Altered expression of C/EBP family members results in decreased adipogenesis with aging

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THE AGING PROCESS IS CHARACTERIZED by a decline in function of many physiological systems leading to a myriad of health problems in humans and other mammals. Among these is loss of fat tissue in very old individuals. Fat mass peaks by middle age or early old age and then declines substantially in advanced old age. Loss of fat tissue predisposes the elderly to chronic skin ulcers, disturbances of body temperature, and decreased energy reserves in the face of chronic illness (38, 57). Loss of fat tissue can also result in glucose intolerance (46, 51), potentially contributing to the paradoxical development of type II diabetes in very old, lean patients. Although fat mass declines, new fat cells are formed throughout the life span (5, 20, 39, 45), resulting in stable or increasing numbers of fat cells in fat tissue of old individuals (4, 31, 32, 36, 52, 61). Because fat cell responsiveness to lipolytic agents decreases with increasing age (16, 26, 31), declining body weight, fat mass, percent body fat, and fat cell size may be principally related to reduced capacity for lipid accumulation.

The capacity of preadipocytes to differentiate and accumulate lipid also declines with aging (17, 33, 36). Preadipocytes isolated from old rats and humans accumulate less lipid and have lower lipogenic enzyme activities when exposed to a variety of conditions that induce differentiation than preadipocytes from younger individuals, even after 3 wk in culture (14, 17, 33, 34). Consistent with their decreased capacity for differentiation, preadipocytes isolated from old rats exhibited decreased expression of several differentiation-dependent genes compared with preadipocytes cultured from young rats (34). These differences were not caused by inherent differences in basal expression of these genes in undifferentiated preadipocytes; rather, they reflect a difference in the extent of expression induced during the differentiation process. Thus whereas factors extrinsic to fat tissue (such as diet, activity, and hormonal milieu) likely contribute to declining fat mass during senescence, decreased capacity for lipid accumulation also involves age-related processes intrinsic to the individual preadipocytes and adipocytes in fat tissue.

We therefore hypothesized that the age-related decline in primary preadipocyte differentiation and adipocyte function resulted from an inability to express adequate levels of CCAAT/enhancer binding protein (C/EBP)-α, an adipogenic transcription factor that regulates adipogenesis. The importance of C/EBPα has been demonstrated in vivo, because transgenic mice unable to express C/EBPα lack adipose tissue (3, 46, 48, 55). In vitro, overexpression of C/EBPα is sufficient...
to induce nonadipogenic cell lines to differentiate into adipocytes, whereas overexpression of antisense C/EBPα inhibits differentiation (40). In addition to its role in initiating preadipocyte differentiation, C/EBPα regulates expression of key genes necessary for maintaining the fat cell phenotype (11, 19, 60). Thus C/EBPα is a “bottleneck” in the chain of events beginning with activation of preadipocyte differentiation and ending with the appearance and maintenance of functional fat cells.

Our studies establish that processes intrinsic to preadipocytes and fat cells that involve alterations in expression of C/EBP family members contribute to decreased adipogenesis with aging. We found that C/EBPα-expression declined substantially with aging in differentiating preadipocytes and fat tissue. Overexpressing C/EBPα restored adipogenesis of preadipocytes from old animals. The decline in C/EBPα was related to increased levels of C/EBPβ-liver inhibitory protein (LIP), which interfere with expression of C/EBPα and other adipogenic transcription factors (6, 8, 48, 53, 58, 60). C/EBPβ-LIP increased with aging both in cultured preadipocytes and in fat tissue in vivo. Additionally, expression of C/EBPδ, which acts with full-length C/EBPβ to enhance adipogenesis (58), decreased with aging during early differentiation. These findings clarify the mechanism of the age-related decline in preadipocyte differentiation and shed light on processes responsible for decreases in fat cell size and ability to assimilate lipids in the elderly.

