Molecules and Cells

Minireview

The Single-Cell Revelation of Thermogenic Adipose Tissue

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https://doi.org/10.14348/molcells.2022.0092
www.molcells.org

The past two decades have witnessed an upsurge in the appreciation of adipose tissue (AT) as an immuno-metabolic hub harbouring heterogeneous cell populations that collectively fine-tune systemic metabolic homeostasis. Technological advancements, especially single-cell transcriptomics, have offered an unprecedented opportunity for dissecting the sophisticated cellular networks and compositional dynamics underpinning AT remodelling. The "re-discovery" of functional brown adipose tissue dissipating heat energy in human adults has aroused tremendous interest in exploiting the mechanisms underpinning the engagement of AT thermogenesis for combating human obesity. In this review, we aim to summarise and evaluate the use of single-cell transcriptomics that contribute to a better appreciation of the cellular plasticity and intercellular crosstalk in thermogenic AT.

Keywords: adipose tissue, metabolism, obesity, single-cell sequencing, single-nucleus sequencing, thermogenesis

INTRODUCTION

The brown adipose tissue (BAT) is the metabolically active organ specialised in heat generation (thermogenesis). The presence of BAT in mammals is believed to confer survival advantages that allow organisms to survive and to be active during low ambient temperatures without hibernation and hypothermia (Cannon and Nedergaard, 2004). Compared with their energy-storing lipid-laden white counterparts, the brown adipocytes are characterised by smaller multilocular lipid droplet and high amounts of mitochondria packed with cristae and iron, where the latter gives BAT its brownish appearance. Similarly, the beige adipocytes, despite having distinct cellular progenitors and developmental lineages, are inducible thermogenic adipocytes arising from the white adipose tissue (WAT) and possess brown-like morphology and transcriptional landscapes upon cold exposure or adrenergic stimulation. Remarkably, these beige adipocytes can be readily interconverted between whitening and browning when exposing to warm and cold stimuli respectively (Nanduri, 2021; Roh et al., 2018). Once activated, the high potential of mitochondrial respiration of beige and brown adipocytes facilitates uncoupling of substrate oxidation and ATP production, dissipating energy in the form of heat through uncoupling protein 1 (UCP1) and contributing to up to 60% of total energy expenditure in small mammals (Carpentier et al., 2018; Cohen and Kajimura, 2021). Alternatively, UCP1-indepedent futile cycling of lipids and amino acids where ATP are consumed in the process of synthesis and degradation of triglycerides and peptides can also fuel heat production (Onogi and Ussar, 2022). Other futile circuits identified in beige adipocytes include the phosphorylation and dephosphorylation of creatine and the exchange of calcium ions between endoplasmic reticulum and cytosol (Ikeda et al., 2017; Kazak et al., 2015). In addition to be praised as an energy-burning furnace, the BAT also serves as the macronutrient sink for buffering circulating lipids, glucose and amino acids and

Received 3 June, 2022; revised 7 July, 2022; accepted 11 July, 2022; published online 25 September, 2022

eISSN: 0219-1032

helps regulate systemic metabolic homeostasis by secreting endocrine molecules known as the batokines (Chondronikola et al., 2016; Hankir and Klingenspor, 2018; Villarroya et al., 2017; Yoneshiro et al., 2019). Moreover, the fact that BAT confers cardiometabolic benefits independent of increasing energy expenditure alludes to its health-promoting potential and functional complexity (Becher et al., 2021; Gu et al., 2021; Kajimura et al., 2015; Mills et al., 2021). In addition to the efforts on leveraging the functionality of thermogenic adipocytes, insights are being made into the interplay and interconversion between the distinct cell types within the thermogenic adipose tissue (AT), which are benefited from the appreciation of cellular diversity and the identification of new but underrepresented cell type(s).

RESOLVING THE ADIPOSE SINGLE CELL MAP - A BRIEF HISTORY SUMMARY

In fact, early attempts in isolation, cloning and sorting of brown/beige adipocytes have hinted at the intrinsic cellular heterogeneity of AT, but such understanding can only scratch the surface due to the probable overrepresentation of highly-proliferating cells and the lack of characterisation into inter-cellular communication (Hagberg et al., 2018; Jespersen et al., 2019; Shinoda et al., 2015; Spaethling et al., 2016). Such challenges have been largely overcome in the era of single-cell genomics, where developments in single-cell RNA sequencing (scRNA-seq) and single-nucleus RNA sequencing (snRNA-seq) have allowed high-throughput profiling of transcriptomic signatures one cell at a time, leading to the appreciation on the diversity of adipose cell populations and accelerating advancements in the field of thermogenic AT research. The field of single-cell genomics has been rapidly evolved from manual picking of a few embryos to liquid-handling robotics by MARS (massively parallel single-cell)-seq enabling thousands of cells to be sampled (Jaitin et al., 2014; Tang et al., 2009). An exponential leap in the magnitude of cell numbers is achieved with the introduction of droplet-based platforms (10× Genomics Chromium, inDrop and Drop-seq) and the most recent *in situ* barcoding, which allows hundreds of thousands of cells to be sequenced with increasing rounds of split pool barcoding (Rosenberg et al., 2018; Zheng et al., 2017). Technological development on scRNA-seq is reviewed elsewhere (Svensson et al., 2018).

