Emerging Role of C/EBPβ and Epigenetic DNA Methylation in Ageing

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Changes in epigenetic DNA methylation are the most promising predictor of biological age and lifespan in humans, but whether methylation changes affect ageing is unresolved. Here, we discuss converging data, which indicate that one mode by which aberrant DNA methylation can affect ageing is via CCAAT/enhancer binding protein beta (C/EBPβ). This basic leucine-zipper (bZIP) transcription factor is controlled by the lifespan regulator mechanistic/mammalian target of rapamycin complex 1 (mTORC1) and plays an important role in energy homeostasis and adipose tissue differentiation. Emerging evidence indicates that access of C/EBPβ proteins to cognate binding sites is regulated by DNA demethylation via ten-eleven translocation (TET) methylcytosine dioxygenases and their adaptor proteins growth arrest and DNA damage-inducible protein 45 alpha (GADD45α) and inhibitor of growth 1 (ING1). We discuss the emerging causal nexus between C/EBPβ, energy metabolism, and DNA demethylation in organismal ageing.

Epigenetic DNA methylation: Driver or Bystander of Ageing?

A multitude of molecular alterations accompany organismal ageing [1,2]. Among them, epigenetic alterations have a great influence on ageing, including histone modifications, exchange of canonical histones by histone variants, and the noncoding RNA expression pattern [3]. One of the best documented changes during ageing involves alterations in DNA methylation (Box 1). However, whether methylation changes can affect ageing is unclear. Recent evidence indicates that access of the C/EBPβ transcription factor to cognate binding sites is regulated by DNA demethylation and that impaired demethylation of C/EBPβ sites can lead to premature ageing [4]. This basic leucine zipper (bZIP) protein (see Glossary) plays an important role in controlling energy homeostasis and is regulated by mechanistic/mammalian target of rapamycin complex 1 (mTORC1), a major regulator of health and lifespan. Here, we provide an integrated view on DNA methylation and ageing together with the role C/EBPβ may play in metabolism and longevity.

Mammalian DNA methylation is an epigenetic mark that occurs mostly on CpG dinucleotides. Globally, DNA methylation and CpG density show a bimodal distribution. CpG islands (CGIs), GC-rich regions with high densities of CpGs found near promoter regions, are generally unmethylated. In contrast, most regions with low CpG density are heavily methylated in normal tissues, notably at repetitive DNA, where the bulk of genomic DNA methylation resides. Besides unmethylated and heavily methylated regions, a third category exists, namely, lowly methylated regions (LMRs), which are non-CGI loci associated with regulatory elements such as transcription factor (TF) binding sites. TF binding is necessary and sufficient to create LMRs [5].

The genome-wide pattern of DNA methylation is tissue and cell state specific. It is the result of the action of cytosine DNA methyltransferases, of which there are three in mammals, Dnmt1, Dnmt3a, and Dnmt3b. However, methylation patterns are also shaped by specific pruning of methyl marks, both by active as well as passive DNA demethylation [6]. Active DNA methylation proceeds by oxidation of methyl groups via the TET family of methylcytosine dioxygenases [7]. TET enzymes oxidize 5mC sequentially to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxycytosine (5caC). Thymine DNA glycosylase (TDG) removes 5fC and 5caC to restore unmethylated cytosine.

DNA methylation is mostly associated with gene silencing, although the correlation of methylation with gene activity is often loose [6]. For example, despite millions of genomic sites that become unmethylated in embryonic stem cells lacking Dnmt1, including at least 6100 promoters, only a few

Highlights

Ageing is closely associated with and influenced by energy metabolism, and C/EBPβ is emerging as a key regulator of energy metabolism and longevity.

DNA hypermethylation in GADD45α/ING1 mutant mice is associated with progeria due to a failure of TET-dioxygenase mediated demethylation of C/EBPβ binding sites.

Accordingly, GADD45α/ING1 mutant mice phenocopy major symptoms of C/EBPβ mutant mice, indicating that a GADD45α–ING1–C/EBPβ axis regulates energy metabolism and ageing.

mTORC1 controls the translation of Cebpb-mRNA into two isoforms, the transcriptional activator liver-enriched activating protein (LAP), and inhibitor liver-enriched inhibitory protein (LIP). C/EBPβ super-mice, in which the inhibitory LIP is inactivated display healthier ageing and prolonged life span.