METHODS

Preadipocyte isolation and culture. Fischer 344 (median survival 26 mo; maximum survival 32 mo) and Brown Norway rats (median survival 32 mo; maximum survival 43 mo) were barrier-reared, specific pathogen-free, raised under conditions of the perirenal depots of 3-, 17-, or 24- to 27-mo-old rats was minced, and those with evidence of pathology were excluded. Animals in respects other than their ages (10, 18, 50). The protocol was approved by the Boston University Institutional Animal Care and Use Committee. Separate groups of animals were used for each experiment. Animals were autopsied and blotted as described previously (44, 62). Membranes were probed with rabbit polyclonal antibodies to C/EBPα, β, or δ mouse monoclonal antibodies to C/EBPβ (Santa Cruz Biotechnology; 14AA, C-19, C-22, and H-7, respectively). Visualization of the binding of the horseradish peroxidase conjugated secondary antibody was performed by chemiluminescence. Specificity of the interaction was assessed using specific blocking peptide. Ten micrograms of protein were loaded in each lane. Total protein contents (pg/cell) were 305 ± 32 and 310 ± 38 in undifferentiated and differentiated preadipocytes from 3-mo-old rats, respectively, and 335 ± 39 and 333 ± 42 in undifferentiated and differentiated preadipocytes from 24-mo-old rats (n = 16 in each group). Equal amounts of protein from undifferentiated or differentiated preadipocytes from young or old rats were loaded in parallel on the same gels. Amounts of each protein loaded were selected so that results were in the linear response range for that protein. Densitometric results were expressed as a percentage of total optical density within each gel and were normalized to reflect differences in cellular protein content.

DNA transfection. Preadipocytes from 24-mo-old rats were cotransfected with pMT2rC/EBPα and pMSV-β-gal (Invitrogen) at a molar ratio of 5:1 (transcription factor/reporter construct) using the calcium phosphate precipitation method without glycerol shock treatment to ensure that transfected

RNA analysis. For RNA blot analyses, RNA was isolated from preadipocytes using the guanidinium thiocyanate-phenol method (9). RNA integrity was verified using 2% denaturing agarose gels. Messenger RNA abundance was measured by RNA blot analysis, as described previously (34). RNA was hybridized to a 1.5-kb fragment complementary to C/EBPα (from plasmid ptet-o-C/EBPα), a 1.5-kb fragment complementary to C/EBPβ (from plasmid ptet-o-C/EBPβ), a 450-bp fragment complementary to β-actin (from plasmid pFHβA-3'UT-HP), or a 1.6-kb fragment complementary to 28S rRNA (from clone pABE) (35). Preadipocyte mRNA levels were measured densitometrically and adjusted for 28S rRNA levels in the same lane on the same gel to correct for differences in RNA loading. We have shown that 28S rRNA levels are the same in undifferentiated and differentiated epididymal and perirenal preadipocytes when equal numbers of cells are analyzed (34).

Protein analysis. For protein analyses, cells were harvested and blotted as described previously (44, 62). Membranes were probed with rabbit polyclonal antibodies to C/EBPα, β, or δ mouse monoclonal antibodies to C/EBPβ (Santa Cruz Biotechnology; 14AA, C-19, C-22, and H-7, respectively). Visualization of the binding of the horseradish peroxidase conjugated secondary antibody was performed by chemiluminescence. Specificity of the interaction was assessed using specific blocking peptide. Ten micrograms of protein were loaded in each lane. Total protein contents (pg/cell) were 305 ± 32 and 310 ± 38 in undifferentiated and differentiated preadipocytes from 3-mo-old rats, respectively, and 335 ± 39 and 333 ± 42 in undifferentiated and differentiated preadipocytes from 24-mo-old rats (n = 16 in each group). Equal amounts of protein from undifferentiated or differentiated preadipocytes from young or old rats were loaded in parallel on the same gels. Amounts of each protein loaded were selected so that results were in the linear response range for that protein. Densitometric results were expressed as a percentage of total optical density within each gel and were normalized to reflect differences in cellular protein content.
cells detected by staining for β-galactosidase (βgal) would also be expressing C/EBPα. Approximately 15% of transfected cells were positive for βgal. Increased C/EBPα-expression in transfected cells was verified by Western immunoanalysis. Transfection efficiency was not affected by aging. Mock-control cells were transfected with pMSV-βgal and pMT2xHGh that consists of the adenovirus major late promoter fused to a reverse-orientation human growth hormone construct of the same size as pMT2r/C/EBPα. After 48 h of culture in differentiation medium, cultures were fixed in 3% formaldehyde and stained with the chromatographic βgal substrate x-gal to identify transfected cells (49). Observers unaware of the origin of the cells assessed extent of differentiation by examining transfected cells for the presence of doubly refractile lipid inclusions using low-power phase-contrast microscopy (33, 56). The lipid nature of these inclusions was confirmed by staining with oil red O. The proportion of transfected cells containing such inclusions was compared with that of parallel mock-control cultures. For C/EBPα-LIP transfection experiments, we used the probe CMV-LIP, kindly provided by Dr. U. Schibler (12), and a transfection and analysis strategy analogous to that described above for C/EBPα except that transfectants from 3-mo-old animals were exposed to differentiation medium for 72 h. Increased C/EBPα-LIP expression in transfected cells was verified by Western immunoanalysis.

