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New models of adipogenic differentiation highlight a cell-autonomous response to temperature

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Abstract: Temperature is a key regulator of brown adipose tissue (BAT) function, acting through central sensory inputs to influence metabolism and energy storage. Although animal models have produced a wealth of information on the pathways, effectors and responses mediating the physiological response of adipose tissue to temperature *in vivo*, the use of cell culture models now offers evidence of an additional cell-autonomous response to temperature changes, in the absence of neural input. In particular, stem cell models provide new insight into the regulation of adipogenic differentiation and the induction of browning features *in vitro*. Here the basis for adipogenic responsiveness to low temperature is discussed, together with different human cell models available to outline the benefits of cell-based approaches for future BAT research.

Abbreviations

WAT	White adipose tissue
BAT	Brown adipose tissue
UCP1	Uncoupling protein 1
TRP	Transient receptor potential
SNS	Sympathetic nervous system
NE	Norepinephrine
β3-ARs	β3-adrenergic receptors
PKA	Protein kinase A
PPARγ	Peroxisome proliferator-activated receptor-gamma
PGC-1α	PPARγ-coactivator-1-alpha
iPS	Induced pluripotent stem cells
BM	Bone marrow
MSC	Mesenchymal stromal cells (MSC)
SERCA2b	Sarco-endoplasmic reticulum Ca^{2+} -ATPase 2b
TRPV1-4	Transient receptor potential cation channel subfamily V member 1-4
TRPM8	Transient receptor potential cation channel subfamily M member 8.

Introduction

Adipose tissue exerts important physiological roles in health and disease, including endocrine, metabolic and thermal control. In mammals, at least three types of adipose tissue exist, white (WAT), brown (BAT) and beige adipose tissue (Cinti, 2012; Chechi *et al.*, 2018). They differ in anatomical location, morphology, and physiological role. WAT acts as the main site of metabolic energy storage and excessive accumulation leads to obesity and associated pathologies such as diabetes type 2, cardiovascular diseases, several cancers and osteoporosis (Bray *et al.*, 2017). In contrast, BAT is a major site of thermogenesis in small mammals, hibernators, and infants. It can be activated by a range of stimuli including cold exposure, nutrients and adrenergic receptor agonist (Himms-Hagen *et al.*, 1994; Klingenspor, 2003; Westerterp-Plantenga *et al.*, 1999). The unique role of BAT is due to the presence of a specific mitochondrial uncoupling protein 1 (UCP1), which uncouples oxidative phosphorylation from ATP synthesis, thus burning stored energy by generating heat (Himms-Hagen, 1990). When

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maximally activated, BAT generates up to 300 times more heat per mass unit than any other organ in the body (Power, 1989; Symonds *et al.*, 2015) which hints at a potential role in energy dissipation that could be useful for obesity regulation.

Alongside their distinct physiological roles, WAT and BAT adipocytes also exhibit different morphological features. Unlike white adipocytes, which are identifiable by a large lipid droplet, flattened nucleus, and a low number of mitochondria, brown adipocytes are characterised by numerous small lipid droplets, centrally positioned nucleus, and abundant mitochondria (Cinti, 2005; Suter, 1969). Clusters of UCP1-expressing cells, so-called beige or brite adipocytes have also been found in some WAT depots, and their activation is referred to as browning (Bartelt and Heeren, 2014; Nedergaard and Cannon, 2014). The most studied physiological mechanism known to induce browning of white adipocytes is based on cold exposure (Van Der Lans *et al.*, 2013; Walden *et al.*, 2012), however, browning can also be induced by nutritional and pharmacological activators such as capsaicin, fibroblast growth factor-21 (FGF21) (Kim *et al.*, 2013) or mirabegron, a highly β3-selective adrenergic agonist (Barbatelli *et al.*, 2010; Finlin *et al.*, 2018; Petrovic *et al.*, 2010). When fully stimulated, beige cells are able to increase lipolysis, lipid droplet number, mitochondrial density and show varying levels of UCP1 in different adipose depots, hinting to a thermogenic capacity typically seen in BAT (Nedergaard and Cannon, 2013; Ohno *et al.*, 2012; Vitali *et al.*, 2012), and could thus serve as a model to study a cell-autonomous response to temperature (Velickovic *et al.*, 2018).

