Fat cells gain new identities

CELL BIOLOGY

Fat Cells Gain New Identities

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ASC-1, PAT2, and P2RX5 are newly identified cell surface proteins that may distinguish brown/beige from white adipocytes in mouse and human adipose tissue (Ussar *et al.*, this issue).

DIFFERENTIATING FAT

Use it or lose it is the modus operandi of brown adipose tissue (BAT); keep it for later is that of white adipose tissue (WAT). Whereas WAT stores lipids in the form of triglycerides, BAT combusts fatty acids to form heat via uncoupling protein 1 (UCP1), bypassing ATP production. The presence of active BAT in adult humans was proven in 2009 when regions that showed high uptake of radioactively labeled glucose on positron emission tomography–computed tomography (PET-CT) scans histologically resembled BAT (*1*–*3*). But what is the physiological relevance of BAT in humans? The notion that obese subjects possess lower amounts of BAT as compared with that of lean subjects suggests a role for BAT in energy expenditure. Indeed, cold acclimation enhances BAT volume and activity and results in reduced fat mass, suggesting that BAT activation is a promising target to combat obesity and associated disorders in humans (*4*, *5*).

In mice, BAT depots are well defined and are composed of cells derived from a Myf5-positive lineage. Human BAT derived from deep neck adipose tissue genetically resembles mouse BAT, although its mitochondrial respiration is lower, and Myf5 expression has not yet been reported (*6*). In mice WAT, UCP1-expressing "beige" (or "brite") adipocytes can be detected after prolonged cold exposure or peroxisome proliferator–activated receptor γ (PPARγ) agonism. Upon stimulation, these beige adipocytes acquire a "brownish" (BAT-like) phenotype with multilocular lipid droplets and high UCP1 expression. In humans, recent studies suggest that BAT is composed of a mixture of brown and beige adipocytes (*6*). Beige adipocytes are also detected in human WAT, especially in patients who are chronically exposed to high levels of catecholamines.

But from where do these beige adipocytes in human BAT and (mouse) WAT derive? There is not yet an answer to this question, but possible explanations are from both differentiation of beige precursor cells and transdifferentiation of white adipocytes (*7*). Thus, beige adipocytes are probably dynamic cell types that can rapidly appear when needed—for instance, when the demand for thermogenesis is high. However, understanding the role of brown and beige adipocytes in substrate utilization and whole-body metabolism is far from complete, especially in humans. Identification of cell-surface markers that are specific to brown, beige, and white adipocytes may provide novel insights into fat cell biology and also facilitate the discovery of new therapeutics that target, for example, BAT. In this issue of *Science Translational Medicine*, Ussar *et al.* (*8*) indeed define three new cell-surface markers that can distinguish the different types of adipocytes and could therefore be used to identify and target these cells in vivo.

CELL-SURFACE MARKERS

To better understand brown and beige adipocyte biology in mice and in humans, proper identification of the different cell types is essential. By performing in silico, in vitro, and in vivo studies, Ussar *et al.* sought to identify markers for white, brown, and beige adipocytes. In the in silico studies, *adiponectin* was used as a model gene for white adipocyte specificity in order to identify previously unknown WAT-specific markers. A similar approach was used for BAT, with the brown adipocyte–specific gene *UCP1*. Genes that correlated with *adiponectin* or *UCP1* expression had a high expression level in adipose tissue, and encoded cell surface proteins were included in the marker search.

Through this approach, Ussar *et al.* selected ASC-1 as the surface marker for white adipocytes and the amino acid transporter PAT2 and the purinergic receptor P2RX5 as markers of brown and beige fat cells (Fig. 1). These markers, selected in silico, were then validated in mouse and human adipose tissue. The authors used a broad validation process, including verification of the cell-surface markers through immunofluorescence in adipose tissue cultures, as well as determination of gene and protein expression levels in lean and obese mouse and human adipose tissue. Furthermore, marker expression was determined upon differentiation of various brown and white adipose tissue cultures. The latter method, especially, may provide valuable insights regarding the possible role of these cell-surface markers for adipose tissue biology, although more studies will be needed to elucidate the precise functional role.

ASC-1, PAT2, AND P2RX5 IN ADIPOCYTE BIOLOGY

With the identification of the cell-surface markers described by Ussar *et al.* (*8*), an important question arises regarding the physiological role of these markers in adipocyte biology. ASC-1, PAT2, and P2RX5 were nearly absent in mouse and human preadipocytes but were induced upon adipocyte differentiation, suggesting a role in adipocyte formation and/or physiology. Consistent with this, expression of *PAT2* and *P2RX5* was lower in BAT of diabetic (*db*/*db*) mice, which is in line with the "whitening" of BAT. Conversely, whereas *P2RX5* expression was enhanced in WAT upon cold exposure (also known as "browning" of WAT), *ASC-1* was down-regulated.

How could PAT2 and P2RX5 be physiologically linked to activation of brown adipocytes? Because PAT2 and P2RX5 are both membrane transporters (amino acid transporter and ion channel, respectively), they may be linked to intracellular signaling pathways involved in BAT activation. A main activation route of brown adipocytes is the β_3-adrenergic pathway, resulting in increased levels of intracellular cyclic cAMP, activation of protein kinase A, and subsequent phosphorylation of downstream targets. Whether the β_3-adrenergic pathway and/or other intracellular pathways are coupled to PAT2 and P2RX5 membrane transporters remains to be determined—for instance, by the study of intracellular signaling cascades in brown adipocytes in vitro upon