Statistical analysis. Results are means ± SE, and significance determination was by paired t-tests or ANOVA as appropriate (28, 29). In transfection experiments, comparisons of numbers of transfecants containing lipid inclusions to mock-transfected cells were made by logistic regression analysis, with P values determined from log likelihood ratios (37).

RESULTS

Expression of C/EBPα declines with aging. To determine whether altered C/EBPα-expression contributes to the age-related decline in the intrinsic capacity of preadipocytes to differentiate, we measured C/EBPα-mRNA levels in undifferentiated and differentiated preadipocytes cultured from perirenal fat depots of young (3 mo), middle-aged (17 mo), and old (24 mo) Fischer 344 rats. Donor age had no effect on C/EBPα-mRNA levels in primary undifferentiated preadipocytes (Fig. 1). However, we observed significant blunting of differentiation-related induction of C/EBPα-mRNA abundance with increasing donor age. C/EBPα-mRNA levels in differentiating cells declined progressively through the life span so that C/EBPα-mRNA abundance was 7.5-fold higher in cells from young compared with old animals (P < 0.01, n = 5). A similar age-related decline in C/EBPα-mRNA induction was observed in each of three experiments (data not shown) in preadipocytes cultured from barrier-reared Brown Norway rats that differed from Fischer 344 rats in several respects (42). Use of a differentiation-inducing medium of composition distinct from that used in the above experiments (34) resulted in the same age-related decline in C/EBPα-mRNA induction (data not shown). Thus a substantial decrease in C/EBPα-mRNA induction during adipogenesis occurs throughout the lifespan, and this change results from events intrinsic to primary preadipocytes.

We then examined the time course of C/EBPα-induction during preadipocyte differentiation in primary preadipocytes from young and old rats, because age-related differences in C/EBPα-abundance could reflect differences in the time course of C/EBPα-induction. Increases in C/EBPα-protein were evident by 4 h in differentiating preadipocytes from both age groups and achieved peak levels within 24 h that were sustained at least 72 h (Fig. 2). Within 8 h after initiation of differentiation, the induced level of C/EBPα-protein was significantly greater in differentiating preadipocytes isolated from young compared with old animals (Fig. 3; 2.4 ± 0.1-fold; P < 0.00001, n = 6; paired t-test). Similar results were found in epididymal preadipocytes (data not shown). C/EBPα-mRNA is alternatively translated into 42- and 30-kDa protein isoforms, with the 42-kDa isoform being more effective at promoting differentiation and the replicative arrest that accompanies differentiation than the 30-kDa isoform (41). Although total C/EBPα-expression declined with aging, relative expression of the two C/EBPα-protein isoforms remained unchanged. Densities of the 42-kDa isoform in cells from 3- and 24-mo-old rats were 2.0 ±
Fig. 3. Aging is associated with decreased C/EBPα-protein abundance. Densitometric analysis of total C/EBPα-protein levels (30- plus 42-kDa isoforms) was performed 8 h after inducing differentiation in preadipocytes from young (3M) and old (24M) animals in parallel in 6 separate experiments. Means ± SE are shown, with values expressed as percentages of optical density (OD) within each experiment normalized for cellular protein content. 0.3 and 2.0 ± 0.2 times greater than the 30-kDa isoform, respectively (n = 6). Therefore, the age-related decline in C/EBPα-mRNA was 1) mirrored by concomitant changes in C/EBPα-protein abundance, 2) evident early in the differentiation program, and 3) reflective of overall diminished C/EBPα-levels rather than a delayed response to differentiation inducers or alterations in the proportions of C/EBPα-protein isoforms.