Several lines of evidence suggest that the browning process could play a role in controlling whole-body energy balance by promoting increased energy consumption at the expense of excessive adipose storage in the form of WAT (Kim and Plutzky, 2016; Quesada-Lopez *et al.*, 2016; Shabalina *et al.*, 2013), thus contributing to better metabolic health. In both human and rodent models, the main areas containing brown adipocytes are recognised for having a greater density of capillaries and noradrenergic fibres when compared to WAT regions (Cinti, 2000, 2005; Zingaretti *et al.*, 2009). Using glucose or fatty acid imaging tracers alongside a morphological and molecular characterization, Zhang and colleagues showed a high similarity in the distribution of brown and beige depots in humans and rodents (Zhang *et al.*, 2018). Human BAT is heterogeneous, consisting of both brown and beige cells; their proportion depends on anatomical location and varies according to age and anatomical region (Bartelt and Heeren, 2014). Classical brown fat depots in infants and rodents are mainly distributed around the interscapular space in the upper back region and perirenal sites (Park *et al.*, 2014). The main BAT fat depots in adult humans are located within the supraclavicular, cervical, axillary, and paravertebral regions, with a predominantly beige molecular signature in the supraclavicular area and a classical brown adipocyte signature in the deep neck. Mouse inguinal adipose tissue is the largest depot able to recruit beige adipocytes upon chronic cold exposure (Walden *et al.*, 2012) while it was recently reported that human brown fat depots contain beige adipocytes showing increased thermogenesis and molecular characteristics similar to murine beige cells (Wu *et al.*, 2012).

All adipocytes originate from a mesodermal progenitor (Park *et al.*, 2014; Seale *et al.*, 2008). Although beige adipocytes share many features with brown adipocytes, they are associated with different marker genes (De Jong *et al.*, 2015; Sharp *et al.*, 2012; Wu *et al.*, 2012) and developmental source (Sanchez-Gurmaches *et al.*, 2012; Timmons *et al.*, 2007). It has been shown that brown adipocytes arise from cells expressing the homeobox gene Engrailed 1 (En1). These En1 expressing cells give rise to brown adipocytes in anterior depots, while En1 negative cells contribute to BAT, muscle and dermis development. Brown adipocytes precursors also express myogenic factor 5 (Myf5) and paired box 7 (Pax7) (Lepper and Fan, 2010). Furthermore, embryonic brown adipocyte precursors express the helix-loop-helix transcriptional factor early B-cell factor 2 (EBF2) and are able to differentiate into brown adipocytes expressing UCP1 and PRDM16 (Seale *et al.*, 2008; Wang *et al.*, 2014). Depending on their level of Pax7 expression, cells can commit to brown adipogenic or skeletal muscle lineage, respectively (Lepper and Fan, 2010). Moreover, beige adipocytes arise from heterogeneous populations of adipogenic precursors and can be identified by the expression of CD137, transmembrane protein 26 (TMEM26), proton-coupled amino acid transporter 2 (PAT2) and P2X purinoreceptor 5 (P2RX5) surface markers. In addition to skeletal muscle and BAT, myogenic factor 5 (Myf5)-positive precursor cells also have the potential to give rise to white and beige adipocytes (Ambele *et al.*, 2020) as recent studies have confirmed that white adipocytes from the interscapular, anterior and retroperitoneal depots originate from Myf5 expressing cells (Sanchez-Gurmaches and Guertin, 2014).

Physiological temperature response

BAT is highly responsive and rapidly adapts to changes in environmental temperature, and cold exposure is considered to be the primary physiological stimuli for BAT activation (Cannon and Nedergaard, 2004; Klingenspor, 2003). Exposure to cold activates transient receptor potential (TRP) channels present in skin sensory nerves, which in turn, send signals to the brain (Wetsel, 2011). A complex process of thermogenic stimulation of BAT then follows, coordinated by the sympathetic nervous system (SNS) (Bamshad *et al.*, 1999). BAT is highly innervated by numerous sympathetic nerve fibres which release norepinephrine (NE), the main neurotransmitter. NE stimulates alpha- and beta-adrenergic receptors. β3-adrenergic receptors (β3-ARs) are considered the most important adrenergic receptors mediating thermogenic action. Coupled to adenylate cyclase, β3-ARs mediate a rise in intracellular cAMP, which in turn activates the cAMP-dependent protein kinase A (PKA) and phosphorylation of the transcription factor response element-binding protein, CREB (Zhang *et al.*, 2004). This canonical pathway leads to increased lipolysis (Himms-Hagen, 1990) and releases fatty acids, which can activate mitochondrial UCP1 and serve as an energy source for thermogenesis (Matthias *et al.*, 2000).

