Plasticity of Thermogenic Adipocytes

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White and brown adipocytes play different roles in metabolism and physiology. However, both cell types appear to be plastic with an ability to switch between metabolic states in response to changes in the cellular environment. Hence, the separation between brown and white adipocytes is less clear than previously thought.

Adipocytes are mesenchymal cells specialized in storing large quantities of lipids in the cytosol as lipid droplets. They are found in adipose tissues such as those located subcutaneously or intra-abdominally, as well as in many smaller depots within or surrounding organs. Examples include the adipose tissue in the bone marrow and the adipose tissue associated with the kidneys and the adrenal gland. In addition, adipocytes can be found interspersed in other tissues, e.g. in muscle fibers and thymus. While the general biochemical function of all adipocytes is related to lipid storage, the specific physiological functions of adipocytes in the different tissues and their cross-talk with other resident cell types are not well understood. Interestingly, an increasing body of evidence indicates that adipocytes display a remarkable plasticity in response to the specific tissue niche as well as physiological stresses such as under- and over-nutrition, environmental temperature, exercise, and age.

White and brown adipocytes

Traditionally, adipocytes have been classified as white or brown. White adipocytes are large unilocular cells specialized in storage and release of fatty acids. Endocrine signals derived from these adipocytes provide continuous information to the brain as well as peripheral tissues on the degree of filling of adipocyte stores. Brown adipocytes contain many smaller lipid droplets (multilocular) and many mitochondria that are partially uncoupled owing to high expression of the uncoupling protein 1 (UCP1). As a result, these adipocytes are specialized in oxidation rather than release of fatty acids and in conversion of metabolic energy into heat (i.e. non-shivering thermogenesis). The major activator of non-shivering thermogenesis is believed to be cold exposure, which leads to release of norepinephrine from the sympathetic nervous system (SNS) and activation of β-adrenergic receptors on brown adipocytes.

Brown-like adipocytes – a new cell type?

More recently a third type of adipocyte, the brown-like (or beige, brite) adipocyte, has been shown to be induced in certain white adipose tissues (WAT) in rodents in response to physiological stresses such as cold (Figure 1). Similar to brown adipocytes, brown-like adipocytes are rich in mitochondria that express UCP1 and can perform non-shivering thermogenesis. During the recent years, these adipocytes have attracted considerable research interest because of their potential to increase WAT energy metabolism, which may increase whole-body energy expenditure and promote clearance of glucose and fatty acids from the plasma. Notably, mice lacking brown adipose tissue (BAT) become obese, while BAT transplantation reverses obesity in obese mice, indicating that, at least in rodents, BAT contributes significantly to organismal energy expenditure. Thus, the hope is that by recruiting brown-like adipocytes in humans one could combat obesity or the metabolic consequences thereof.

So far more than hundred signals/factors promoting browning of white adipocytes in rodents have been identified (Figure 1). In humans, browning of WAT has been demonstrated in patients with pheochromocytoma, i.e. with chronically high levels of norepinephrine, as well as in cancer cachexia. These examples show that browning of human

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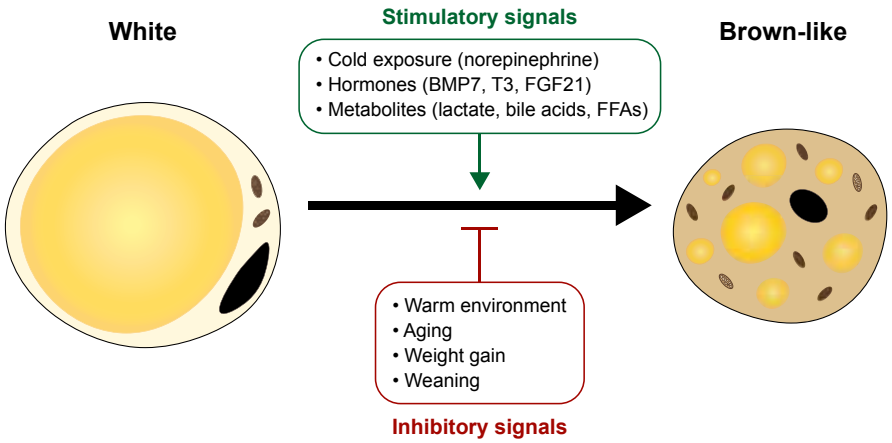

Figure 1: *Physiological signals controlling browning of white adipocytes. Cold exposure which leads to release of norepinephrine from the sympathetic nervous system is the most prominent activator of adipocyte browning; however, numerous circulating hormones and metabolites have also been implicated in browning. By contrast, several physiological conditions, such as warm environment, aging, weight gain and weaning, are known to reduce browning.*

WAT is possible; however, the extent to which browning of WAT occurs in humans during non-diseased physiological conditions is currently not clear.