Due to the unique physical characteristics of AT, scRNA-seq is exclusively applied for investigating the relatively dense stromal-vascular fraction (SVF) of freshly harvested AT, as the floating fraction of buoyant adipocytes with various sizes cannot be dissociated into uniform single-cell suspension. Conversely, snRNA-seq, which bypasses harsh enzymatic dissociation, permits the recovery of major adipose cell types from either frozen or fresh tissues, and helps shed light on the exquisite networking between adipocytes and SVF components, especially the immune milieu. However, snRNA-seq can suffer from significant loss of reads, which may compromise the accuracy in distinguishing different cell types when based on transcripts only. Nevertheless, both technologies can face the challenge of contamination from ambient RNA (McLaughlin et al., 2022). A systematic comparison between different single-cell/nucleus sequencing platforms has been made elsewhere (Ding et al., 2020). With a focus on the single-cell toolbox, this review is aimed to offer the readers with the updated understanding on the heterogeneity of thermogenic adipocytes at single-cell resolution. Although prior attempts have been made to summarise the adipose single-cell atlas, specifically the adipose progenitors (Duerre and Galmozzi, 2022; Sun et al., 2021; Wang et al., 2022), efforts into delineating the crossroad between immunity and metabolism are lacking. A comprehensive discussion on each immune cell type is beyond our scope and has been covered by Trim and Lynch (2022), nonetheless, our review is aimed to highlight the pro-thermogenic inter-cellular crosstalk between immune cells and brown/beige adipocytes and their respective dynamics as revealed by single-cell transcriptomics (Tables 1 and 2). Future promises of single-cell technology and considerations when applying single-cell genomics data for human metabolic diseases will be discussed.

HETEROGENEITY OF BEIGE ADIPOCYTES

Perhaps one of the most prominent features of WAT plasticity is the dynamic interconversion of beige adipocytes by browning or whitening in response to environmental cues, but questions may be raised on: Are there multiple inputs that are capable of provoking beiging independently? Do different inputs signal different types/functionality of beige adipocytes? What are the modalities, in addition to histone modifications, that permit the beige adipocytes to swiftly adapt to the constantly evolving metabolic landscape? To answer these, Wang et al. (2016) analysed the morphologies and transcription signatures of *Ucp1*+ beige adipocytes following treatment of inhibitor of PPARγ phosphorylation (roscovitine), PPARγ agonist (rosiglitazone) and β3-adrenergic receptor (ADRB3) agonist (CL316,243) respectively. Although mice treated with the three browning agents are equally protected from diet-induced obesity (DIO) independent of BAT thermogenesis, roscovitine gives rise to a population of beige adipocytes with larger and fewer lipid droplets or paucilocular morphology compared with the classic multilocular morphology of adrenergic-induced beige adipocytes. Remarkably, when comparing to brown adipocytes, the beige adipocytes recruited from different browning agents constitute distinct populations with non-overlapping transcriptome (Wang et al., 2016). This work is later expanded by the identification of glycolytic beige adipocytes that originate from *MyoD*+ myogenic progenitors and devour glucose to produce heat under cold exposure and deprivation of ADRB3 signalling (Chen et al., 2019). Further insight into the thermogenic subpopulations comes from a recent report on the single-cell atlas of human and mice WAT scrupulously constructed by Emont et al. (2022). In human WAT, a subtype of potentially thermogenic adipocyte, which is unexpectedly enriched in visceral depot, is identified to express beige/brown markers *PPARGC1A* and *EBF2*, where the latter is documented to be a pro-browning transcription factor in mice inguinal WAT (iWAT) and BAT (Angueira et al., 2020; Wang et al., 2014). This subtype is also observed to have gene enrichment for mitochondrial respiration and axon guidance and have probable interactions with AT endothelial