The results indicate a causal nexus between C/EBPβ, energy metabolism, and DNA demethylation in organismal ageing.

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Box 1. Epigenetic DNA Methylation Clocks

Statistical analysis of publicly available human DNA methylation data led to the proposition of signature methylated CpGs, designated ‘epigenetic clocks’, whose methylation level tightly correlates with biological age. Hannum’s clock is applicable to whole blood and interrogates 71 CGs. Horvath’s clock is a multitissue predictor and interrogates 353 CGs [65]. Hannum’s and Horvath’s clocks have correlation coefficients >0.9 with chronological age and average errors below 5 years. Horvath’s clock is the most widely used and it permits comparing the biological age of different tissues from the same individual. Epigenetic age according to Horvath’s clock is close to zero for embryonic cells and shows biphasic behavior, ticking fast during childhood, and from ~20 years of age the clock continues at a slower constant rate. These epigenetic clocks predict biological age and inform about positive or negative deviation of biological from chronological age, as well as predicting time to death. For example, DNA from tumour tissue shows accelerated biological age compared with chronological age of the patient. HIV infection, known to induce premature biological aging, also accelerates the epigenetic clock [66]. Conversely, super-centenarians may have decelerated epigenetic age [66].

In vitro, DNA methylation age increases with cell passage number while reprogramming can reverse DNA methylation age [65,67]. Recently improved multitissue clocks for the first time also reveal age acceleration in DNA from Hutchinson Gilford progeria syndrome patients [68] and strongly predict time to death and time to heart disease [69].

There is a propensity of clock CpGs to be found near genes involved in development and differentiation [16] and this appears consistent with the tendency of polycomb target genes DMRs to locate at bivalent chromatin domain promoters harboring Polycomb target genes [20–22]. Since Polycomb and bivalent promoters frequently regulate developmental control genes, age-related hypermethylation ultimately may reflect changes in tissue homeostasis and cell differentiation [16]. Other than this rather general conclusion, there is no clear biological process, including expression level of genes nearby clock CpGs or ageing DMRs, which show consistent correlation with the methylation level. Hence a key question remains: is DNA methylation a driver or a passenger of the ageing process?

Glossary

Autophagy: cellular mechanism eliminating and recycling unnecessary or defective cell components.

Basic leucine zipper (bZIP): superfamily of transcription factors; the leucine zipper mediates the required dimerization for DNA binding specified by the basic region at duplex DNA sequences, as homodimers or heterodimers.

Bivalent chromatin domains: regions of regulatory chromatin bound by histones modified by repressive and activating marks.

Calorie/dietary restriction (CR): reduced calorie intake barring malnutrition, which alters metabolic function and is associated with increased lifespan in various organisms.

Cellular senescence: phenomenon whereby normal cells cease to divide after a limited number of cell divisions and display an array of altered molecular features.

ChlPseq: method combining chromatin immunoprecipitation with massively parallel DNA sequencing in order to map global binding sites for any protein of interest.

DNA-damage response: network of cellular pathways that sense, signal, and repair DNA lesions.

Ligand activation and lipolysis. metabolic process whereby fat is generated or degraded, for metabolic energy storage and release, respectively.

Mammalian or mechanistic target of rapamycin complex 1 (mTORC1): protein complex composed of mTOR itself, regulatory-associated protein of mTOR (Raptor), mammalian lethal with SEC13 protein 8 (MLST8), proline-rich Akt substrate 40kD (PRAS40), and DEP domain-containing mTOR-interacting protein (DEPTOR). mTORC1 functions as a nutrient/energy/redox sensor and controls various processes including protein synthesis.

Memory: naive T-cell: ratio between memory T cells that act in response to re-exposure to antigens and naive T cells that form the pool of T cells that are activated in response to unknown antigens. The ratio increases during ageing and is a measure of immunological ageing.