One of the much debated questions in the field is whether brown-like adipocytes should be considered brown adipocytes, or whether they represent a distinct cell type. It has been argued that brown-like adipocytes express specific brown-like defining marker genes; however, none of these marker genes have been linked to important biochemical functions and many appear to derive from anatomical position. Furthermore, no markers are unique for any single adipocyte type (brown, white or brown-like) (1).

Importantly, brown-like adipocytes appear to perform the same biochemical functions as classical brown adipocytes and to be activated by the same browning agents. In this respect, it is also worth noting that the sympathetic neuronal pathways stimulating WAT and BAT have identical origins in the brain. Thus, the distinction between brown and brown-like adipocytes appears somewhat artificial.

Another controversial question is which cells give rise to the brown-like adipocytes, i.e. do they primarily arise by phenotypic switching of existing white adipocytes into brown-like adipocytes (transdifferentiation), or are they recruited by *de novo* differentiation of progenitors? Lineage-tracing studies, where cells are permanently "labelled" due to their expression of a particular lineage-specific gene, have recently provided evidence that both pathways play a role in browning of rodent WAT and that the relative importance of these pathways depends on the tissue and the environmental challenge. Notably, however, such lineage-tracing studies are inherently biased towards cells that express one particular gene, and further insight into this question awaits future more unbiased approaches such as single-cell sequencing.

Adipocyte heterogeneity and plasticity

A key question that to date remains unanswered is whether the ability to undergo browning is restricted to a subpopulation(s) of progenitor cells and mature adipocytes in WAT, or whether every white adipocyte has the ability to differentiate into a brown-like adipocyte. In support of adipocyte heterogeneity, a recent study identified two adipocyte populations in subcutaneous adipose tissue in mice – one brown-like subpopulation that expressed high levels of medium-chain acyl-CoA dehydrogenase (MCAD) and UCP1 but low levels of fatty acid synthase (FASN), and another white subpopulation that expressed high levels of FASN, and low levels of MCAD and UCP1 (2).

Furthermore, another recent study observed that around 50% of former UCP1-positive brown-like adipocytes became UCP1-positive again when re-exposed to cold after an intermediate whitening period (3), suggesting that adipocytes that once were brown-like constitute a subpopulation with an epigenetic memory of being brown.

The above reports clearly support adipocyte heterogeneity of WAT that has been exposed to browning signals. The question is how this heterogeneity arises in the first place. Interestingly, after re-exposure to cold temperatures, brown-like adipocytes primarily localize to islands of brown-like adipocytes within the WAT, indicating that the microenvironment is a crucial factor for determining adipocyte fate. In particular, the proximity to norepinephrine fibers might be a determining factor, since these fibers correlate with the number of brown-like adipocytes, and since the lack of β3-adrenoreceptors depressed browning (4). Thus, the apparent heterogeneity may arise as a result of proximity to norepinephrine fibers.

Interestingly, during conditions of thermoneutrality or chronic positive energy balance, brown and brown-like adipocytes convert to white adipocytes, a process called whitening. In addition, in several mammals as well as humans there is a general decrease in the amount of BAT following weaning, and during aging. This indicates that the thermogenic phenotype of brown and brown-like adipocytes is reversible and requires sustained β-adrenergic stimulation to maintain the thermogenic signature.

Thus, depending on how one defines a cell type, one could view brown-like

adipocytes as products of cellular plasticity of progenitors and mature adipocytes, and the interconversion of white to brown-like adipocytes as phenotypic transitions allowing the organism to adapt to physiological stresses. These emerging intricacies call for a revision of the classification of adipocytes.

Progenitor heterogeneity and plasticity

Intriguingly, progenitors isolated from different adipose depots retain their depot-specific characteristics in culture (5), suggesting that the microenvironment has preprogrammed the progenitors to a particular adipocyte sub-lineage and that this preprogramming is relatively stable. The question is whether there in a given WAT are distinct populations of white and brown-like progenitor cells, or whether progenitors are mostly bipotent with cellular fate decisions being determined stochastically or by conditional signals.