Cold exposure triggers dynamic structural and functional alterations in BAT, including UCP1 activation (Klaus *et al.*, 1991), hyperplasia and hypertrophy (Bukowiecki *et al.*, 1986), increased mitochondriogenesis (Puigserver *et al.*, 1998; Suter, 1969), but also enhanced BAT vascularisation (Hausman and Richardson, 2004) and innervation (Geloen *et al.*, 1992). At the molecular level, these changes are under the control of numerous transcriptional factors and coactivators, particularly peroxisome proliferator-activated receptor gamma (PPARγ) and PPARγ-coactivator-1-alpha (PGC-1α) (Petrovic *et al.*, 2010; Uldry *et al.*, 2006). A reduction in environmental temperature can also promote beige cell activation (Barbatelli *et al.*, 2010; Himms-Hagen *et al.*, 2000). Moreover, BAT activation by cold exposure has been reported in several human studies (Hanssen *et al.*, 2016; Law *et al.*, 2018; Saito *et al.*, 2009; Van Der Lans *et al.*, 2013; Van Marken Lichtenbelt *et al.*, 2009) underlining the important role of temperature on brown adipocyte thermogenic activation as an evolutionarily conserved feature.

Numerous small animal models, such as chemical and surgical denervation (Festuccia *et al.*, 2010; Takahashi *et al.*, 1992) have demonstrated a crucial role of SNS in BAT thermogenesis and WAT browning, which involve different adrenergic receptors (Cinti *et al.*, 2002; Jimenez *et al.*, 2003; Nedergaard and Cannon, 2014). Although it is widely accepted that the predominant β-adrenoceptor subtype in brown and beige adipose tissue is the β3-adrenoceptor (Cinti *et al.*, 2002; Himms-Hagen, 1990; Ramseyer and Granneman, 2016), recent studies shown that, in contrast to rodents, human BAT thermogenesis occurs through β2-AR signalling both *in vivo* and *in vitro* (Blondin *et al.*, 2020; Jocken *et al.*, 2007; Schiffelers *et al.*, 2001). Along with SNS input, the parasympathetic nervous system is involved in the regulation of energy expenditure and energy intake (Bartness *et al.*, 2010), for which two small BAT depots, mediastinal and pericardial, are parasympathetically innervated (Giordano *et al.*, 2004; Schafer *et al.*, 1998). Thus, there is a complex interplay between SNS and sensory nerves mediating the response of adipocytes to temperature.

BAT is the main site of non-shivering thermogenesis, and since thermogenesis is impaired in UCP1 deficient mice, the role of UCP1 could be interpreted as indispensable in this process. However, several studies carried out in UCP1-deficient mice hint at the possible existence of an alternative thermogenic mechanism to maintain body temperature (Liu *et al.*, 2003; Olsen *et al.*, 2017; Ukropec *et al.*, 2006). Besides cold exposure, other factors have been observed to affect browning including calcium, creatine and N-acyl amino acids, mediating a UCP1-independent thermogenic process (Bertholet *et al.*, 2017; Ikeda *et al.*, 2017; Kazak *et al.*, 2015; Long *et al.*, 2016; Ukropec *et al.*, 2006). Although the mechanisms involved in UCP1-independent thermogenesis are unclear, they could involve an ATP-consuming process specific for beige adipocytes (Anunciado-Koza *et al.*, 2008; Ikeda *et al.*, 2017; Kazak *et al.*, 2015). The possibility that temperature changes could also induce an adipogenic response in a cell-autonomous manner is an open question, which requires *in vitro* models to explore the thermogenic responsiveness in adipocytes in the absence of sympathetic and sensory inputs/outputs.