Table 1. Single-cell studies on cellular heterogeneity of BAT

Species	Tissue depot, fraction	Techniques	Main findings			Reference
			Adipocytes	APC/preadipocytes	Immune cells	
E17.5 CD-1 mice	iBAT, mature	*In vitro* clonal and RNA-seq	Considerable variations in the expressions of brown marker genes (Ucp1, Adrb3, Cidea, Ppargc1a) between nine brown adipocytes.			Spaethling et al., 2016
10-week-old C57BL/6J male mice	iBAT, mature	scRNA-seq	Adipoq^{low}Ucp1^{low} low-thermogenic adipocytes marked by Fabp4/5, Cd36, Cldn5, Cav1/2.			Song et al., 2020
7-week-old AdipoCre-NucRed transgenic mice	iBAT, mature	snRNA-seq	10 adipocyte subpopulations identified in CE, RT, and TN. Thermogenesis-regulatory adipocytes marked by Cyp2e1, Aldh1a1, Nrip1, Auts2.			Sun et al., 2020
16 patients (4 males, 12 females), aged 49.2 ± 19.0 y, BMI 24.8 ± 4.7 kg/m^2	Deep-neck BAT, whole	snRNA-seq	Eight adipocyte subpopulations identified with a greater enrichment of CYP2E1+ ALDH1A1+ adipocytes compared to mice.			Sun et al., 2020
8-week-old, male C57BL/6J mice	iBAT, SVF	scRNA-seq and *in vitro* clonal	Eif5, Tcf25, Bin1 each mark three subtypes of brown adipocytes with varying expressions of UCP1 and different degrees of adrenergic sensitivity.			Karlina et al., 2020
C57BL/6N male mice	Thoracic aorta PVAT, SVF	scRNA-seq		Adipogenic progenitors (fibroblast) marked by Pdgfra and Pparg in neonates. Adipogenic progenitors (SMCs) marked by Myh11, Trpv1 in adults.		Angueira et al., 2021
3 male patients, aged 64 y, BMI 28.2 kg/m^2	Peri-aortic PVAT, whole	snRNA-seq		Fibroblastic preadipocytes marked by PPARg, COL15A1/COL4A4. Adipogenic SMC-like cells marked by PPARg, PDGFRb.		Angueira et al., 2021
9-week-old male C57BL/6J mice	iBAT, SVF	scRNA-seq		VSM-derived adipogenic progenitors marked by Trpv1 are recruited by cold exposure.		Shamsi et al., 2021

Table 1. Continued

Species	Tissue depot, fraction	Techniques	Main findings			Reference
			Adipocytes	APC/preadipocytes	Immune cells	
C57BL/6 J male mice	iBAT, SVF	scRNA-seq and MacSpectrum			Increased recruitment of Ly6^{low} patrolling monocytes and expansion of ATM with pre-activation states.	Rosina et al., 2022
C57BL/6 mice	iBAT, SVF	scRNA-seq			Three clusters of monocytes marked by different levels of Ly6c expressions and four clusters of macrophages involved in lipid handling and matrix remodelling. Adipocyte-specific ATGL deletion triggers increased monocyte recruitment and proportion of ATM with lipid-handling phenotype.	Gallerand et al., 2021

APC, adipose progenitor cells; iBAT, interscapular brown adipose tissue; CE, cold exposure; RT, room temperature; TN, thermoneutrality; BMI, body mass index; SVF, stromal vascular fraction; PVAT, perivascular adipose tissue; SMC, smooth muscle cell; VSM, vascular smooth muscle; ATM, adipose macrophages; ATGL, adipose triglyceride lipase.

cells (ATECs) and adipose macrophages (ATMs), especially during obesity (Emont et al., 2022). Somewhat surprising is the observation that mouse white adipocyte populations do not cluster in depot-specific manner and are not analogous to human adipocytes, where the thermogenic human adipocytes cannot be mapped to their mouse counterparts. Aligned with previous research, two subclusters within a mouse adipocyte population defined by expressions of *Ucp1, Prdm16* and *Ppargc1a* are relatively enriched in iWAT of female mice (Emont et al., 2022). An increasing appreciation on the species-dependent differences in thermogenic potential of WAT depots is needed when extrapolating animal data.

HETEROGENEITY OF BROWN ADIPOCYTES

The realisation that not all brown adipocytes are created equal but have varying degrees of *Ucp1* expressions and thermogenic potential can be traced back to 1985 by Cadrin et al. (1985). But it was not until 2016 when Spaethling et al. (2016) became one of the first to attempt the single-cell characterisation of cellular heterogeneity using nine mature brown adipocytes isolated from E17.5 mice embryos. Despite being identified as thermogenesis-capable, the levels of *Ucp1* expression can have more than 1,000-fold difference across these cells. The considerable variability of gene expression between individual adipocytes is also observed for *Adrb3*, implying different degrees of responsiveness to sympathetic innervation (Spaethling et al., 2016). This work is subsequently validated using a thermosensitive dye (ERthermAC)-based functional assay (Kriszt et al., 2017). Furthermore, the authors also reported that at least one tenth of the brown adipocyte populations failed to respond to adrenergic stimuli.

With the subsequent boom in scRNA-seq research and databases, several lines of evidence begin to peel back the layers of heterogeneity within brown adipocytes. The coexistence of distinct subpopulations of brown adipocytes is first alluded by Song et al. (2020) showing that brown adipocytes with high adiponectin expression constitute less than 40% of the adipocyte pool in BAT. scRNA-seq of isolated adipocytes revealed that the *Adipoq*^{low} brown adipocytes have significantly lower expressions of genes involved in mitochondrial respiration and thermogenesis. By contrast, these supposedly low-thermogenic adipocytes express genes enriched in fatty acid uptake, tight junctions, cell-cell trafficking and futile creatine cycling, which may help rescue the suppressed UCP1-dependent thermogenesis. Interestingly, the low-thermogenic adipocytes can be converted to high-thermogenic. But such functional plasticity seems to be lost during ageing, and cell population dynamics of the ageing AT have yet to be captured (Song et al., 2020).

