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Research Advance

Heat shock factor 1 in fat biology: *comments on 'Local hyperthermia therapy induces browning of white fat and treats obesity'*

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Beige fat, a newly discovered adipose tissue featuring high functional flexibility, exhibits the characteristics of white fat at rest, but can be activated to function like brown fat under cold or β-adrenergic stimulation via a process termed 'browning of white fat'. This activation enables beige fat to greatly promote heat production and energy consumption, as well as to improve systemic glucose and lipid metabolism. Beige fat also exists in supraclavicular and paraspinal regions in human adults, which endows its great potential for intervention of obesity and metabolic diseases.

The clinical application of cold or β-adrenergic agonist treatment for beige fat activation is hindered due to various reasons, e.g. limited long-term effectiveness and potential adverse effects on cardiovascular systems (Bhadada et al., 2011). Notably, aside from cold, previous reports have shown that heat or hyperthermia therapy (HT), i.e. hot tub bathing, sauna, and heat blanket wrapping, also renders metabolic benefits in humans and mice (Hooper, 1999), although whole-body HT in a heated environment may lead to undesired side effects including heightened sympathetic tone and increased cardiovascular risks (Masuda et al., 2019). This inspired us to implement hyperthermia locally to beige fat by applying a thermal source on supraclavicular fat depots of human subjects or utilizing polyethylene glycol cross-linked polydopamine nanoparticles combined with near-infrared illumination to achieve local hyperthermia therapy (LHT, 41°C ± 0.5°C) in human or mice beige fat. Comprehensive analyses of various genetically engineered mice and adeno-associated virus mediated beige fat-specific overexpression or knockdown mice eventually led to the innovative discovery that beige fat could sense local hyperthermia and induce thermogenesis via heat shock factor 1 (HSF1), which effectively ameliorates metabolic disorders including obesity, insulin resistance, and hepatic steatosis without obvious side effects. Furthermore, via chromatin immunoprecipitation–sequencing (ChIP–seq), we performed integrative study of the HSF1 direct target genes on a genome scale in beige adipocytes, which provided a full picture for the HSF1 regulatory network in adipose tissues and newly identified the HSF1–Hnrnpa2b1 (A2b1) transcriptional axis in beige fat activation. Finally, we revealed the association of HSF1 single nucleotide polymorphism (SNP) with human metabolic traits in >10000 population and found that HSF1 gain-of-function variant Pro365Thr increased A2b1 transcripts in beige adipocytes and beige fat (Li et al., 2022).

Of note, we highlighted heat as a new inducer of beige fat activation via hyperthermia at 41°C ± 0.5°C. It is well known that sensors for heat signaling include HSF1 and transient receptor potential vanilloid 1 (TRPV1), which were both reported to play critical roles in fat biology and energy homeostasis. Interestingly, using specific small interfering RNAs, we found that the mild hyperthermia used in our study (41°C) requires HSF1, but not TRPV1, which is inconsistent with a reported threshold of 43°C for TRPV1 activation. Notably, another recent report used 45°C transdermal photothermal–pharmacotherapy in adipose tissue to ameliorate obesity and metabolic disorders and found that TPRV1 may mediate the effects of this relatively high temperature by increasing the intracellular calcium concentration and engaging in downstream thermogenic gene programs (Zan et al., 2022), though it did not exclude the possible involvement of HSF1 in this process. Indeed, we have previously shown that HSF1 responds to heat of 42°C–45°C and induces PGC1α expression. HSF1 and PGC1α then physically interact with each other and co-transactivate heat shock proteins (HSPs) including Hsp70 to maintain proteostasis and achieve cellular protection from thermal insults (Xu et al., 2016). Nevertheless, Zan's study and ours both

