimensional (2D) multiplexed approaches are laborious and error-prone to implement, with multiple targeted mass inclusion and trigger lists as well as a number of filters that need to be specified manually.

Tomahto is an application programming interface (API)-based algorithm developed by Yu et al. (5) that overcomes previous limitations of 2D multiplexed proteomics experiments. Key to the Tomahto workflow is the use of real-time instrument control and decision making to adjust acquisition parameters for the mass spectrometer on the fly. This results in a more automated and simplified experimental setup. Benchmarking Tomahto reveals equivalent performance to that of untargeted proteomics experiments, but with more than one-log improvements to both sample input requirements and analysis times.

Yu et al. (5) applied their workflow to the large-scale targeted proteomic analysis of aging, a fundamental biological process characterized by decline of cellular and tissue homeostasis (10). Although many transcriptomic approaches have been applied to the aging process across diverse tissues and model organisms (11, 12), proteome-wide measurements have been more limited and, when performed, focused only on specific tissues with limited biological replicates at a time (13, 14). Consequently, how tissue proteomes change across an organism during aging and whether specific cell-type or tissue proteomes are more sensitive to age-associated changes have remained open questions. By comparing nine distinct tissues with five biological replicates per group, Yu et al. (5) surprisingly identify white fat as a unique tissue that exhibits dramatic age-dependent changes at the level of the proteome. These changes include a broad suppression of oxidative metabolism pathways and increased inflammatory processes with age. Other proteome-level changes include lower ribosome levels and reduced lipolysis with concomitant up-regulation of lysosome and phagosome pathways. By contrast, brown adipose tissue proteomes were largely unchanged, as were the proteomes for the other eight analyzed tissues. Taken together, these experiments

[a]Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305; and [b]Stanford Chemistry, Engineering & Medicine for Human Health (ChEM-H), Stanford University, Stanford, CA 94305

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[1]Email: jzlong@stanford.edu.

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identify and contextualize white adipose tissue proteome changes as a dominant tissue-level signature of organismal aging.

White Fat at the Crossroads of Energy Metabolism and Aging

This study from Yu et al. (5) contributes to a growing body of work linking obesity, lipid metabolism, and fat tissues to the aging process (15). Individuals with normal body mass index tend to live longer and healthier lives than those who are obese (16). However, some evidence paradoxically suggests that elderly individuals specifically appear to benefit from being slightly overweight (17). Yu et al. (5) demonstrate that, at least in mice, "youthful" white fat and "aged" white fat are fundamentally distinct across multiple biological pathways. Some of these age-associated molecular differences in fat tissue may explain the disparate epidemiological associations between body mass index and longevity.

Could white adipose tissue itself directly influence the aging process? It has long been recognized that beyond energy storage, white fat also functions as a dynamic endocrine organ (18). One provocative hypothesis is that white fat represents the primary site for age-associated molecular changes, which is then amplified and transduced to other tissues via secretion of adipose-derived factors. These fat-derived secreted factors may also be present in the youthful or aged blood plasma milieu that has been well established to exert rejuvenating or proaging effects, respectively (19, 20). Revisiting adipose-derived circulating factors during aging could uncover molecular mechanisms that coordinate local nutrient and energy metabolism with systemic age-associated dysfunction. The Tomahto workflow developed by Yu et al. (5), which enables highly sensitive, rapid, and multiplexed proteomic measurements, would be especially suitable for these additional lines of future inquiry.

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