Instead of being thermogenic professional, the notion that some brown adipocytes could be specialised regulatory cells is elaborated by Sun et al. (2020) using snRNA-seq of brown adipocytes. They identify a rare adipocyte population in mice interscapular BAT (iBAT) clustered by marker genes *Cyp2e1* and *Aldh1a1*, whose number is observed to increase and decrease upon thermoneutrality and cold challenge respectively (Sun et al., 2020). The *Cyp2e1+ Aldh1a1+* adipocytes also

Table 2. Single-cell studies on cellular heterogeneity of beige adipose tissue/adipocytes

Species	Tissue depot, fraction	Techniques	Main findings			Reference
			Adipocytes	APC/preadipocytes	Immune cells	
13 healthy patients, 10 females, 3 males	SAT and VAT, whole	snRNA-seq	Seven adipocyte subpopulations with depot-specific enrichment and correlations with BMI. Thermogenic adipocyte subpopulation marked by EBF2, PPARGC1A, ESRRG is found to be exclusively enriched in VAT.			Emont et al., 2022
19-week-old C57BL/6J female and male mice	iWAT and eWAT, whole	snRNA-seq	Six adipocyte subpopulations with diet-dependent enrichment. Two subclusters within mAd1 identified as thermogenic and marked by Prdm16, Ppargc1a, Ucp1, Cidea.			Emont et al., 2022
C57BL/6J male mice	iWAT, SVF	scRNA-seq		SMC-like APC marked by Cd81, Pdgfra, Sca1 give rises to beige adipocytes independent of stimuli. Number of CD81+ APC is inversely associated with metabolic syndrome in humans.		Oguri et al., 2020
Human	Abdominal SAT preadipocytes	*In vitro* clonal and scRNA-seq		Two-cluster separation of preadipocytes after 7- and 14-day differentiation driven by genes in protein synthesis, ECM remodelling and metabolism.		Ramirez et al..2020
8- to 10-week-old C57BL/6 mice	iWAT, mature and SVF	snRNA-seq and scRNA-seq	14 clusters of adipocytes. Adipocyte cluster nine characterised by thermogenic markers Adrb3, Lipe, Plin1 at baseline and increased expression of Ucp1, Ppargc1a and Cidea following cold/CL treatment.		Adipose-resident T- and B-cells produce IL10 antagonising IL10Ra+ thermogenic adipocytes.	Rajbhandari et al., 2019
8-week-old C57BL/6 mice	iWAT, SVF	scRNA-seq		Mesenchymal stem cell cluster two may represent adipogenic progenitor marked by Fabp4, Pdgfra.	13 immune cell clusters. Cold exposure and CL treatment favour the expansion of lymphoid- and myeloid-derived immune cells respectively, where cold and CL influences type I interferon response differently.	Rabhi et al., 2020
8-week-old male C57BL/6J WT and iAdFASNKO mice	iWAT, SVF	scRNA-seq			Increased M2 polarisation of ATM marked by Mgl2, Cd163 and Lyve1 and increased ratio of M2/M1 ATM.	Henriques et al., 2020

APC, adipose progenitor cells; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; BMI, body mass index; iWAT, inguinal white adipose tissue; eWAT, epididymal white adipose tissue; ECM, extracellular matrix; IL, interleukin; CL, beta-3 adrenergic agonist; WT, wild type; ATM, adipose macrophages.

have reduced *Ucp1* expression and smaller mitochondria with disorganised cristae that has also been observed in brown adipocytes from *Ucp1*-KO and *ChREBP*-KO mice, suggesting diminished mitochondrial oxidative capacity (Cinti, 2018; Sakiyama et al., 2021). Remarkably, despite being the minority (2.9%) within iBAT, these adipocytes can become major contributors to systemic energy expenditure in cold-exposed mice after cell-specific deletion of *Aldh1a1*, whose expression is also negatively correlated with oxygen consumption rate (OCR) and *Ucp1* levels. Moreover, loss or gain of *Aldh1a1* in brown adipocytes is also observed to modulate levels of acetate, which signals through G-coupled protein receptor 43 (GPR43) to suppress BAT thermogenesis (Sun et al., 2020). Note that previous study using immortalised brown adipocytes suggests beneficial influences of acetate-GPR43 signalling on *Ucp1* expression, mitochondrial biogenesis and OCR during cell differentiation (Hu et al., 2016). Intriguingly, expression of *Cyp2e1* diminish in the white adipocyte pools following high-fat diet (HFD), which is accompanied with enrichment in cell cycle genes (*Sept9* and *Cdkn1a*) (Emont et al., 2022). Additionally, in both rodent and human BAT, the *Cyp2e1+* adipocytes are observed to acquire both unilocular and multilocular morphologies resembling white and classical brown adipocytes respectively. Together, these may raise the question on whether the expressions of *Cyp2e1* and *Aldh1a1* represent a transient cell state or a *bona fide* adipocyte subpopulation. Moreover, given that acetate has shown to have immunomodulatory actions on other tissues (Daïen et al., 2021; Macia et al., 2015), one may propose that the adipocyte acetate-GPR43 signalling could have ripple effects over adipose immune reservoir, which in turn, can turn up or off the heat.