In vitro models for BAT research

Although research on BAT has largely relied on *in vivo* models to study functional responses to physiological stimuli, the number of translational cell models available for research is expanding. Samples extracted from pheochromocytomas, an adrenal tumour type associated with increased BAT propensity (Vergnes *et al.*, 2016; Wang *et al.*, 2011), have provided a source of human mesenchymal-like stromal progenitors with demonstrated inducible browning ability (Di Franco *et al.*, 2014). More recently, a non-pathological human alternative to the pheochromocytoma model has emerged through pluripotent stem cells. Yao *et al.* (2019) reported a detailed treatment for the production of BAT-like cells from induced pluripotent stem cells (iPS) originating from neural cells (Hafner *et al.*, 2018; Yao *et al.*, 2019). These cells were used to identify new brown lineage markers such as PR domain containing 16 (PRDM16), Iodothyronine Deiodinase 2 (Dio2) and paired box gene 3 (Pax3) (Mohsen-Kanson *et al.*, 2014), and highlighted differences in the signals required by primary tissue-derived cultures to achieve lineage commitment, such as the inhibitory effect of the transforming growth factor β (TGFβ) pathway on brown adipogenesis (Hafner *et al.*, 2016). Adipose tissue itself has produced several culture models for BAT studies. Cells harvested from neck tissue, known to harbour BAT-like cells within the supraclavicular region, have been successfully cultured and differentiated to a brown phenotype (Lee *et al.*, 2014; Liu *et al.*, 2019). Human subcutaneous WAT samples were also used to yield multipotent progenitor cultures induced to acquire molecular and functional brown features over a 2-week treatment *in vitro* (Elabd *et al.*, 2009). This process was found to involve PPARγ signalling, as also observed in mouse cultures (Merlin *et al.*, 2018; Petrovic *et al.*, 2008). Different tissue sources beside traditional adipose depots have been used to isolate cells with inducible browning features. Mesenchymal stromal progenitors from the bone marrow (BM) can acquire brown-like features *in vitro* (Velickovic *et al.*, 2018), adding to the repertoire of cell models for BAT research. The mammary gland may offer a further source of cells with browning capacity, as recently hinted for mouse mammary alveolar epithelial cells (Giordano *et al.*, 2017). Progenitors isolated from separate anatomical locations might, however, vary in their differentiation potential or signalling requirements, highlighting the need to compare cultures prepared from WAT, BAT, BM, and other sources to identify potential tissue-specific regulators of BAT induction.

In vitro evidence outlining a cell-autonomous effect of low temperature on beige/brown adipogenic differentiation and activation

Evidence of cell-autonomous responses to temperature has arisen from studies carried out in a range of cell types, including adipocytes but also myocytes, chondrocytes and osteoblasts, which share a common mesenchymal origin (Caplan, 1991; Pittenger *et al.*, 1999; Prockop, 1997). Typically, mesenchymal stromal cells (MSC) culture *in vitro* is performed at 37°C, chosen for optimal growth for most mammalian cells (Watanabe and Okada, 1967). Both