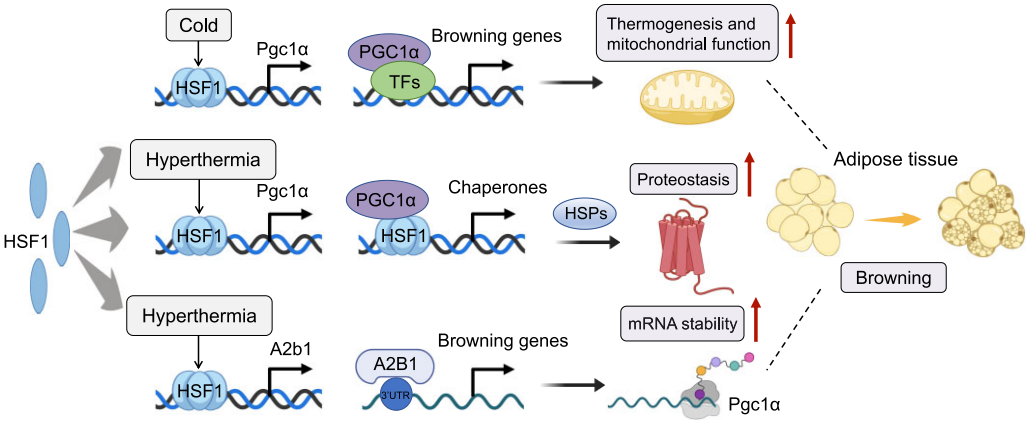

Figure 1 HSF1 regulatory network in fat biology. HSF1 plays key roles in metabolic regulation via its ability of sensing diverse thermal stresses including cold and hyperthermia in adipose tissues. Under cold exposure, HSF1 directly activates Pgc1α transcription for enhanced thermogenesis and mitochondrial functionality. In hyperthermia, Pgc1α is transcriptionally induced by HSF1, which leads to increased PGC1α level to cooperate with HSF1 and they synergistically induce the expression of chaperones for proteostasis in a positive-feedback loop. On the other hand, hyperthermia also causes HSF1 to directly induce the expression of A2B1, which increases the mRNA stability of metabolic gene transcripts including Pgc1α by binding to their 3′-untranslated region. The close cooperation between HSF1 and PGC1α ensures the browning of white fat under thermal changes for metabolic benefits.

supported the notion that hyperthermia is sufficient to induce metabolic reprogramming of white fat browning in beige fat for thermogenesis and energy expenditure with a relatively wide temperature range (Li et al., 2022; Zan et al., 2022), although for a safe and comfortable intervention, a lower and milder heat would be more preferable. The present study provides a proof-of-concept of LHT on beige fat in rodents and humans, while future investigation to define the temperature, endurance, and frequency for optimal LHT-treating efficacy and energy homeostasis, as well as novel delivery strategies for LHT on beige fat, is warranted.

HSF1 is a transcription factor and master regulator of heat shock response (HSR), an effective adaptive mechanism that enables organisms to cope with a wide variety of environmental stresses. A critical step in HSR is the transcription of HSPs, which function as chaperons to facilitate protein refolding and maintain cellular homeostasis during stresses (Gomez-Pastor et al., 2018). However, recent studies have revealed a broader array of HSF1 target genes in different tissues and cell types, other than its classic function of chaperon regulation. For example, HSF1 drives transcriptional programs for cell cycle regulation, signaling, metabolism, adhesion, and translation to support malignancy of human cancers, which are distinct from its function in heat shock (Mendillo et al., 2012). By cross-analyzing our in-house ChIP–seq dataset from mouse beige adipocytes with dataset from human adipocytes differentiated from mesenchymal stem cells (GSE24326, Lo et al., 2011), we found that metabolic process is the top pathway affected by HSF1 in adipocytes. Most HSF1 targets in fat are involved in the modulation of protein homeostasis, including classic HSF1 target chaperons and ubiquitin B (UBB), a peptide critical for protein ubiquitination and degradation, which are consistent with a role of HSF1 in ensuring proteostasis by simultaneously inducing chaperons for protein refolding and degrading defective proteins. Notably, the analysis revealed an RNA-binding protein HNRNPA2B1 (A2B1) as a novel HSF1 target in beige adipocytes. Subsequent *in vitro* and *in vivo* studies further revealed the importance of A2B1 in beige fat activation by enhancing the stability of metabolic gene transcripts including Pgc1α and Ucp1. Considering that we previously found Pgc1α as a direct target of HSF1 during cold stimulation (Ma et al., 2015), it is particularly interesting that HSF1 regulates Pgc1α at both transcriptional and post-transcriptional levels. PGC1α, in turn, facilitates HSF1 for transcriptional activation, thus forming a positive feedback loop for efficient and ample HSF1 downstream signaling. These studies concerning HSF1 collectively suggested that the HSF1–PGC1α axis may serve as a cell-autonomous sensor for temperature fluctuation and a functional keeper of beige adipocytes (Figure 1).

HSF1 is modified by several post-translational modifications including phosphorylation that alters DNA binding and transcriptional activities on target genes in concert with cofactors under different physiological and pathological conditions. HSF1 is subjected to multiple phosphorylation events mediated by various kinases. HSF1 hyper-phosphorylation leads to either its increased activity or the phosphorylation-regulated degradation mediated by its ubiquitylation. Of note, we have found a gain-of-function HSF1 missense variant p.Pro365Thr with improved human metabolic traits. Threonine (Thr) is an amino acid residue frequently subjected to phosphorylation modification and the Pro365Thr SNP is near two reported HSF1-suppressing phosphorylation sites Ser363/Thr367. It would thus be interesting to study

how the amino acid change of Pro365Thr affects HSF1 activities. Moreover, humanized transgenic mouse models are needed to provide genetic evidences of HSF1 Pro365Thr contribution to metabolic performances for future potential gene therapy in humans.

In conclusion, other than its traditional role in the regulation of HSPs, HSF1 has more multifaceted functions than meeting the eye, especially in the realm of metabolism. Facing the obesity pandemic, it would be critical to unraveling the HSF1 network in metabolic organs for novel targets and strategies for metabolic intervention.

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