Ageing by Reduced Access to C/EBPβ Binding Sites

Recent evidence for a causative role of DNA hypermethylation in ageing comes from a mouse model that is a homozygous mutant for two stress response genes, Gadd45a and Ing1, and displays a
premature ageing phenotype (hereafter referred to as double knockout; DKO) [4]. GADD45a and ING1 cooperate as adapter proteins in TET/TDG-mediated DNA demethylation, targeting the demethylating enzymes to specific genomic loci [4,23–26]. Consequently, DKO mouse embryonic fibroblasts (MEFs) display mostly hypermethylated DMRs [4]. Both the hypermethylated DMRs and GADD45a binding sites are located next to superenhancers that also have signatures of C/EBPb binding sites. Indeed DKO cells show reduced binding of C/EBPb to cognate sites. Consistently, DKO mice phenocopy symptoms of mutants of Cebpb or the related Cebpa gene; for example, with defects in adipogenesis, fat browning, increased catabolism, and female infertility. Ing1 appears to be the driver of these phenotypes, as for example, single Ing1 mutant MEFs already display mild adipogenesis defects, which are exacerbated in DKOs. ChIPseq reveals that Ing1 protein in MEFs binds to promoters, which show a gene ontology signature for signaling by the insulin receptor, a top prolongevity gene, and motifs for E2F TFs. E2Fs are prominent regulators of metabolism, including adipogenesis and insulin signaling [27], and they are well established downstream mediators of C/EBPβ and C/EBPα during cell differentiation [28]. Moreover, binding sites of E2F1 show a strong enrichment among ageing-associated genes [29].

Most of the Cebp mutant-like symptoms observed in Gadd45a/Ing1 DKO mice are also characteristic for aged or progeroid mice (Figure 1), indicating that the premature aging of the DKO mice, in fact, reflects impaired C/EBP function. This suggests a model where GADD45a–ING1 promote enhancer demethylation to permit C/EBPβ binding and transactivation of target genes, which maintains energy homeostasis and prevents organismal ageing. While these results indicate that aberrant DNA methylation can drive premature ageing, it remains to be shown if the hypermethylated CpGs identified in the DKO mice also show corresponding changes in physiologically aged mice and whether they overlap with CpGs defined in mouse epigenetic clocks. Curiously, DNA methylation actually enhances C/EBPβ binding to canonical TTGCGCAA sites in vitro [30], while in vivo unmethylated sites are preferred [31], possibly due to interference by methyl-CpG-binding domain (MBD) proteins.

Hypermethylation of C/EBPβ-dependent superenhancers in the DKO model is manifest already in naive embryonic fibroblasts. This suggests that demethylation of C/EBPβ sites occurs during embryogenesis and poises enhancers to fire upon adequate stimuli in adult life. Thus, epigenetic ageing may in fact start during fetal life, which is supported by the finding that the DNA methylation clock
Cebpa gene is replaced by a second Cebp gene have an increased median lifespan of 20% and show

GADD45α and ING1 have previously been linked to ageing and both interact with several longevity regulators. GADD45α is a multifunctional protein involved in cell cycle regulation, apoptosis, stress response, and DNA repair [36]. As a stress response gene, Gadd45α is transcriptionally activated by p53 and FoxO3a. Lamin A, a protein which is mutated in Hutchinson–Gilford progeria interacts with Ing1 [37]; Ing1 and GADD45z both regulate senescence [38,39]. HSF1 is a well-established longevity gene and GADD45z acts downstream in an HSF1–FOXO3–SOD2/CAT/GADD45z cascade implicated in stress response and lifespan extension [40]. In Drosophila, overexpression of D-GADD45 increases longevity, with long-lived animals showing resistance to stress [41]. GADD45β, a close homologue of GADD45α, mediates systemic gene expression responses in mice during calorie restriction (CR) [42], which is associated with longevity. Deletion of Gadd45a prolongs lifespan in telomerase-deficient mice [43]. Mechanistically, GADD45α promotes demethylation at the subtelomeric regions of short telomeres and its deficiency promotes chromatin compaction and attenuates initiation of a DNA damage response at short telomeres of telomerase-deficient cells.

The finding that impaired C/EBPβ function emerged as a cause of progeria in Gadd45a/Ing1 DKO is tantalizing in light of the important nexus between energy metabolism and ageing since C/EBPs are key regulators of glucose and fat metabolism (Box 2), as discussed below.

