Regulation of Collagenase Expression during Replicative Senescence in Human Fibroblasts by Akt-Forkhead Signaling*

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The expression of collagenase (matrix metalloproteinase 1) in human fibroblasts increases during aging both in vivo and in vitro. This age-associated increase in collagenase expression has been postulated to contribute to the age-related decline in tissue function by increasing proteolysis of matrix components, but little is known regarding the regulation of collagenase expression. We examined the role that the serine/threonine kinase Akt plays in collagenase expression during in vitro senescence of WI-38 normal human lung fibroblast cells. Our results indicate that Akt-mediated signals, acting through the forkhead transcription factor FKHRL1, can regulate collagenase expression in WI-38 fibroblasts. Dominant negative forms of Akt increase collagenase promoter activity in early passage WI-38 fibroblasts, whereas an active form of Akt suppresses steady state levels of collagenase mRNA in senescent WI-38 fibroblasts. In addition, the activity of a synthetic promoter containing forkhead-specific binding sites, as measured by luciferase activity, is much higher in senescent cells compared with early passage WI-38 fibroblasts. These results indicate that members of the forkhead family of transcription factors play a role in the regulation of the collagenase promoter and that increased activity of forkhead transcription factors may underlie the increase in collagenase expression observed during replicative senescence.

Normal human diploid fibroblasts have a limited proliferative capacity when placed into tissue culture. Following a defined number of population doublings, these cells gradually lose the ability to proliferate. There is some cell death, and the process eventually results in a population of cells that is viable but is nonreplicating (1). This process has been termed replicative senescence (2, 3) and has provided an important model for the study of the biology of aging. A compilation of the published changes that occur during replicative senescence has been published (3). Included in these changes are alterations in size, morphology, gene expression, the regulation and timing of the cell cycle, and the turnover of the extracellular matrix. The expression of the extracellular matrix-degrading enzymes, interstitial collagenase (matrix metalloproteinase 1) and stromelysin-1, are increased in senescent fibroblasts compared with early passage cells (4, 5), leading to a shift in the relative ratio of collagenase to collagen production between early passage and senescent cells such that there is a relative overproduction of collagenase (6). The observation that there is a relative overproduction of collagenase in aging cells has led to the proposal that senescent cells display a matrix-degrading phenotype. During aging in vivo, there is a shift in the fibroblast from a matrix synthesizing to a matrix-degrading phenotype, resulting in an elevated proteolytic environment, which may contribute to age-related loss of tissue function (7, 8). Despite the potential importance of increased collagenase expression during aging and the identification of changes in transcription of the collagenase gene during replicative senescence (9), no definitive data regarding the mechanisms that underlie the age-related increase in collagenase production are available.

Previous studies in our laboratories have shown that inhibition of the intracellular enzyme phosphatidylinositol (3)kinase (PI3-kinase) and the serine/threonine kinase Akt-1/PKBα, referred to here as Akt. Three members of the forkhead transcription factor family most closely related to Daf-16 (FKHR, FKHRL1, and AFX) have been shown to be directly phosphorylated by Akt (16–18). This phosphorylation serves to negatively regulate the activity of these transcription factors, reducing their ability to enhance transcription from target promoters and promoting nuclear exclusion (19, 20).

In this study, we have examined the regulation of collagenase expression at the level of the promoter to identify the basis for the increased expression of collagenase during replicative senescence. We find that the collagenase promoter can be regulated by the activity of the serine/threonine kinase Akt in both early passage and senescent WI-38 fibroblasts. The effect of Akt on collagenase gene expression may be mediated by members of the forkhead family of transcription factors.

EXPERIMENTAL PROCEDURES

Materials—All cell culture materials were purchased from Meditech Inc. with the exception of the keratinocyte serum-free medium, 2 The abbreviations used are: PI, phosphatidylinositol; IGF, insulin-like growth factor; FBS, fetal bovine serum; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase kinase; FHRE, synthetic forkhead promoter.

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which was purchased from Life Sciences Inc. Antibodies to Akt and phospho-Akt were purchased from Upstate Biotechnology Inc (UBI) and Cell Signaling, respectively. The Akt kinase assay kit was produced by UBI. The chemical inhibitors LY294002, PD98059, and wortmannin were purchased from BIOMOL. Detection reagents and secondary antibody were obtained from Amersham Biosciences, Inc. Plasmids and reagents for luciferase measurements were purchased from Promega.