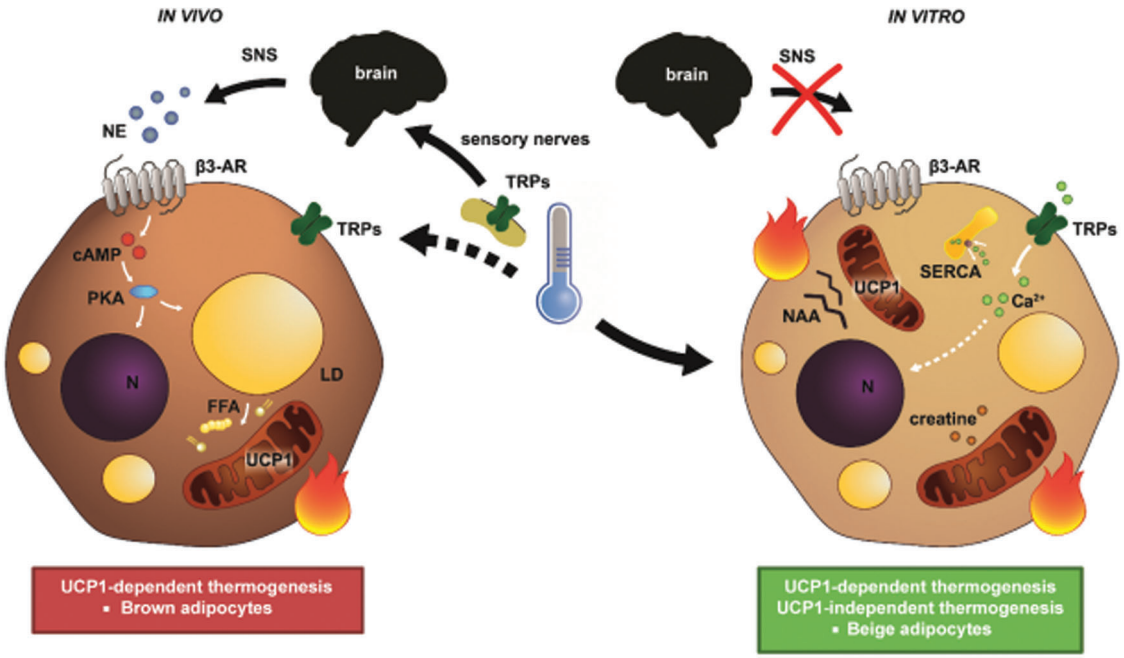

FIGURE 1. Cell thermogenesis mechanisms.
In vivo, the UCP1-dependent pathway involves cold-activated TRPs on sensory neurons, which transmit information on temperature changes to the brain and activate the sympathetic nervous system (SNS). Norepinephrine (NE) release and adrenergic activation increases cAMP levels and promotes a cascade of signals leading to the release of free fatty acids (FFA). Although several TRPs are involved in the TRPs-SNS-BAT axis in vivo, possible TRPs direct activation in brown adipocytes in BAT remains to be clarified (dashed arrow). In addition, beige cells are able to respond to temperature changes in cell-autonomous manner, with an alternative UCP1-independent thermogenic mechanism affecting adipogenic gene expression. NAA, nonessential amino acids; LD, lipid droplet, N, nucleus.

hyperthermia and hypothermia can affect cell survival; however, cells appear more sensitive to hyperthermia (Kalamida *et al.*, 2015). While hyperthermia shows mostly deleterious effects (Chen *et al.*, 2017; Dickson and Shah, 1972; Rodriguez-Luccioni *et al.*, 2011), hypothermia effects on cell growth are considered largely cell line dependent with cell type-dependent effects on ATP levels, cell respiration and glucose uptake (Chuppa *et al.*, 1997; Hendriks *et al.*, 2017; Muckle and Dickson, 1971; Velickovic *et al.*, 2018; Vergara *et al.*, 2018).

Beside the aforementioned temperature responsiveness mediated through sympathetic regulation, a small number of *in vitro* studies have highlighted an adipocyte-autonomous response (Fig. 1). An explant model using human adipose tissue depleted of blood vessels, cultured at lower temperatures than controls (32–34.5°C) produced a reduction in leptin secretion (Peino *et al.*, 2000). Several hours of cold exposure (31°C) induced thermogenic gene markers UCP1 and PGC-1α, increased respiration, and 20% more uncoupled respiration in 3T3-F442A white-adipose tissue-derived mesenchymal stromal cells, with no obvious change in adipocyte markers (i.e., adipocyte protein 2, PPARγ and adiponectin). Longer periods (10 days) at 33°C showed an increase in a whole array of thermogenic genes expression and interestingly, this activation was independent of the canonical cAMP/PKA/CREB pathway. Raising the temperature to 39°C in the same study indicated that thermogenic induction is not a nonspecific response to temperature stress (Ye *et al.*, 2013). As reported there, the temperature-induced thermogenic program was found to be specific for white and beige cells (mouse cell lines, mouse primary white/beige adipose tissue-derived mesenchymal stromal cells, and human primary subcutaneous adipose tissue-derived mesenchymal stromal cells) but not classical brown adipocytes.

In a more recent study from our group (Fig. 2), the functional bioenergetic changes resulting from the cold exposure of beige-like adipocytes in culture were confirmed by measurements of oxygen consumption rate and uncoupled respiration using the Seahorse assay (Divakaruni *et al.*, 2014). This study compared BM-derived mesenchymal stromal progenitors differentiated at 32°C (hypothermic) with 37°C cultures (control) (Velickovic *et al.*, 2018). Under hypothermic conditions, adipogenesis was enhanced, with a rise in smaller lipid droplets accompanied by increased UCP1 and beige-selective gene expression, suggesting a brown-like response (Velickovic *et al.*, 2018). PGC-1α protein expression was increased in adipocytes cultured at hypothermia, as observed in BAT *in vivo* (Klingenspor, 2003; Puigserver *et al.*, 1998), with nuclear localization (Velickovic *et al.*, 2018).