IMMUNE-ADIPOCYTE CROSSTALK IN THERMOGENESIS

In fact, while thermogenic adipocytes may take the lead on stage, there is increasing appreciation on the regulatory actions of adipose immune cells during thermogenesis through intercellular crosstalk. Several thermogenesis-supportive candidates have been identified, including the γδT-cells, type 2 innate lymphoid cells (ILC2), invariant natural killer T-cells (iNKT), eosinophils and the Slit3-expressing M2-like macrophages (Brestoff et al., 2015; Kohlgruber et al., 2018; Lee et al., 2015a; Lynch et al., 2016; Rao et al., 2014; Wang et al., 2021). The pro-thermogenic actions of these immune cells are often significant and incontrovertible in the context of beigeing, while they are found to have little or no impact on BAT thermogenesis (Brestoff et al., 2015). On the other hand, more efforts are required to help understand the depot-specific difference in immunometabolism. Nevertheless, previous studies were predominantly relying on flow cytometry and cell sorting, which might have offered a relatively myopic perspective on the AT immune dynamics. Instead, by combining single-cell and single-nucleus sequencing on mice iWAT collected after cold or adrenergic stimuli, Rajbhandari et al. (2019) elegantly depicted the immune-adipose crosstalk as a regulatory mechanism of beige thermogenesis. They demonstrated that a distinct population of adipocytes characterised by gene enrichment in fatty acid metabolism and norepinephrine signalling (*Adrb3, Lipe, Plin1, Pnpla2*) can become highly responsive to adrenergic or cold stimuli through upregulating brown/beige markers, underscoring their high-thermogenic potential (Rajbhandari et al., 2019). Specifically, mice with adipocyte-specific knockout of interleukin (IL)10 receptor (*AdIL10Ra*-KO), which are obesity-resistant, acquire significant expansion of the *Adrb3*^{high} adipocytes in their subcutaneous AT especially during adrenergic stimulation, which is accompanied with alterations in genes involved in cell trafficking and lipid uptake. The authors subsequently revealed that lymphocytes, specifically T- and B-cells are the major producers of IL10 in response to ADRB3 agonism, which preferentially antagonise the activity of the *Adrb3*^{high} adipocyte through IL10-IL10R signalling (Rajbhandari et al., 2019). However, adrenergic stimulation seems to be only one of the missing puzzles behind thermogenesis, since BAT from mice with triple adrenergic receptor knockout still preserves its sympathetic innervation and can generate heat through alternative purinergic induction (Razzoli et al., 2015). Similarly, the ADRB3 agonist mirabegron has shown to have little or no influence over resting energy expenditure, lipid/carbohydrate oxidation and BAT fat fraction in South Asians, whereas cold exposure induces significant metabolic and thermogenic responses in the same participants (Nahon et al., 2020). In favour of the argument against adrenergic-induced thermogenesis, Rabhi et al. (2020) performed scRNA-seq on beige fat stromal vascular fraction and demonstrated that cold and ADRB3 agonist treatment led to different patterns of immune remodelling of AT. An expansion of adipose immune cells with myeloid origins and upregulation of type-1 interferon signalling and extracellular matrix remodelling is observed upon agonism of ADRB3, whereas cold induces a shift of immune populations to lymphoid origins, including B-cells and T-cells, and suppression of interferon signalling and Stat1 phosphorylation (Rabhi et al., 2020). In fact, both Lynch's group and Spiegelman's group have shown that cold exposure predominantly nudges the PLZF+ *Vγ6+* γδT cells to expand their populations and to produce IL17, which directly acts on the IL17-receptor of brown adipocytes to favour thermogenesis and sympathetic innervation (Hu et al., 2020, Kohlgruber et al., 2018). By contrast, interferon produced by Th1 and CD8+ T-cells has been shown to suppress HFD-induced thermogenesis but spare cold-induced thermogenesis of brown adipocytes (Zhou et al., 2021). Moreover, despite cold is associated with a shift from myeloid to lymphoid identity, an exquisite neuro-immune pathway governing sympathetic arborisation and axonal growth in iWAT has been proposed, where cold induces the activation of IL5-producing ILCs and the subsequent recruitment of eosinophils producing nerve growth factor (Meng et al., 2022). By combining snRNA-seq and scRNA-seq, further study could help uncover the diversity of sympathetic neurons innervating BAT and their corresponding interactions with the SVF and adipocytes during thermogenesis.

Another fascinating piece of work comes from Rosina et al. (2022), who demonstrated that ATM and monocytes are key BAT housekeepers for removing damaged mitochondrial components released by the cold-stressed brown