Plasmid Constructs—Expression plasmids for Akt including the active form (Akt-myr) and the dominant negative form (Akt K179M) contained in SR α were provided by Dr. Philip Tschilis (Thomas Jefferson University, Philadelphia, PA). The expression plasmids for Ras containing an activated Ras cDNA (valine 12 mutation, RasV12) in pBabe was purchased from Life Sciences Inc. Antibodies for Western blot analyses were obtained from Amersham Biosciences, Inc. Plasmids and reagents for luciferase measurements were provided by Dr. Marcus Thelen (University of Geneva, Geneva, Switzerland). The luciferase constructs containing 3470 bp of the collagenase promoter (pGL3470) and a deletion mutant of this construct (pGL3470Δ) were provided by Dr. Judith Cameron (Cambridge University, Cambridge, MA). The expression plasmids containing the active (p110-CAX) and wild type PI 3K (p110) were provided by Dr. Marcus Thelen and Dr. Gary Nolan (Stanford University, Stanford, CA).

Cell Culture—Stock cultures of WI-38 fetal human diploid fibroblasts were grown in minimum essential medium, supplemented with 2 mM L-glutamine and 10% fetal bovine serum (FBS) as previously described (21). Cultures were considered to be at the end of their replicative lifespan when they were unable to complete one population doubling during a 4-week period that included 3 consecutive weeks of re-feeding with fresh medium containing 10% FBS. WI-38 cells at early passage were less than 30 population doublings, and those at the end of their replicative lifespan were greater than 95% lifespan completed. This translates to a population doubling of 58–65, depending upon the subline. Cultures were made quiescent by culturing in serum-free Dulbecco’s modified Eagle’s medium for 72 h before FBS stimulation. In experiments involving LY294002 (50 μM), PD98059 (25 μM), or rapamycin (100 nM), cells were incubated with each respective inhibitor for 24 h.

The packaging cell line used for retroviral production and subsequent infection of WI-38 cells was the Phoenix cell line provided by Dr. Gary Nolan (Stanford University, Stanford, CA).

Transfections and Luciferase Assays—Transient transfections were performed as follows. WI-38 cells were seeded at 1 x 10^4/cm^2. 24 h after seeding, cultures were transfected with both a control plasmid, pRL-CMV (containing the luciferase enzyme derived from Renilla reniformis) under the control of the cytomegalovirus promoter) and the plasmid of interest using LipofectAMINE (Invitrogen) according to the manufacturer’s instructions. Following the incubation period described in individual experiments, cultures were washed twice with phosphate-buffered saline and lysed using a lysis buffer provided by Promega. Luciferase assays were performed using the Promega dual luciferase assay system, according to the manufacturer’s protocols. Transfection efficiency was normalized to the control luciferase plasmid, pRLCMV, that can be measured in the same assay mix. Thus, the highly sensitive luciferase measurement can be used for both experimental and internal control measurements.

Western Blot Analysis—Western blot analysis was performed as follows. Total cellular protein was isolated by scraping cells into lysis buffer (50 mM Hepes, pH 7.4, 150 mM NaCl, 1.5 mM MgCl2, 1 mM EGTA, 10% glycerol, 1% Triton X-100). Equal amounts of protein were loaded onto a 6% SDS-polyacrylamide gel for separation. Following transfer to nitrocellulose filters, total protein was visualized by staining the filters with Ponceau S Red to assure equal loading of the samples. Western analysis was carried out using antibodies that recognize Akt (UBI), the phosphorylated form of Akt (Cell Signaling), and β actin (Sigma). Western blots were carried out using 5% dry milk as a blocking reagent in 10 mM Tris, pH 8.0, 150 mM NaCl, containing 0.5% Tween 20. Bound antibody complexes were visualized using the Amersham ECL Western blotting system (Amersham Biosciences, Inc.).

Akt Enzyme Assays—Enzymatic assays for the activity of the serine/threonine kinase Akt were carried out using an Akt assay kit available from UBI following the manufacturer’s instructions. Briefly, cells were lysed as for Western blotting followed by immunoprecipitation of the Akt enzyme using the antibody provided by UBI. (The effectiveness of the immunoprecipitation reaction was verified by Western blotting.) Activity of the Akt enzyme was determined by incubating the immunoprecipitated proteins with a synthetic peptide substrate (RPRAATF), which is based upon the Akt phosphorylation site of glycogen synthase kinase 3 and γ-32P-ATP in reaction buffer. The entire reaction was spotted onto glass fiber discs and washed extensively with phosphoric acid, followed by a wash with ethanol/ether. Filters were then dried and immersed for 15 min in scintillation counting solution.