Thus, based on results from several groups, there is strong evidence that adipocytes can respond to cold directly in a cell-autonomous manner. It seems likely that temperature-sensitive induction of a thermogenic response is not a hallmark of all adipocyte types, as beige and white adipocytes possess this ability while brown adipocytes are not capable of sensing temperature changes *in vitro* (Ye *et al.*, 2013). According to the literature, the dynamics of the cell-autonomous adipogenic and thermogenic response appears to depend on numerous factors, including the duration of the stimulus and the type of adipocyte

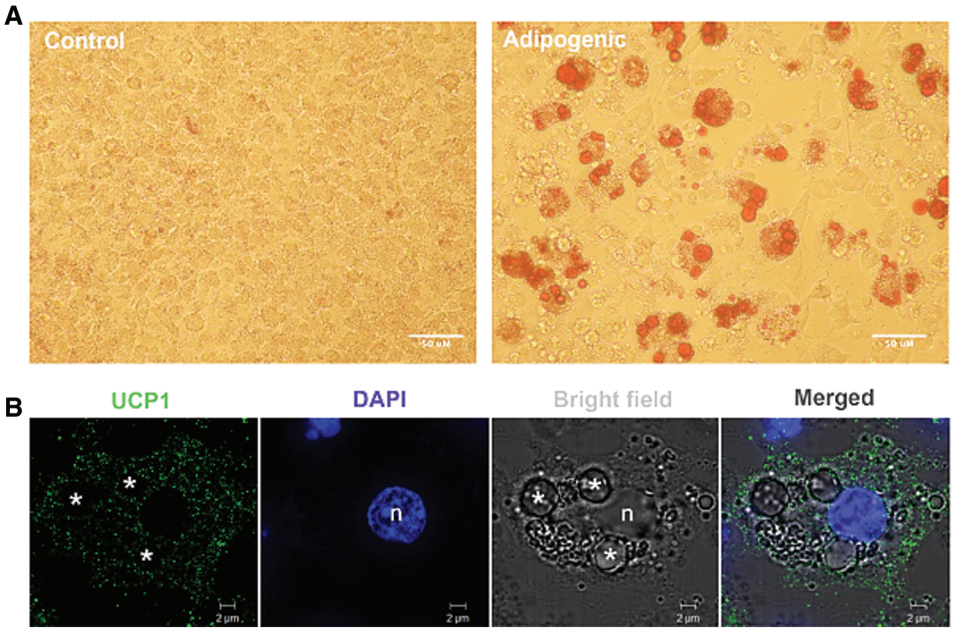

FIGURE 2. *In vitro* model of MSC adipogenic differentiation.
(A) Oil red O staining of differentiated mouse MSCs compared to undifferentiated cells (control) after 7 days of treatment at 37°C. Scale bar: 50 μm. (B) Super-resolved structured illumination microscope image showing presence of UPC1 (green) in adipogenic treated mouse MSCs after 7 days of treatment at 37°C with nuclear counterstain (n, blue) and lipid droplets (asterisks). Scale bar: 2 μm.

(Kern *et al.*, 2014; Vargas *et al.*, 2015; Velickovic *et al.*, 2018; Ye *et al.*, 2013).