**mTORC1 Controlled C/EBPβ- Isoform Expression in Health and Lifespan Regulation**

C/EBPβ was postulated to play a role in health span and lifespan determination ever since studies revealed its role in the regulation of metabolism and energy homeostasis. Transgenic mice in which the Cebp gene is replaced by a second Cebp gene have an increased median lifespan of 20% and show
reduced fat storage and increased mitochondrial biogenesis in white adipose tissue (WAT) [44,45], suggesting that C/EBP\(_b\) acts as a pro-longevity factor. Cebpb is a recurring top hit in screens identifying candidate transcriptional regulators of ageing-associated genes [29,46,47]. Besides its transcriptional activities on superenhancers, the regulation of C/EBP\(_b\) protein isoform expression by the mTORC1 signaling pathway provides another link to lifespan regulation [48]. The mTORC1 pathway integrates intracellular nutrient and energy availability with growth factor and/or hormonal signals. Its activation stimulates the conversion of nutrients and energy into macromolecules for cell growth while at the same time inhibiting the recycling of cellular constituents back into nutrients through autophagy. Inhibition of the mTORC1 pathway in response to CR is seen as a key event that mediates the health- and lifespan-extending effect of CR. Accordingly, reduced signaling through the mTORC1 pathway itself caused by pharmacological inhibition (e.g., by rapamycin) or by genetic mutations of pathway components delays the onset of age-related diseases and extends lifespan in different model organisms [49,50]. Notwithstanding its key role in ageing, little is known of the factors downstream of mTORC1 that mediate the physiological response in terms of gene regulation.

mTORC1 controls the translation of the Cebp-mRNA into its two functionally different protein isoforms, the transcriptional activator liver-enriched activating protein (LAP) and the transcriptional inhibitor liver-enriched inhibitory protein (LIP), and thereby adjusts metabolic gene regulation downstream of C/EBP\(_b\) to energy/nutrient availability [48]. mTORC1 stimulates the expression of the inhibitory C/EBP\(_b\)-LIP isoform through a translational mechanism that requires an upstream open reading frame (uORF) within the Cebp-mRNA that serves as a cis-regulatory element. Thus, reduced mTORC1 activity results in lower C/EBP\(_b\)-LIP expression and thereby in reduced inhibitory C/EBP\(_b\) function. Since the activating C/EBP\(_b\)-isoform C/EBP\(_b\)-LAP is not affected by mTORC1, the net effect of mTORC1 inhibition is an increase in C/EBP\(_b\) transcriptional activity (Figure 2).

Strong support for a role of differential C/EBP\(_b\) isoform expression in health and lifespan regulation comes from knock-in mice that contain a mutation of the cis-regulatory Cebp-uORF required for mTORC1-mediated LIP expression. In these Cebp\(_{uORF}\) mice C/EBP\(_b\)-LIP expression is abrogated and as a consequence the transcriptional C/EBP\(_b\) function is increased (C/EBP\(_b\) super-mice), which mimics reduced mTORC1 signaling at the level of C/EBP\(_b\). These C/EBP\(_b\) super-mice display an

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**Figure 2. mTORC1-Controlled Translation of Cebp-mRNA.**

Cebp-mRNA is translated into the protein isoforms C/EBP\(_b\)-LAP and C/EBP\(_b\)-LIP. The transcriptional activator C/EBP\(_b\)-LAP is translated by regular translation initiation. Translation into the transcriptional inhibitory protein C/EBP\(_b\)-LIP requires an initial translation of the uORF in cis and subsequent translation re-initiation at the downstream AUG codon. This process is stimulated by mTORC1 signaling, which in turn is stimulated by nutrient availability and growth factors and suppressed under calorie restriction or pharmacological inhibition by rapamycin. Abbreviations: C/EBP\(_b\), CCAAT/enhancer binding protein \(\beta\); LAP, liver-enriched activating protein; LIP, liver-enriched inhibitory protein; mTORC1, mammalian/mechanistic target of rapamycin complex 1; uORF, upstream open reading frame.
**Key Figure**