adipocytes. In their study, scRNA-seq combined with MacSpectrum analysis, which is an algorithm designed to resolve macrophage activation states and functional profile (Li et al., 2019), demonstrates an increased expansion of ATM with less-inflammatory pre-activation phenotype following cold exposure, which are differentiated from *Ly6c*^{low} patrolling monocytes and show higher expressions of genes involved in NRF2 response and production of reactive oxygen species. These pre-activated ATM are observed to internalise adipocyte-derived extracellular vesicles carrying mitochondrial debris and oxidative wastes, hence preventing the deterioration of cold-induced thermogenesis and sustaining BAT antioxidant defence (Rosina et al., 2022). This study beautifully illustrates that cold exposure is also a potent stimulus for eliciting widespread reconditioning of BAT immune reservoir. Future research may help uncover detailed mechanisms on cold-mediated signalling in immune cells and whether the immune cells have intrinsic 'cold sensor'. In addition to the canonical catecholamine-mediated thermogenesis, a macrophage-dependent but innervation-spared pathway for thermogenesis by beige adipocytes has been identified in mice with adipocyte-specific ablation of fatty acid synthase (iAdFASNKO). Based on results from scRNA-seq and FACS of ATM populations, the authors observed an increased polarisation of ATM to the *Cd163+ Lyve1+* M2-like ATM, whose signalling is indispensable for beiging in iAdFASNKO mice. Moreover, the macrophage-adipocyte crosstalk rescues the suppressed thermogenesis by sympathetic denervation, where the beige adipocytes upregulate cAMP/PKA signalling independent of ADRB3 activation (Henriques et al., 2020). A key question to be asked is how ATM enables the beiging reprogramming in adipocytes deprived of catecholamines. Generally, there is pervasively lack of appreciation on the functionality shift of adipose immune cells across varying beiging/browning cues. scRNA-seq studies nevertheless offer a compelling piece of evidence that thermogenesis, independent of stimuli, is accompanied with and sometimes dependent on the remodelling of adipose immune repertoire. Besides thermogenesis, it should not be forgotten that the adipocyte-immune axis is also crucially involved in BAT tissue homeostasis, where monocyte recruitment to BAT support healthy tissue expansion during lipolysis ablation without instigating overt inflammation and formation of crown-like structures. Likewise, the diversity of ATM and monocyte populations and their roles warrant genetic engineering targeting specific cell type and possibly single-clone transplantation assay for functionality assessment (Gallerand et al., 2021).

HETEROGENEITY OF THERMOGENIC PRECURSOR

In addition to potentiating thermogenesis of existing brown adipocytes, cold or adrenergic stimulation of BAT also facilitates local proliferation and adipogenic commitment of thermogenic precursor and progenitors (APCs) that licenses greater heat-generating capacity (Bukowiecki et al., 1986; Nedergaard et al., 2019). Lineage-tracing study has shown that BAT de novo adipogenesis is achieved mainly through recruiting the *Pdgfra+* progenitors, whose populations expand by 4-fold during cold stress and differentiate into brown adipocytes located near the edges of BAT (Lee et al., 2015b). Subsequently, single-cell genomics offers the glimpse into the heterogeneity of APCs. By combining scRNA-seq and *in vitro* clonal, Karlina et al. (2020) were able to interrogate distinct developmental lineages of brown pre-adipocytes, which give rise to brown adipocytes with varying degrees of *Ucp1* expression and adrenergic sensitivity. Interestingly, the authors found that cell clustering based on scRNA-seq alone cannot differentiate cellular states and lineages, where genes controlling preadipocyte differentiation are overrepresented (Karlina et al., 2020). In addition to *Pdgfra+* mesenchymal progenitors, a novel population of smooth muscle cell (SMC)-derived APCs expressing *Trpv1* has been shown to contribute to 7.2% of brown adipocyte pool and 10.5% of the beige population in iWAT during cold exposure, where the identity and functionality of *Trpv1+* APCs are identified and validated using scRNA-seq coupled with trajectory analysis and lineage-tracing labelling (Shamsi et al., 2021). The expression of *Trpv1*, which encodes the vanilloid receptor sensing heat and pain, may imply the role of local sensory network in BAT governing thermogenesis and *de novo* adipogenesis independent from central innervation (Szallasi et al., 2007). Similarly, by studying the perivascular AT (PVAT), which is a brown-like depot, Angueira et al. (2021) discovered two distinct APC lineages during the neonatal and adult periods, including the fibroblastic preadipocytes expressing *Pdgfra* and *Pparg* and SMC-like APCs marked by *Trpv1* and *Myh11*, where the latter is found specifically in adult stage. Such finding contrasts with previous study reporting the selective expression SMC markers in beige but not brown adipocytes (Long et al., 2014). Nevertheless, human PVAT is also found to harbour fibroblastic and SMC-like preadipocytes analogous to the mice counterparts, which hints at the clinical significance of thermogenic preadipocytes (Angueira et al., 2021).