RNA Isolation and Northern Blot Analysis—Total RNA was isolated following the guanidinium isothiocyanate method of Chomczynski and Sacchi (24). Northern analysis was carried out using 10 μg of total RNA in glyoxal agarose gels. Size fractionated RNA was transferred to Nytran filters electrophoretically in 1x Tris acetate EDTA buffer (TAE). Probes were labeled by random priming and hybridization was carried out using a solution of 7% SDS, 0.25 mM NaPO4, 5.6 mM Na3P2O7, 2 mM EDTA. Washing was carried out under standard conditions.

RESULTS

Previous studies in our laboratory indicate that inhibition of PI 3-kinase causes an increase in the steady state level of collagenase mRNA in early passage WI-38 human fibroblasts (10). To determine whether transcription of the collagenase gene was affected by an inhibition of PI 3-kinase in early passage WI-38 cells, a plasmid construct containing a 3.4-kb fragment of the collagenase promoter (pGL3470; Ref. 25) driving the expression of the firefly luciferase cDNA was introduced into early passage WI-38 cells in a transient transfection system. The cells were then exposed to inhibitors of PI 3-kinase or an inhibitor of MEK. The activity of the collagenase promoter, as measured by luciferase activity, was increased in the presence of two separate inhibitors of PI 3-kinase, LY 294002 and wortmannin. An inhibitor of MEK (PD98059) actually decreased the activity of the collagenase promoter, but the effect was not statistically significant (Fig. 1A).

To confirm the results obtained using the chemical inhibitors of PI 3-kinase, expression constructs that contained a cDNA encoding either an active (p110-CAX) or a wild type (p110) form of the 110-kDa subunit of PI 3-kinase (26) were introduced into the WI-38 cells along with the collagenase promoter reporter plasmid, pGL3470. The wild type form of the p110 subunit acts as a dominant negative because it competes with the activated p85/p110 holoenzyme for substrates. Consistent with the results obtained using the chemical inhibitors, the constitutively active form of p110 (p110-CAX) had no effect on the collagenase promoter activity, whereas the wild type p110 subunit increased the activity of the collagenase promoter (Fig. 1B). Introduction of an activated form of Ras also had no effect on collagenase promoter activity. These results, taken together with the lack of an effect on collagenase promoter activity by the addition of the MEK inhibitor (Fig. 1A), indicate that fluctuations in mitogen-activated protein kinase have little influence on the collagenase promoter.

Among its many effects, activation of PI 3-kinase leads to an increase in the activity of the serine/threonine kinase Akt (27). Because Akt is known to affect the activity of a number of transcription factors including CREB, this enzyme is a possible mediator of PI 3-kinase-dependent regulation of the collagenase promoter. To assess the role of Akt in the regulation of the collagenase promoter, we introduced both an active (Akt-myr) and a dominant negative (Akt K179M) form of the enzyme into early passage WI-38 cells in the presence of the collagenase promoter–luciferase reporter construct. The activity of the collagenase promoter was measured in a transient transfection assay. The introduction of a dominant negative form of Akt increased the activity of the collagenase promoter (Fig. 1C). Greater collagenase promoter activity was obtained as greater quantities of the dominant negative Akt construct were introduced into the WI-38 cells (Fig. 1C). The fact that a dominant negative form of Akt increases collagenase promoter activity is consistent with the published effect of the PI 3-kinase inhibitor (10) and the fact that an inactive form of the p110 catalytic

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subunit of PI 3-kinase increased the activity of the collagenase promoter (Fig. 1B). Increased collagenase promoter activity in the presence of a dominant negative form of Akt implies that Akt activation plays a negative role in the regulation of the collagenase promoter, i.e. activation of Akt leads to a reduction in transcription.

The forkhead transcription factors are part of a large family of winged helix/loop helix transcription factors for which the symbol Fox (Forkhead box) has been proposed to indicate the presence of the characteristic winged helix domain (28). Several members of this superfamily of transcription factors are negatively regulated by insulin/IGF signaling through the activation of Akt (16–20). This negative regulation has been linked to the phosphorylation of specific sites in the three forkhead family members (FKHR1, FKHR1L, and AFX), which are the closest mammalian homologs of the C. elegans transcription factor Daf-16 (28). These sites exist within consensus sites for Akt phosphorylation RXRXX(S/T) (29). Because the forkhead tran-
scription factor FKHRL1 is the most ubiquitously expressed of the three Daf-16 homologs (30), we chose to examine the possibility that this isoform can mediate the effect of Akt on the collagenase promoter. The FKHRL1 isoform may play a role during apoptosis, and activation of Akt decreases FKHRL1 transcriptional activity (31). To examine the possibility that changes in forkhead activity contribute to the increased expression of collagenase during replicative senescence, the activity of the collagenase promoter was measured in early passage and senescent WI-38 fibroblasts in the presence or absence of an expression construct that contains the FKHRL1 cDNA (Fig. 2). The introduction of the wild type FKHRL1 increases the activity of the collagenase promoter in both early passage and senescent WI-38 cells. A triple mutant of FKHRL1 (FKHRL1-TM) in which the three Akt phosphorylation sites had been converted to alanine (T32A, S253A, S315A) activated the collagenase promoter more effectively than the wild type FKHRL1 (Fig. 2).