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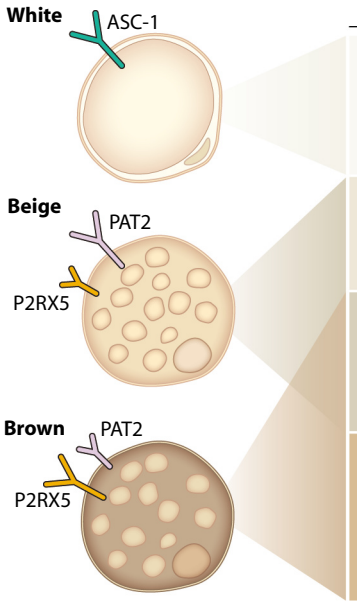

Marker	Adipocyte type	Plasma membrane? (YES/NO)
Leptin	White	NO
Adiponectin	White	NO
ASC-1	**White**	**YES**
HOXC8	White	NO
HOXC9	White	NO
SP100	Beige	NO
CD40	Beige	YES
Ear2	Beige	NO
CD137	Beige	YES
UCP1	Beige/brown	NO
PGC1α	Beige/brown	NO
PAT2	**Beige/brown**	**YES**
P2RX5	**Beige/brown**	**YES**
TMEM26	Beige/brown	YES
PRDM16	Brown	NO
CIDEA	Brown	NO
Eva1	Brown	YES
Ebf3	Brown	NO
Hspb7	Brown	NO
ZIC1	Brown	NO

Fig. 1. Distinguishing white, beige, and brown. The table shows a selection of identified markers of different adipocytes. Several markers can be present on both beige and brown adipocytes. A subset of markers is present at the plasma membrane, including ASC-1, P2RX5, and PAT2 (boldface entries). Of these, Ussar *et al.* suggest that PAT2 is more of a beige cell marker, whereas P2RX5 is more of a brown cell marker (*8*).

stimulation with ligands for these transporters or evaluation of brown adipocyte function after BAT-specific knockout of PAT2 or P2RX5. This combined approach might yield pharmacological ligands of these transporters capable of stimulating BAT activity in vivo.

CLINICAL IMPLICATIONS

Enhancing BAT activity could combat type 2 diabetes and obesity. As such, it will be important to determine whole-body distribution of BAT and WAT—for instance, using the cell-surface markers newly identified by Ussar *et al.* (*8*). Many research groups have paved the way in the quest to identify white, beige, and brown adipocytes in human adipose tissue on the basis of molecular markers. Several markers for beige/brown adipocytes have been linked to the high number of mitochondria present in these cells (such as PGC1α and PRDM16) (Fig. 1). The list of additional markers presented to distinguish adipocytes is extensive and includes several genes and microRNAs. Molecular markers identified within various studies—namely, ZIC1, EVA1, CIDEA, TMEM26, CD137, TBX1, FBXO31, and EBF3—are not necessarily specific for classical brown or beige cells. This could be explained by the fact that brown adipose tissue samples can be collected at different locations (for example, the superficial or deep neck region or the supraclavicular region) (*6*, *9*). Moreover, some "established" markers can be detected in other nonadipose tissues as well. For example, CD137 is expressed on immune cells and human primary tumors. Importantly, most markers are not expressed at the cell surface, eliminating the possibility for antibody-based purification (Fig. 1). In their study, Ussar *et al.* have thus handed the scientific community new tools to distinguish white, beige, and brown adipocytes according to surface expression (*8*). These markers could be used for detecting fat cells, for distinguishing brown from beige adipocytes, or for targeting therapeutics.

The current gold standard for BAT detection in adult humans is the cold-induced ^{18}F-fluorodeoxyglucose (FDG) PET-CT scan, which relies on the visualization and quantification of the glucose analog FDG. Brown adipocytes only take up glucose upon activation (with cold); as such, use of a cooling protocol before conductance of the PET-CT scan is necessary. Imaging methods that rely on constitutively expressed cell surface proteins on differentiated brown adipocytes would allow for quantification of BAT without cold stimulation. This method may be especially useful in assessing the response of BAT volume to pharmacological stimuli because cold exposure before imaging may actually mask the true effect of the intervention.

Considering the specificity of PAT2 and P2RX5 for BAT visualization, a few statements could be made. As compared with PAT2, expression of P2RX5 appeared to be lower in WAT, making it more BAT-specific (Fig. 1). Conversely, P2RX5 was also detected on brown preadipocytes. This could limit the use of this marker to the assessment of BAT volume because brown preadipocytes are evidently also present in BAT, resulting in an overestimation of BAT volume. Brown and beige adipocytes may be distinguished in vivo by means of the cell-surface markers PAT2 and P2RX5; however, Ussar *et al.* indicate that P2RX5 may be more of a brown adipocyte marker and PAT2 a beige adipocyte marker, making the clear distinction between the two cell types difficult at present. Thus, future studies are needed to assess whether these markers discretely represent the two BAT subtypes, primarily by determining the physiological role of these markers in BAT biology. Furthermore, targeting molecules should be designed to extracellular epitopes of PAT2 and P2RX5 in order to use these extracellular markers for imaging purposes.

Therapeutically, it is tempting to speculate whether drugs targeting markers such as PAT2 and P2RX5 may be used to activate BAT in obese individuals in vivo. An exciting area of current research is the effectiveness of enhancing human BAT activation, resulting in increased energy expenditure and reduced adipose tissue mass accumulation, also taking into account possible differences in ethnic background. For instance, South Asian people have lower BAT volume and activity as compared with those of Caucasians (*10*). Currently, our understanding of BAT biology and metabolism is vastly increasing, and previously unidentified markers such as PAT2 and P2RX5 will provide new steps toward successful targeting and activating of BAT in humans. Combined with the identification of white adipocytes by using ASC-1, a distinction can be made between white, beige, and brown adipocytes. This may eventually result in generation of a detailed distribution pattern of these adipocyte types and identification of people who may benefit most from a BAT-targeted strategy in order to combat obesity and related disorders, such as type 2 diabetes.

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