Subclonal analyses of cells from the subcutaneous WAT suggested that there are distinct subpopulations of brown-like and white progenitors. Interestingly, however, there is also evidence of bipotent progenitor cells that have the potential to differentiate into brown-like adipocytes upon β-adrenergic stimulation and into white adipocytes following feeding with high-fat diet. These results indicate that despite differential preprogramming of progenitor cells in the different adipose tissues, at least some progenitor cells display plasticity in response to the appropriate signals.

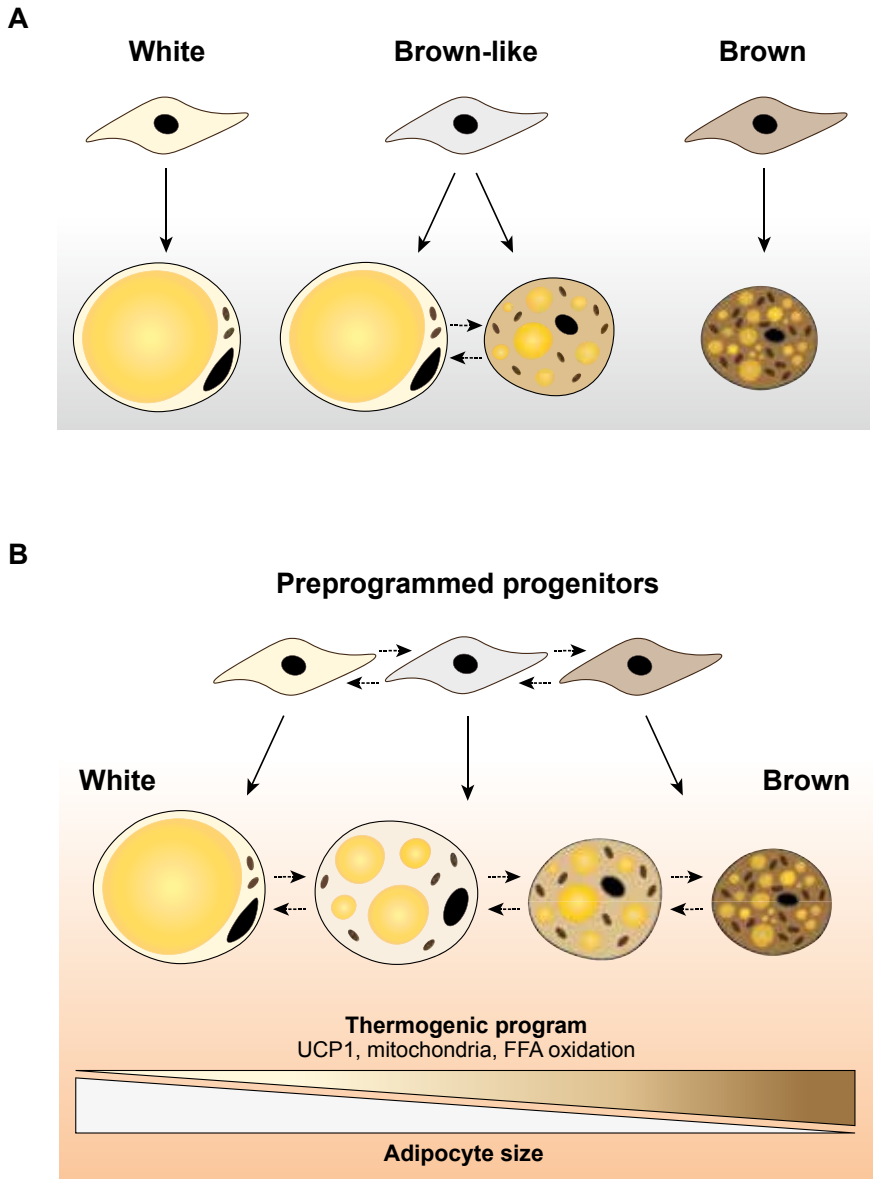

Figure 2: *Adipocyte plasticity. Two competing models explaining the relationship between different types of adipocytes. (A) In the first model, all progenitor cells are preprogrammed to a specific lineage (white, brown-like or brown), and only brown-like and dormant white adipocytes have the potential to undergo browning and remain plastic. (B) In the second model, adipocytes and their progenitors constitute a continuum of states. The inherent memory of the state in each cell works to conserve the state, whereas plasticity is induced in response to certain physiological signals.*