To determine whether the age-related decline in C/EBPα-expression during preadipocyte differentiation is maintained in fat cells in vivo, we measured C/EBPα-mRNA abundance in collagenase-isolated inguinal fat cells from young, middle-aged, and old Brown Norway rats. We observed an age-related decline in isolated fat cell C/EBPα-mRNA abundance similar to that in differentiating cultured preadipocytes (Fig. 4A). An analogous, progressive, age-related decline in total C/EBPα-protein levels was also observed in epididymal, perirenal, and inguinal fat tissue from young and old Fischer 344 rats (2.3 ± 0.6-fold; n = 3 young and 3 old rats, 3 sites; P < 0.005; ANOVA), with no change in relative expression of the 42- and 30-kDa isoforms (Fig. 4B). Decreased C/EBPα-protein abundance was also apparent in adipocytes isolated from the epididymal depots of 27- compared with 3-month old Brown Norway rats (data not shown). Thus the decline in C/EBPα-expression with aging in differentiating cultured preadipocytes is reflected in fat cells in vivo, occurs in different rat strains, and is an intrinsic feature of fat cells from all depots tested.

Our data show that the age-related decline in adipogenesis is associated with decreased C/EBPα-expression. To determine whether augmenting C/EBPα-expression in preadipocytes from old animals can restore capacity to accumulate lipid, we transiently transfected epididymal preadipocytes from 24-month old rats with a rat-specific C/EBPα-expression vector, pMT2rC/

Fig. 4. C/EBPα-expression decreases with age in fat tissue. A: C/EBPα-mRNA abundance in collagenase-isolated inguinal (I) fat cells from 3M and 27M barrier-reared male Brown Norway rats was measured by Northern analysis. A blot representative of 3 experiments is shown. β-Actin mRNA was used as a loading control. B: C/EBPα-protein abundance in epididymal (E), perirenal (P), and I fat tissue from 3M compared with 24M F344 rats was determined by Western blot analysis. An experiment representative of 3 separate experiments is shown.
We observed that aging had no effect on C/EBPβ-mRNA (Fig. 6). The total amount of C/EBPβ-protein measured by densitometry was also not affected by age [ratio of total C/EBPβ-protein in preconfluent preadipocytes from 3-mo-old to 24-mo-old animals = 0.98 ± 0.20 and in preadipocytes treated with differentiation-inducing medium for 48 h, 0.99 ± 0.09 (Fig. 7)]. LAP/LIP ratios were similar in preconfluent preadipocytes from young and old animals [(LAP/LIP)young/(LAP/LIP)old = 1.0 ± 0.3, P = not significant]. However, 4 h after initiating the differentiation program, the ratio of LAP to LIP in preadipocytes from young rats was twice that in cells from old animals [(LAP/LIP)young/(LAP/LIP)old = 2.0 ± 0.4, P < 0.02; n = 10].

The age-related increase in relative expression of the inhibitory 19-kDa C/EBPβ-LIP isoform in differentiating preadipocytes was observed both in whole fat tissue and collagenase-isolated fat cells from epididymal, inguinal, and perirenal fat depots (Fig. 8). Thus the increase in inhibitory C/EBPβ-LIP expression with aging is a common feature of both primary preadipocytes and fat cells in all depots tested. A 14-kDa C/EBPβ-isofrom, which may be a proteolytic cleavage product of C/EBPβ (1), was found in whole fat tissue but not in cultured preadipocytes. However, abundance of the 14-kDa isoform was not affected significantly by aging (abundance in young/old = 1.03 ± 0.18; P = 0.75 by ANOVA; n = 3 depots in 3 animals).

This increase in C/EBPβ-LIP isoform expression likely contributes to decreasing adipogenesis with aging. To test this prediction, we transiently transfected a truncated C/EBPβ-vector, CMV-LIP, that overexpresses only C/EBPβ-LIP (12) into perirenal preadipocytes isolated from young animals. These cells normally differentiate much more extensively than cells from old animals or cells isolated from other fat depots of young animals (33). The βgal-reporter construct...
RESULTS were confirmed using a second monoclonal antibody.

Expression was also higher in fat cells isolated by collagenase digestion from the E and P depots of older than of young rats (not shown). Expression was also higher in fat cells isolated by collagenase digestion from the E and P depots of older than of young rats (not shown). Results were confirmed using a second monoclonal antibody.

We also observed a transient increase in C/EBP expression during differentiation to augment adipogenesis (58). C/EBP acts in concert with full-length C/EBP to inhibit adipogenesis in young primary rat preadipocytes as occurs in cell lines (60), supporting our contention that increasing C/EBP expression with aging contributes to decreased capacity for adipogenesis in primary preadipocytes.