Nexus between C/EBPβ, DNA Methylation, and Longevity Pathways

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**Figure 3.** The activating C/EBPβ–LAP and inhibitory C/EBPβ–LIP transcription factors bind to hypomethylated superenhancers to modulate gene expression. The LIP:LAP ratio is regulated by mTORC1 signaling, which integrates nutrient, energy, and growth factor signaling. mTORC1 signaling and C/EBPβ–LIP expression are suppressed under conditions of nutrient and/or energy deprivation, such as calorie restriction. Occupancy of C/EBPβ sites is determined by DNA demethylation of superenhancers residing in chromatin loops with E2F-associated promotores. TET/TDG-mediated demethylation of C/EBPβ binding sites is targeted by GADD45α associated with C/EBPβ binding sites and ING1 bound at E2F-associated promoter sequences. Connections to other longevity proteins including SIRT1, p53, and FoxO3 is depicted. The involvement of SIRT1-mediated deacetylation is hypothetical, and based on SIRT1–C/EBPα regulation. Abbreviations: AAT, amino acid transporter; Ac, protein lysine-acetylation; AKT, also known as protein kinase B (PKB); AMPK, AMP-activated protein kinase; 4E-BP, initiation factor eIF4E binding protein; C/EBPβ, CCAAT/enhancer binding protein β; eIF4E, translation initiation factor 4E; FoxO3, Forkhead box O3; GADD45α, growth arrest and DNA damage protein 45α; IGF-1, insulin-like growth factor 1; ING1, inhibitor of growth family member 1; LAP, liver-enriched inhibitory protein, C/EBPβ protein isoform; LIP, liver-enriched inhibitory protein, C/EBPβ protein isoform; LKB1, liver kinase B1, also known as serine/threonine kinase 11 (STK11); mTORC1, mammalian/mechanistic target of rapamycin complex 1; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase; RTK, receptor tyrosine kinase; SIRT1, sirtuin 1, silent mating type information regulation 2 homolog 1; TDG, thymine DNA glycosylase; TET, ten-eleven translocation. Open lollipops, unmethylated CpGs at C/EBPβ sites.
improved metabolic phenotype with features observed under CR [51], including leanness, increased insulin sensitivity and glucose tolerance, enhanced fatty acid oxidation, reduced steatosis, and higher adiponectin levels [48]. At gene regulation level, C/EBPβ super-mice show an increased expression of genes connected to both de novo lipogenesis and lipolysis in WAT, while in liver lipogenesis, genes tend to be repressed and genes involved in β-oxidation are upregulated. The same gene expression pattern is observed in mice under CR suggesting that, in C/EBPβ super-mice, a similar metabolic roundabout is induced as in mice under CR. In CR-fed mice, ingested carbohydrates are first converted into fat that is subsequently broken down again through lipolysis into fatty acids, which undergo β-oxidation in peripheral tissues for energy production. In other words, mice under CR burn more fat than they eat [51]. Although this metabolic detour with increased fat production at first sight seems not to agree with the reduced food availability under CR conditions, it is proposed to be at least partially responsible for the beneficial effects of CR [51] and therefore could also contribute to the increased metabolic health in C/EBPβ super-mice.

Female C/EBPβ super-mice show a markedly increased median lifespan of 20%, that is partially due to a decrease and delay in cancer incidence. Aged C/EBPα super-mice appear rejuvenated, with better motor coordination, a more juvenile memory:naïve T cell ratio, and a reduction in ageing-associated interindividual variation in gene expression, particularly of genes related to fatty acid metabolism and oxidative phosphorylation. Since these genes are either known or anticipated C/EBPα target genes, their increased variation in expression levels upon ageing in wild-type mice might be due to ageing-associated fluctuation in C/EBPβ–LIP/LAP isoform ratios, which is prevented in the C/EBPβ super-mice. C/EBPβ–LIP expression is indeed elevated in aged liver and fat tissue [52–54], suggesting that ageing-associated deregulation of C/EBPβ–LIP may contribute to the risk of developing age-related conditions. This idea is supported by a mouse model that only expresses the C/EBPβ–LIP isoform but lacks C/EBPβ–LAP isoform expression as these mice display an earlier onset of ageing-associated tumorigenesis and a reduction in lifespan [55]. Altogether, the studies demonstrate that an increased transcriptional function of C/EBPβ promotes health and lifespan in mice.

**Potential Role of C/EBPα in Health and Lifespan Regulation**

Similar to C/EBPβ, C/EBPα is an important metabolic regulator with partially overlapping target genes and functions with respect to C/EBPβ. Similar to Cebpβ, the Cebpα-mRNA contains a cis-regulatory uORF and is translated into different protein isoforms, termed C/EBPα-p42 and C/EBPα-p30. As for C/EBPβ–LIP, the expression of C/EBPα-p30 is under control of mTORC1 signaling [56], indicating a role for C/EBPα in mTORC1 downstream effects.