Interplay between plasticity and heterogeneity

The above discussion highlights that adipocyte phenotype is the result of the balance between fate-conserving and plasticity-inducing mechanisms. According to the more classical model (Figure 2A), dedicated white progenitor cells exclusively differentiate into white adipocytes, brown progenitor cells into brown adipocytes and brown-like progenitor cells into brown-like adipocytes. According to this model, brown-like adipocytes convert back to dormant "white" adipocytes when thermogenesis is no longer a priority. The dormant "white" adipocytes and classical white adipocytes are viewed as different cell types defined by specific marker genes, as discussed above.

The second model (Figure 2B) proposes that although progenitor cells and mature adipocytes are preprogrammed toward a specific lineage, they still retain some degree of plasticity and can change this preprogramming in response to physiological signals. At this point none of the models can be excluded, but data to support the second model is accumulating.

Concluding remarks and future perspectives

Thermogenic adipocytes have been intensively studied during the recent years because of their alleged potential to counteract the development or consequences of obesity. However, there are still many open questions regarding the origin, plasticity and regulation of these cells. These include questions such as: What drives thermogenic adipocyte plasticity? What niche-specific

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approach viable even in subjects with insulin-resistance. Proof-of-principle studies in mice have actually shown that increasing BAT mass by transplantation can improve glucose tolerance and insulin sensitivity in models of obesity and type I diabetes. Together this makes BAT a desirable target for anti-diabetic treatment.

Sequestering of signaling metabolites in BAT

Lipids and glucose are the classic metabolites associated with uptake in brown adipocytes, but brown adipocytes also take up other metabolites. This is of particular interest because multiple metabolites have been shown to not only function as energy sources and anabolic substrates, but also as intra- and extracellular signaling molecules [5].

Recently, we identified that cold-activated BAT can accumulate substantial amounts of the Krebs cycle metabolite succinate [6]. We showed that activated BAT actively take up succinate from the circulation, a hitherto undescribed phenomenon, which also provides an integrating paradigm for the longstanding observation of variable and substantial concentrations of succinate in the blood stream. The source tissue for succinate is still undetermined, but at least under cold conditions BAT acts as a specific sink for circulating succinate.

The uptake and accumulation of a metabolite like succinate is interesting for multiple reasons. First, we observed that succinate in itself was an activator of thermogenesis in brown adipocytes, and that just adding succinate to the drinking water of mice could increase their energy expenditure and counteract diet-induced obesity. Hence, our findings suggest that dietary interventions that involve acute elevation of systemic succinate levels may protect against metabolic disease, but only for individuals with a sufficient endowment of thermogenic adipose tissue.

Furthermore, it has been suggested that chronically elevated succinate can promote an inflammatory response. Therefore, our findings imply that a major anti-inflammatory mechanism of BAT activity may involve sequestration of circulating succinate to antagonize systemic inflammation. Since the metabolic disruptions coinciding with obesity are also associated with a chronic low-grade inflammation, using BAT to sequester potential pro-inflammatory metabolites, like succinate, could be of substantial interest. The discovery that a single mitochondrial metabolite is a molecular driver of adipocyte thermogenesis opens many interesting avenues of investigation into the selective uptake of metabolites not only in BAT, but also other metabolically active tissues.

Perspectives

Exposure to environmental cold effectively drives BAT activation and improves metabolic profiles in both mice and humans. Curiously, it seems that BAT has a unique ability to sequester multiple different metabolites from the circulation, many of which when present in excess amounts have deleterious effects on health. The sink and drain effect, where activated BAT not only takes up these metabolites, but also has an efficient way of disposing of them via uncoupled respiration is quite unique. To take advantage of this effect we need to establish efficient ways of boosting BAT activity, and preferably find alternative ways of activating metabolite uptake besides just letting mice and people freeze. Being able to control the flow of metabolites in the blood stream could pose huge advantages to treating obesity, diabetes, heart disease, inflammation, fatty liver disease, and many others.

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signals regulate plasticity? And are there fundamental functional differences between the brown and brown-like thermogenic program, or do brown-like adipocytes represent shades of brown? Resolving these fundamental questions will require a combination of the use of intelligent lineage-tracing mouse models, single cell sequencing and advanced molecular studies.

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