C/EBP expression declines with aging. In cell lines, C/EBP acts in concert with full-length C/EBP early during differentiation to augment adipogenesis (58). We also observed a transient increase in C/EBP-abundance early during primary preadipocyte differentiation that peaked at about 4 h (data not shown). A decline in C/EBP-protein abundance with aging was found 4 h after induction of differentiation in preadipocytes from 3- compared with 24-month-old rats (Fig. 9; C/EBP in young/old = 1.7 ± 0.3; n = 5; P < 0.05; paired t-test). Thus decreased induction of C/EBP expression early during differentiation may, together with increased C/EBP-LIP, contribute to impaired adipogenesis with aging.

DISCUSSION

Most cell dynamic research on aging has been focused on effects of aging on cell replication. Less is known about effects of aging on the capacity of cells to acquire specialized function through differentiation. However, there may be a general tendency for aging to be associated with impaired capacity for differentiation. Besides the age-related impairment in preadipocyte differentiation, declines have been documented in adrenocortical precursor, cultured keratinocyte, and intestinal crypt cell differentiation (21, 22, 25, 27, 47).

Until our work in preadipocytes, the mechanisms responsible for this impairment in differentiation with aging were not well understood.

Our work in primary preadipocytes shows that changes in expression of the adipogenic regulators C/EBPα, C/EBPβ-LIP, and C/EBPβ contribute to blunted differentiation with aging. These orchestrated changes involve increases as well as decreases in expression of key adipogenic regulators both at the mRNA and protein levels, leading to decreased adipogenesis. For example, the differentiation-dependent profile of C/EBPα-mRNA expression declined with age, whereas that of C/EBPβ did not. Conversely, the relative abundance the antiadipogenic C/EBPβ-LIP-protein isoform increased with donor age, whereas that of C/EBPβ-isoforms was unchanged. Restoration of C/EBPα-levels in preadipocytes from old donors by transient transfection allowed them to complete the differentiation program, suggesting that the primary defect was the inability to maintain adequate levels of this important adipogenic regulator.

We anticipated that changes in adipogenic transcription factor expression would impair not only the ability of preadipocytes to differentiate, but might also exhibit age-related changes in expression in the fat cells that develop from them. This was, in fact, what we observed. Reduced abundance of C/EBPα- and elevated C/EBPβ-LIP expression were found both in cultured differentiating preadipocytes and in fat cells in vivo, indicating that our findings in vitro are relevant in vivo. These changes in adipogenic transcription factor expression would be anticipated to impair not only preadipocyte differentiation, but also to influence the function of the fat cells that develop from them. For example, in addition to reduced fat cell size, blunted C/EBPα-expression in adipocytes can contribute to impaired glucose tolerance through effects on Glut4 ex-

Fig. 8. Expression of C/EBPβ-LIP increases with age in fat tissue. C/EBPβ-protein expression was determined in E, I, and P fat depots from 3M and 24M F344 rats. A Western analysis representative of 3 separate experiments is shown. C/EBPβ-LIP expression was higher, relative to that of C/EBPβ-LAP, in fat from old compared with young rats (P < 0.005; ANOVA). In 2 further experiments, C/EBPβ-LIP expression was also higher in fat cells isolated by collagenase digestion from the E and P depots of old than of young rats (not shown). Results were confirmed using a second monoclonal antibody.

Fig. 9. Decreased C/EBPβ-expression occurs with aging early during preadipocyte differentiation. C/EBPβ-protein abundance was measured in preadipocytes cultured in parallel from 3M and 24M rats 4 h after initiation of differentiation. A Western immunoblot representative of 5 experiments is shown above. Below are means ± SE of densitometric analyses of C/EBPβ-protein abundance in these experiments, with values expressed as percentages of optical density within each experiment normalized for cellular protein content.
expression (15), among other mechanisms. We also found that abundance of adipocyte lipid binding protein (aP2), the expression of which is induced by C/EBPα, is lower in fat cells from old than from young rats (7). Furthermore, decreased C/EBPα in concert with increased C/EBPβ-LIP expression may be a general aging phenomenon in organs involved in lipid metabolism. For example, C/EBPα-abundance declines with age in freshly isolated whole mouse liver tissue and, after treatment with lipopolysaccharide, more C/EBPβ-LIP is expressed in this tissue in old than in young mice (24).