Conversely, the modalities of cold-induced beige fat recruitment in mice are somewhat controversial. Beiging has been proposed to occur either through de novo adipogenesis from beige progenitors, trans-differentiation of pre-existing adipocytes or a combination of both (Shao et al., 2016; Wang et al., 2013). Although ADRB3 agonist treatment preferably activates trans-differentiation of white adipocytes (Himms-Hagen et al., 2000; Lee et al., 2015b), the presence of diverse beige populations as discussed previously may favour the argument supporting beige adipogenesis, and findings from scRNA-seq further elaborate on the heterogeneity of beige APCs arising to different stimuli. Through scRNA-seq of non-immune SVF fraction, Oguri et al. (2020) identified *Cd81* as a marker for SMC-like beige APC (*Acta2+, Sm22+, Pdgfra+),* where CD81 also functions as a critical regulator in cold-induced beiging through mediating irisin-induced integrin-FAK signalling and whole-body metabolism. Importantly, the number of *CD81+* APCs is negatively correlated with metabolic syndrome in humans (Oguri et al., 2020). Although scRNA-seq of human subcutaneous preadipocytes has failed to identify APCs expressing beige markers, which can be attributed to the chronic exposure of thermoneutrality in humans, Ramirez et al. (2020) nevertheless identify a cluster expressing *Tbx15*, which is a marker of glycolytic adipocyte and has shown to be crucial for beige thermogenesis