Besides mTORC1, another nutrient-sensitive regulator connected with the control of health and lifespan, the NAD+-dependent deacetylase SIRT1, affects C/EBPα activity [57]. Fluctuations in NAD+ levels due to nutrition, exercise, circadian rhythm, or ageing regulate SIRT1 activity. Initially, the homologous yeast Sir2 was described as extending lifespan, but further studies in worms, flies, and mice have challenged this interpretation and led to the current prevailing view that SIRT1 activity plays a role in health maintenance and stress response [58–60]. Increased expression of the SIRT1 homologues in yeast, flies and Caenorhabditis elegans results in lifespan extension, and mammalian SIRT1 activity plays a role in health maintenance and stress response, acting to extend lifespan by rescuing from the effects of life-shortening stress [58–60]. SIRT1 deacetylates C/EBPα, which is required for the elevation of mitochondrial mass and respiratory function in response to glucose deprivation, suggesting that C/EBPα is a critical downstream mediator of SIRT1 function. This is supported by the finding that expression of a deacetylation-mimicking C/EBPα mutant is sufficient to increase the expression of mitochondrial genes and enhance mitochondrial respiration in the absence of SIRT1 or nutrient deprivation [57]. Mouse models addressing these mTORC1- or SIRT1-dependent modes of C/EBPα regulation should give more evidence for a possible role of C/EBPα in health and lifespan regulation in the future.

**Other C/EBPs in Ageing**

It is likely that other C/EBP proteins also play a role in ageing, given their overlapping function and binding site specificity. C/EBPβ mediates DNA-damage response, and similar to GADD45α and
ING1, C/EBPβ deficiency sensitizes mice to ionizing radiation [61]. Loss of C/EBPβ exacerbates cognitive decline in aged mice exposed to radiation due to impaired oxidative stress response [62]. Relatedly, the worm orthologue of CEBPβ regulates a neuronal gene clusters in ageing nematodes [63]. Furthermore, coexpression screening of a seed list of genes overexpressed with age yielded Cebpα, Cebpb, and Cebpδ as the top hits, and C/EBP motifs were found to be enriched in the promoters of ageing-associated genes [46]. It is important to consider that different C/EBP family members form heterodimers and share DNA recognition sites, but differ in expression patterns and upstream regulatory pathways. Due to this complexity, little is known about an integrated C/EBP function coming from the combined action of the different C/EBP family members and its involvement in the ageing process. This will be an important topic of further studies.

Concluding Remarks

Whether changes in epigenetic DNA methylation can drive organismal ageing is an unresolved question. Progeric Gadd45a Ing1 DKO mice show impaired binding of C/EBPβ due to a defect in TET-mediated DNA demethylation of C/EBPβ superenhancers. This suggests a GADD45α–ING1–C/EBPβ axis regulating energy metabolism and in turn longevity. Hence, changes in DNA methylation are not only excellent markers for biological age but actually can cause ageing when interfering with normal C/EBPβ activity. Independent evidence for C/EBPβ regulating ageing comes from mouse mutants deficient in the inhibitory C/EBPβ–LIP isoform (C/EBPβ super-mice) that show CR-type metabolic adaptations paired with a prolonged health and lifespan, which highlights that increased transcriptional function of C/EBPβ delays the ageing process. C/EBPβ and β are crucial transcriptional regulators of energy metabolism, a pivot in the control of organismal ageing. Effectors changing the ratio of the inhibiting/activating LIP/LAP C/EBPβ and possibly p30/p42 C/EBPβ isoforms therefore may delay organisimal ageing. C/EBPβ as well as GADD45α and ING1 are all part of gene regulatory networks with well-established roles in ageing, including mTORC1, SIRT1, p53, and FOXO3 (Figure 3, Key Figure).

Notwithstanding open questions (see Outstanding Questions), there may be enough ground to build on strategies manipulating C/EBPβ activity to attenuate ageing-related diseases. For example, adifovir dipivoxil reduces C/EBPβ–LIP levels and induces fatty acid β oxidation in cell culture as a potential CR mimetic [64]. Progeria resulting from site-specific demethylation defects raises the possibility that drugs might promote healthy ageing. Furthermore, the likely involvement of other C/EBP proteins besides C/EBPβ might provide additional therapeutic targets to promote healthy ageing.

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