The age-related increase in preadipocyte C/EBPβ-LIP expression is triggered by events during initiation of differentiation, because expression of C/EBPβ-LIP relative to C/EBPβ-LAP was the same in undifferentiated preadipocytes from young and old animals but was higher in preadipocytes from old animals within 4 h of exposure to enriched medium. As cells become differentiated fully, the effect of aging on C/EBPβ-LIP expression is increasingly evident, so that C/EBPβ-LIP protein was very abundant in fat tissue from old animals and not detectable in fat tissue from young animals. This implies that abundance of the C/EBPβ-LIP isoform relative to C/EBPβ-LAP is regulated.

Two potential mechanisms responsible for the relative abundance of C/EBPβ-LIP and C/EBPβ-LAP have been proposed. The first involves RNA processing. There are two potential translation initiation sites in the C/EBPβ-mRNA that are upstream and in frame of the site from which C/EBPβ-LIP is translated. C/EBPβ-LIP may be produced because of leaky ribosomal scanning resulting in bypassing these sites in favor of the C/EBPβ-LIP initiation site (12). This alternative translation process appears to be regulated. An RNA binding protein, CUG triplet repeat binding protein, may be involved in switching among translation initiation sites to produce C/EBPβ-isoforms (54). The second potential mechanism proposed to regulate C/EBPβ-isoform abundance is that proteases, possibly caspases, cleave full-length C/EBPβ-protein, as can occur in macrophages and liver tissue (1, 2). Proteases result in production of 19- and 14-kDa carboxy terminal C/EBPβ-isoforms, the 14-kDa isoform being a prominent product (1). However, we did not detect the 14-kDa isoform in any preadipocyte preparation, although the antibody used would have detected such a product. Whereas we did find a 14-kDa C/EBPβ-isoform in whole fat tissue preparations, its abundance was not affected significantly by aging in the same experiments as those in which the 19-kDa isoform increased. Thus the age-related increase in C/EBPβ-LIP expression we found in adipose tissue is very unlikely to be a result of artifactual proteolytic cleavage during protein extraction. Whatever the responsible mechanism, it is regulated both by aging and by differentiation, and the age-related increase in C/EBPβ-LIP expression results from processes intrinsic to adipose cells.

Besides the C/EBP family members, PPARγ is another transcription factor important in preadipocyte differentiation (3, 48). Expression of C/EBPα or of C/EBPβ and C/EBPδ together augments PPARγ-expression in cell lines (58, 59). In addition to inhibiting activity and expression of C/EBPα, C/EBPβ-LIP causes reduced PPARγ-expression (S. Farmer, personal communication). Because expression of C/EBPβ-LIP increases with age and that of C/EBPα and δ decreases, an age-related decline in PPARγ-expression would be anticipated to occur in fat. Indeed, an age-related decline in PPARγ-mRNA abundance has been observed in subcutaneous whole fat tissue from monkeys (23). Thus decreased expression of PPARγ may also contribute to decreased adipogenesis with aging.

**Perspectives**

Perhaps decreasing adipogenic transcription factor expression with aging serves a protective function. Through middle age, the combination of increasing fat cell size and number contributes to enlargement of fat mass. Because fat cell turnover occurs slowly (in excess of 140 days in the rat), changes in cell characteristic in old age necessarily reflect alterations that occur in preadipocyte function earlier in life (7, 32, 39). Because preadipocyte and fat cell numbers remain stable or increase in old age, if the age-related decline in adipogenic transcription factor expression were not to occur, massive obesity could ensue in later life. Indeed, reduced preadipocyte differentiation capacity may account for the stable or increasing number of preadipocytes in fat depots with aging, despite declining preadipocyte replicative potential (33), and reduced C/EBPα-expression in fat cells may contribute to the decrease in fat cell size that occurs between middle and old age.

Our findings are consistent with the hypothesis that the wide array of phenotypic effects characteristic of aging result from its influence on expression of specific regulatory proteins at early points in pathways controlling expression of many downstream genes (13, 23, 30, 43). Although intervening to alter expression of such proteins may not affect the underlying process causing senescence itself, our findings demonstrate the feasibility of restoring specific functions to senescent cells through such interventions.

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