The activity of a synthetic forkhead promoter (FHRE) that contains three insulin response element sequences driving the expression of the firefly luciferase cDNA (31) was also examined in the early passage and senescent WI-38 cells. This promoter is activated by introduction of the FKHRL1 protein (31). Included as a control in this experiment was a deletion mutant of the collagenase promoter (pGL3470Δ). The activity of this promoter does not increase in senescent cells compared with early passage cells (data not shown), so it was used as a control for comparison of the collagenase promoter activity between early passage and senescent cultures. In Fig. 2, the luciferase activity has been normalized relative to the pGL3470Δ (which was given a value of 1 in both early passage and senescent cells) to allow a direct comparison of promoter activity between early passage and senescent cultures, but it is important to note that the absolute value of the promoter activity of the pGL3470Δ collagenase promoter construct did not vary by more than 10% between early passage and senescent cultures in our assays. Interestingly, the activity of the pGL3470Δ collagenase promoter construct was not affected by PI 3-kinase inhibitors wortmannin or LY294002.

The FHRE promoter had very low activity in early passage cells but the activity was enhanced by the co-transfection of FKHRL1-TM (Fig. 2). Consistent with an increase in forkhead-mediated transcription in the senescent cells, the FHRE promoter has much higher activity in the senescent cells (Fig. 2) when it is introduced into the senescent cells alone, but the activity of the FRE could be induced in the early passage cells through treatment with the PI 3-kinase inhibitor LY294002 (Fig. 2C). The activity of the FHRE promoter increased in the senescent cells when the FKHRL1 constructs (wild type or triple mutant) were co-transfected, but the increase was not as dramatic as in the early passage cells. The results of multiple experiments indicate that the collagenase promoter shows a 2–3-fold increase in activity in senescent cells compared with early passage WI-38 cells and the activity of the FHRE promoter shows a 3–4-fold increase in senescent cells compared with early passage cells (Fig. 2). To verify that the increase in collagenase promoter activity seen in senescent cells is dependent upon forkhead transcription factors, a series of co-transfections were performed that included the pGL3470 construct and increasing concentrations of a double-stranded oligonucleotide that contains the forkhead binding site contained in the Fas ligand promoter (TTAATAAATAA) (30). The inclusion of this oligonucleotide decreased the activity of the collagenase promoter in a dose-dependent manner. Again, we would emphasize that the differences between the early passage and senescent cells in terms of both collagenase and FHRE promoters have been consistent in multiple experiments. These results indicate that the activity of both the collagenase promoter and a synthetic forkhead-responsive promoter are increased in senescent cells.

