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The differential regulation of PPARγ co-activator 1α (PGC-1α) expression between white and brown preadipocyte cell lines is caused by different complexes of basic-leucine zipper (bZIP) transcription factors binding to the cAMP-response element (CRE)

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Brown adipose tissue (BAT) has recently been identified in human subjects and the conversion of white adipocytes with abundant mitochondria and increased energy expenditure is a plausible strategy for combating obesity[1]. PGC-1α, a key gene involved in mitochondrial biogenesis in adipocytes, is cAMP dependent in BAT and augments cAMP-mediated transactivation of the BAT specific gene uncoupling protein 1 (UCP1)[2]. Activation of PGC-1α and UCP1 expression is an essential step in the commitment of preadipocytes to the BAT lineage. It has been demonstrated that forskolin-sensitive PGC-1α expression normally observed in brown HIB-1B cells can be induced in white 3T3-L1 cells by overexpression of CCAAT-enhancer-binding protein-β (C/EBPβ) acting through the CRE of the PGC-1α proximal promoter[3]. The present paper establishes that overexpression of C/EBPβ in 3T3-L1 cells induces PGC-1α expression by altering the combination of stimulatory and inhibitory bZIP transcription factors acting on the PGC-1α-CRE.

3T3-L1 and HIB-1B cells were co-transfected with a reporter construct containing the PGC-1α proximal promoter and expression plasmids for C/EBPβ, CRE-binding protein (CREB), activating transcription factor 2 (ATF-2) and CHOP10. Knock-down of CHOP10 in 3T3-L1 cells was achieved by RNA interference (RNAi). Chromatin immunoprecipitation (ChIP) assays were performed using anti-C/EBPβ, anti-CREB, anti-ATF-2 and anti-CHOP10 in HIB-1B and 3T3-L1 preadipocytes transfected with C/EBPβ and treated with forskolin to stimulate cAMP. Knock-down studies of C/EBPβ in HIB-1B cells are in progress.

Overexpression of C/EBPβ and CREB significantly ($P<0.05$) up regulated, and overexpression of ATF-2 and CHOP10 significantly decreased ($P<0.05$), forskolin-stimulated PGC-1α promoter in 3T3-L1 cells. Co-transfection studies demonstrated that CHOP10 and the truncated isoform of C/EBPβ significantly inhibited the stimulatory ($P<0.05$) effect of C/EBPβ overexpression on PGC-1α promoter in 3T3-L1 and HIB-1B cells, while ATF-2 had a less inhibitory effect. ChIP assays demonstrated that overexpression of C/EBPβ results in strong binding of CREB and C/EBPβ on the PGC-1α-CRE, while diminishing binding of ATF-2 and CHOP10 in response to cAMP, giving a bZIP transcriptional factor-binding profile similar to that of HIB-1B cells. RNAi knock-down of CHOP10 in 3T3-L1 cells allowed PGC-1α to respond to cAMP, and when C/EBPβ was overexpressed the response was further increased.

The results suggest that overexpression of C/EBPγ induces a brown preadipocyte pattern of gene expression in white 3T3-L1 preadipocytes by displacing the repressive CHOP10 and ATF-2 from bZIP heterodimers bound to the CRE on the PGC-1α promoter.

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