

METHODS IN MOLECULAR BIOLOGY

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Thermogenic Fat

Methods and Protocols

Edited by

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Preface

The rediscovery of thermogenic adipocytes in human adults has reignited a great deal of enthusiasm towards these unique metabolic cells that convert chemical energy into heat, therefore holding great promise for helping to counteract obesity and its associated metabolic disorders. It is now appreciated that at least two types of thermogenic adipocytes exist in rodents and humans, classical brown and inducible beige adipocytes. These two types of fat cells arise from distinct developmental origins and position in different anatomical locations. In contrast to white adipocytes that store surplus energy, brown and beige fat cells dissipate chemical energy through uncoupling protein 1 (UCP1), together with other mechanisms. In addition to their respective roles in mediating energy balance, all adipocytes function as endocrine organs and directly control systemic metabolism through adipocyte-secreted hormones (so-called adipokines) and metabolites. It is now considered settled science that adipose tissue as a whole plays a key role in maintaining metabolic homeostasis. Emerging evidence suggests that the mass and activity of thermogenic fat influence body weight, insulin sensitivity, glucose tolerance, and other key metabolic parameters. Further investigation and deeper understanding of the development and function of these thermogenic fat cells may lead to novel therapeutic strategies against metabolic syndromes.

Targeting both experts in this area who want to compare notes with colleagues in the field and newcomers that look forward to learning assays and starting their own investigation of brown and beige fat function, this volume collects protocols that describe methodologies to study thermogenic fat biology from various angles. The first part focuses on how to establish *in vitro* culture systems. Historically, many adipocyte-related studies have been carried out using immortalized cell lines, for example the famous 3T3-L1 cell line that was originally established by Dr. Howard Green's group. While cell line work remains to be an indispensable approach, much of the thermogenic fat specific function can only be evaluated in primary cultures. Several chapters introduced methods on how to isolate, culture, and differentiate primary fat cells from both laboratory mice and humans. It has been reported that immune cells within the subcutaneous fat tissue regulate thermogenic fat functions through paracrine mechanisms. Several chapters presented flow cytometry methods to isolate various subpopulations of precursors within the stromal vascular fraction (SVF) of the adipose tissue, which contains both preadipocytes and immune cells. In the second part of the book, we first introduced multiple means to genetically manipulate and evaluate brown and beige fat *in vivo*. Since one of the most prominent functions of thermogenic fat is to mediate energy consumption through mitochondrial respiration, two chapters were dedicated to discuss methods on bioenergetics analyses both *in vitro* and *in vivo*. We further presented how to experimentally study ion channel function and cell-cell communication in these cells. Lastly, we discussed how to evaluate thermogenic fat content and activity in humans, how to culture and assay these cells through interdisciplinary approaches, including 3-D adipospheres and microfluidic systems, and how to use thermogenic fat cell lines to carry out drug screens.

The editor would like to thank all the contributing authors who shared their “trade secrets” here without reservations so that readers did not have to “reinvent the wheel” and learn these key assays with much less challenge. I am also very grateful to Dr. John Walker, who commissioned this timely book and has provided much invaluable advice along the way.

Ann Arbor, MI, USA

Jun Wu

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