Role for TRP channels in temperature sensing in vitro

The mechanism by which cooling can modify the activity of adipocytes in culture remains to be elucidated. Using β-less mice Ye and colleagues demonstrated that beige cell-autonomous response to cold exposure can be β-AR-independent, with the sarco-endoplasmic reticulum Ca^{2+}-ATPase 2b (SERCA2b) found to be required for beige fat thermogenesis in both the presence and the absence of UCP1 (Ye *et al.*, 2013). At present, *in vitro* studies point to a role for TRP channels as molecular sensors for temperature in adipocytes and suggest they may mediate cell-autonomous adipogenic function (Ma *et al.*, 2012; Uchida *et al.*, 2018; Ye *et al.*, 2012). These channels respond to a range of stimuli including Ca^{2+}, temperature, osmotic pressure, cyclic nucleotides, and dietary compounds. They could therefore represent a beneficial intervention route to regulate energy metabolism, adipogenesis and related pathologies (Zheng, 2013). Among TRP channels, members of transient receptor potential cation channel subfamily V member 1-4 (TRPV1-4) and transient receptor potential cation channel subfamily M member 8 (TRPM8) are the best-characterized, showing an important role in proliferation, differentiation and thermogenesis (Bishnoi *et al.*, 2013; Khare *et al.*, 2019; Uchida *et al.*, 2018; Ye *et al.*, 2012; Zhai *et al.*, 2020). Ye and colleagues reported that TRPV1/TRPV4 double-knockout mouse adipocytes (primary cells) respond to cold temperature exposure (31°C) and increase UCP1 mRNA expression (Ye *et al.*, 2013), suggesting a temperature sensing mechanism that could be independent of TRPV1 and TRPV4. However, the possible involvement of TRPV1 was suggested in brown-like adipocytes from BM-derived mesenchymal stromal cells after treatment at 32°C (Velickovic *et al.*, 2018), with increased TRPV1 gene expression and TRPV1/UCP1 protein co-expression, as well as strong cytoplasmic Ca^{2+} signal consistent with TRPV1 activation (Wetsel, 2011). TRP channels are the major class of Ca^{2+}-permeable channels (Uchida *et al.*, 2017), and evidence regarding the importance of both extracellular and intracellular Ca^{2+} levels for adipogenesis (Ikeda *et al.*, 2017; Jensen *et al.*, 2004; Uchida *et al.*, 2017) hint that TRP functions in adipogenesis and thermogenic activation could be at least in part mediated by alterations in intracellular calcium levels and signalling pathway.

TRP can also be activated by specific agonists and other mechanisms, as observed for TRPV2, that can respond to mechanical force (Sun *et al.*, 2016), whilst the TRP cation channel subfamily A member 1 (TRPA1) responds to the alkamide *trans*-pellitorine, inhibiting lipid accumulation in adipocytes (Lieder *et al.*, 2017). TRPM8 can be activated by menthol stimulating thermogenic gene expression in primary mouse (Jiang *et al.*, 2017) and human white adipocytes (Rossato *et al.*, 2014). Modulation by compounds selective for TRP channels such as capsaicin (Saito, 2015), menthol (Jiang *et al.*, 2017) and other dietary components (Watanabe and Terada, 2015) has thus been considered as a potential intervention route to regulate obesity and associated comorbidities. Moreover, capsaicin has been widely investigated on the basis of its ability to decrease body temperature, increase satiety and energy expenditure in different species, including humans (Belza *et al.*, 2007; Hori, 1984; Westerterp-Plantenga *et al.*, 2005).

Whilst the role of TRP channels has been analysed in tissues such as skin and the gastrointestinal tract (Caterina and Pang, 2016; Holzer, 2011), their precise role and

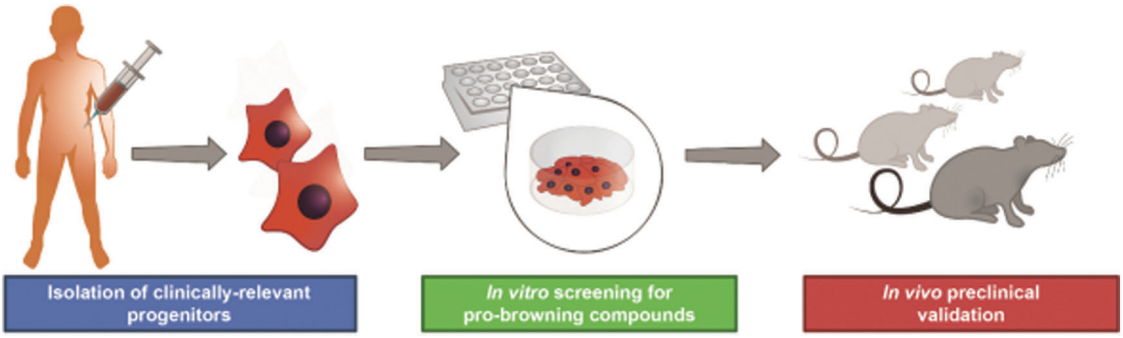

FIGURE 3. Clinical exploitation of patient-derived cellular models.
New cell-based approach could be used to generate patient-specific adipogenic models as tools for selective compound screening (drugs, molecules, nutraceuticals) before *in vivo* validation.

underlying mechanism in adipocytes still needs to be clarified. The fact that TRPs are expressed in numerous tissues in the human body, which are not exposed to temperature changes (Kunert-Keil *et al.*, 2006), suggests different roles and could open a new research avenue for adipogenesis research, as their activation may represent a promising option to combat obesity.