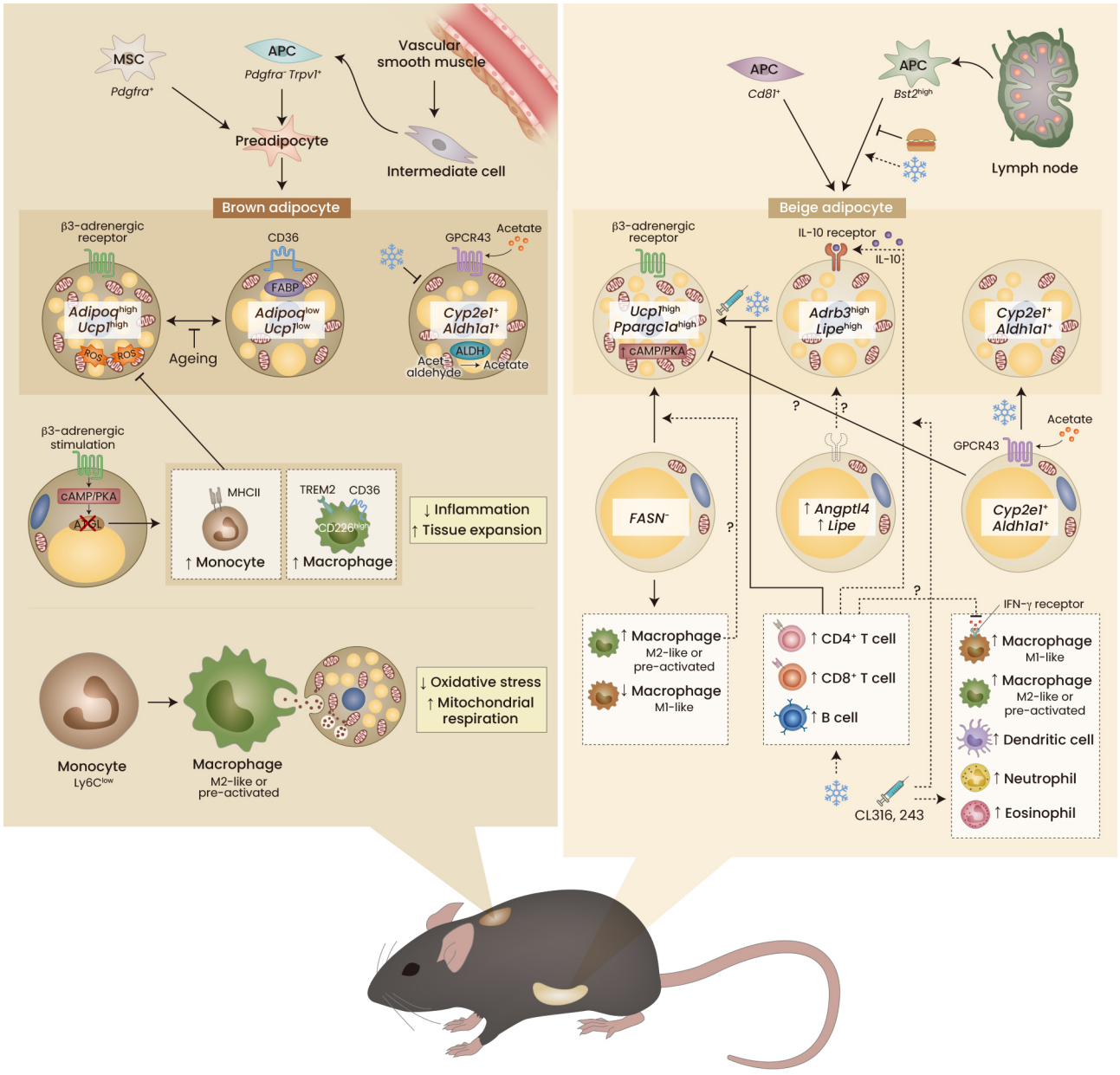

Fig. 1. Intercellular heterogeneity and cell-cell communication of brown (left) and beige (right) AT revealed by single-cell transcriptomics. Left panel: there are at least three subpopulations of brown adipocytes with different degrees of thermogenic potential and adrenergic sensitivity, with the *Cyp2e1*$^{+}$ adipocyte playing a regulatory role. Deletion of ATGL in brown adipocytes is accompanied with enrichment of MHCII-expressing monocytes and *CD226*^{high} macrophages that could support tissue expansion and minimise overt inflammation. Conversely, monocyte depletion favours the multilocular morphology of brown adipocytes. Macrophages derived from *Ly6C*^{low} monocytes serve as scavengers of damaged mitochondria and cell wastes excreted by brown adipocytes, which help prevent oxidative stress and sustain mitochondrial respiration. Right panel: cold and CL treatment lead to distinctive immune landscapes characterised by enrichment of either adaptive or innate immune cells. The lymphoid cells can produce IL10 that downregulates the thermogenic reprogramming of beige adipocytes, while the adipose lymph node is suggested to support beige adipogenesis from *Bst2*^{high} precursors. Note that the question mark labels proposed intercellular interaction that is yet to be validated. ALDH, aldehyde dehydrogenases; ATGL, adipose triglyceride lipase; UCP1, uncoupling protein-1; ROS, reactive oxygen species; FABP, fatty acid binding protein; FASN, fatty acid synthase; PKA, protein kinase A; CL, beta-3 adrenergic agonist; APC, adipose progenitor cells; GPCR, G-protein coupled receptor; IL, interleukin; IFN, interferon; TREM2, triggering receptor expressed on myeloid cells-2. Fig. 1 was created with BioRender.com.

through interaction with *Prdm16* promoter (Ramirez et al., 2020; Sun et al., 2019). Recently, by comparative analysis between single cell atlas of lineage negative cells from visceral and subcutaneous WAT, the elegant work from Kim's group characterized *Bst2*^{high} APCs as beige adipocyte precursors whose biogenesis is regulated by lymph node (Nahmgoong et al., 2022). Moreover, ATMs have been shown to facilitate adipogenesis through clearing of dead adipocytes and participating in extracellular remodelling in visceral AT (Burl et al., 2018), but their interactions with the thermogenic APCs are yet defined. Whether the different thermogenic APC populations can communicate with and even interconvert between one another can be an area of future research.

CONCLUDING REMARKS

The era of high-dimensional single-cell genomics has revolutionised our understanding of the heterogeneity of thermogenic AT and its elegant adipocyte-immune network (Fig. 1). Nonetheless, great promises of sc(n)RNA-seq come with many pitfalls. Specifically, statistical robustness and accuracy can be afflicted by the ever-expanding but inconsistent single-cell datasets. Technical and statistical challenges faced by single-cell genomics has been eloquently discussed in recent reviews (Lähnemann et al., 2020; Sun et al., 2021). Additionally, it is critical for studies to apply consensus markers for cell type annotation. Computational platforms, like SingleR, have enabled the automatic definition of cell subcluster, but such tools are often dependent on data availability of specific cell markers (Aran et al., 2019). Concurrently, stringent quality control should be applied to minimise noise-to-signal ratio and cell-free RNA contamination, while allowing high-throughput and the identification of rare populations. Distinguishing between acquired cell states responding to certain stimuli and cell populations with different ancestors using benchmark dataset is critical for developing cell-targeted therapy. Importantly, one should not miss the forests for the trees, as single-cell transcriptomic is often the tip of the iceberg, where inference of cell trajectory, cell-cell communication and their functionality dynamics requires integration from multiple types of data, including proteomics, metabolomics, and multi-omics, where the latter has been recently shown to hold great potential in teasing apart the epigenetic regulatory network licencing specific cell fates (Argelaguet et al., 2019). Likewise, the computer algorithm MEBOCOST combining scRNA-seq transcriptome with the Human Metabolome Database has recently shown to be a promising tool in interrogating intercellular metabolite-sensor communication in BAT (Zheng et al., 2022). Furthermore, despite the relatively scant discussion on BAT immunometabolism, recent report has strikingly laid out the divergence between thermogenesis and metabolic health, where the inflamed BAT promotes systemic insulin sensitivity through enhancing glucose uptake by other metabolic organs, while protecting against lipotoxicity and DIO at the expense of its energy-burning capacity (Huang et al., 2022). Additionally, there is emerging recognition on the central involvement in BAT thermogenesis, where the oestrogen receptor-expressing or heat-sensing neurons has shown to modulate BAT activity and whole-body metabolic rates (Makwana et al., 2021; Ye et al., 2022). Conversely, the BAT has sensory nerve outflow projecting the hindbrain, midbrain and forebrain regions and is also proposed to mediate the appetite-suppressing actions of secretin via the BAT-brain crosstalk (Ryu et al., 2015; Sun et al., 2022). In consideration of these, a more holistic approach resolving and integrating spatial single-cell multi-omics in brain and AT may help generate new insights into cell-cell interactions, functionality compartmentalisation and interorgan crosstalk governing the functional diversity of thermogenic AT.

ACKNOWLEDGMENTS

This work is supported by the National Natural Science Foundation of China (NSFC) - Excellent Young Scientists Fund (Hong Kong and Macau) (81922079) and General Research Fund (17121520) (to X.H.H.).

AUTHOR CONTRIBUTIONS

Y.Q. wrote the original draft and contributed to the tables and figure. X.H.H. revised and edited the manuscript.

CONFLICT OF INTEREST

The authors have no potential conflicts of interest to disclose.

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