Perspectives for translational research

Due to its high metabolic capacity, BAT has become an attractive new target for therapeutic interventions seeking to manage WAT-related conditions, such as obesity and diabetes. Culture models represent a potential resource to develop drug discovery approaches and high throughput screening, with a view to identifying agents with pro-browning potential *in vitro* before taking them through *in vivo* validation (Nie *et al.*, 2017; Qiu *et al.*, 2018). Whilst the physiological role of SNS and β3-ARs in the UCP1-dependent pathway is well established, other ways to activate and/or recruit beige cells, including through a cell-autonomous pathway, is of particular importance due to the prevalence of beige adipocytes and β2-ARs in adult humans (Blondin *et al.*, 2020; Ikeda *et al.*, 2017). However, many questions remain unanswered regarding the precise role of TRP receptors and their activation in beige and brown adipocytes. Moreover, beige adipocytes arise from different developmental lineages dispersed heterogeneously in certain depots, which might possess lineage plasticity and behave according to specific factors and stimuli (Sanchez-Gurmaches and Guertin, 2014). More research is also required to establish the conditions required for the prolonged retention of active beige adipocytes and their detection. In humans, 18F-fluorodeoxyglucose (18F-FDG) positron emission tomography-computed tomography (PET-CT) presents a gold standard for the measurement of metabolically active BAT and it has been shown that correspond to histologically confirmed BAT depots (Cypess *et al.*, 2009). Since PET-CT reveals glucose metabolism in BAT and other tissues, and not primary BAT fuel (fatty acids) (Nedergaard *et al.*, 2007; Shreve *et al.*, 1999), methods to detect metabolically active and inactive BAT, as well as beige depots, are still missing. Furthermore, the potential use of BAT activation for obesity management still requires a better understanding of the physiological factors conditioning an individual's browning capacity such as age, gender and health condition (Kim *et al.*, 2016; Pfannenberg *et al.*, 2010; Valencak *et al.*, 2017; Valle *et al.*, 2007). Some studies point to a declining in BAT with age (Cypess *et al.*, 2009; Pfannenberg *et al.*, 2010; Saito *et al.*, 2009; Villarroya *et al.*, 2009), other factors may have an impact on these parameters and thus modify the clinical translation of *in vitro* findings.

With the continued need to address obesity and related conditions for public health, the drive for practicable interventions to improve metabolic regulation could benefit from cell-based models amenable to compound screening (Fig. 3). Apart from cold exposure as a typical model of cell-autonomous response to temperature, this could be done by using a molecule, either a drug or a natural compound (Lin *et al.*, 2015; Quesada-Lopez *et al.*, 2016; Sato *et al.*, 2020), which can modulate brown/beige cells activity, directly or using delivery systems, such as nanoparticles or lipid nanocarriers (Xue *et al.*, 2016; Zu *et al.*, 2018). As a proof of concept and example, we recently identified caffeine as a compound promoting browning features in stem cell cultures, before confirming that coffee consumption can activate BAT metabolism in healthy volunteers (Velickovic *et al.*, 2019). This illustrates how tissue culture models could facilitate the identification of novel small molecules, drugs or dietary nutraceuticals promoting BAT function (Okla *et al.*, 2017; Rodriguez Lanzi *et al.*, 2018).

Another potential translational application could involve cellular therapies using *in vitro* differentiated and metabolically activated brown/beige adipocytes for transplantation, as a means to improve metabolic homeostasis. Finally, the availability of new tissue-derived and potentially patient-derived cellular models opens the possibility for more specialised BAT models. It could include the study of BAT response in cells representing specific pathologies, physiological background or conditions such as diabetic or metabo-deficient phenotypes, which are accessible from patients through primary cell isolation or personalised iPS models and can enable the finer analysis of adipogenic and thermogenic traits.

Availability of Data and Materials: The data used to support the findings of this study are available from the corresponding author upon request.

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