To understand the molecular mechanism that underlies the increase in forkhead promoter activity observed in the senescent cells, we examined the activity of Akt in both senescent and early passage cells. The activity of Akt has been reported to increase following mitogenic stimulation of serum-deprived mouse fibroblast cells (27), and this activation is dependent upon site-specific phosphorylation mediated in part by the action of the serine/threonine kinase PDK1 (32, 33). Phosphorylation at both serine 473 in the C-terminal region and threonine 308 in the catalytic region of Akt is required for activation (34). Upon activation, Akt translocates to the nucleus in BALB/c 3T3 cells (35), and it has been shown that Akt can phosphorylate several transcription factors including CREB (36) and members of the forkhead family (16–18, 31). Given these observations, decreased Akt expression or defects in either activation or nuclear translocation of Akt could explain the increase in collagenase expression observed in senescent fibroblasts. We first examined activation of Akt by measuring both enzyme activity and site-specific phosphorylation of the protein. In both early passage and senescent cells, serum stimulation increased cytoplasmic Akt activity (Fig. 3A). This increase in Akt activity was accompanied by increased phosphorylation at serine 473 (Fig. 3B). From these results, we conclude that Akt activation is similar in early passage and senescent cells. The nuclear abundance of active Akt was examined using a phosphospecific antibody to detect the active form of the enzyme. In the cytoplasmic extracts of both early passage and senescent cells, the phosphorylated (active) form of Akt increased following serum stimulation. However, examination of nuclear extracts revealed that only early passage cells exhibited an increase in the amount of active Akt in the nucleus. In the senescent cells, there was very little active Akt in nuclear extracts following serum stimulation (data not shown). This suggests a failure to translocate active Akt to the nucleus in senescent cells. As a control, we examined the nuclear abundance of activated, phosphorylated Erk, which has been reported to decrease in senescent cells relative to early passage cells (37, 38). The fact that the amount of the active form of Akt is lower in the nucleus of senescent cells implies that the increased expression of collagenase is mediated, in part, by a relative decrease in Akt activity. This leads to the prediction that an increase in Akt activity in senescent cells should decrease collagenase mRNA levels in the senescent fibroblasts. To test this prediction, the active form of Akt, Akt-myr, was introduced into near senescent fibroblasts to determine whether the forced activation of Akt can affect collagenase expression in senescent cells. Following infection with retrovirus particles and the time required for neomycin selection, the cultures expressing the active Akt enzyme were greater than 90% life-span completed. At this time in the life-span of these cultures, the expression of collagenase is increased relative to early passage cells (4). However, the senescent cultures that express the active form of Akt had lower levels of collagenase mRNA than control cultures that were infected with the retroviral construct without a cDNA insert in three separate experiments and a representative Northern blot is presented in Fig. 4. Densitometric analysis of these Northern blots indicates that the level of collagenase mRNA is decreased by 66% (1.38 ± 0.28 versus 0.46 ± 0.007 p = 0.05). These results indicate that forced activation of Akt can decrease collagenase mRNA levels in senescent fibroblasts.

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2 J. Campisi, personal communication.
FIG. 2. Activity of the collagenase promoter and a synthetic forkhead-responsive promoter in early passage and senescent fibroblasts. Early passage or senescent WI-38 cells were transiently transfected with a wild type collagenase promoter construct (pGL3470) (shown in panel A) or a synthetic promoter (FHRE) that contains three insulin response elements (shown in panel B). Included in these transfections were either a wild type forkhead expression vector (FKHRL1) or a triple mutant of FKHRL1 that contains mutations in the three Akt phosphorylation sites (threonine 32, serine 253, and serine 315) (FKHRL1-TM). 24 h following transfections, the relative activity of the collagenase promoter was assayed by measuring luciferase activity. A deletion mutant of the collagenase promoter (provided by Dr. Judith Campisi), which has very similar activity in early passage and senescent cells (less than 10% difference in multiple experiments), was used to normalize the activity of both the full-length collagenase and FHRE promoters in the early passage and senescent cultures to facilitate a comparison between early passage and senescent cells. Thus, all values have been normalized to the activity of the collagenase deletion mutant, which was given a value of 1. The experiment shown is a representative experiment of a minimum of three independent experiments. Values that are statistically different from the relevant controls (p < 0.05) are indicated by an asterisk. In panel A, a single asterisk indicates the value is statistically different from the value for the collagenase promoter in early passage cells, whereas a double asterisk indicates that the value is statistically different from the value for the collagenase promoter in the senescent cells. In panel B, an asterisk indicates that the value is statistically different from the value for the FHRE promoter in early passage cells, whereas a double asterisk indicates that the value is statistically different from the value for the FHRE in senescent cells. Statistical significance (p < 0.05) was determined using Student’s t test. In panel C, early passage WI-38 cells were transiently transfected with the synthetic promoter containing the three insulin response elements (FHRE) controlling luciferase expression. Cultures were subsequently treated with either LY294002 to inhibit PI 3-kinase or PD98059 to inhibit MEK activity. Additionally, the wild type forkhead expression vector (FKHRL1) was included in some transfections. The relative FHRE promoter activity following a 24 h incubation with the inhibitors is presented. The values shown are the means of three independent values from one experiment with standard deviations, and these values have not been normalized as in panels A and B. Values that are statistically different from the activity of the FHRE alone in early passage cells are indicated with an asterisk. Statistical significance was determined using Student’s t test.
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**DISCUSSION**

The results presented in this study indicate that there is an increase in forkhead activity in senescent human fibroblasts. This interpretation is supported by the observations that (i) inhibition of Akt in early passage cells increases collagenase promoter activity, (ii) activation of Akt in senescent cells decreases collagenase mRNA levels, (iii) a forkhead-responsive promoter shows strong activity in senescent cells, and (iv) the collagenase promoter is strongly activated by the expression of a mutant form of one of the forkhead isoforms, FKHRL1. This mutation eliminates three consensus phosphorylation sites for Akt within the FKHRL1 protein, rendering it unresponsive to Akt (31).

We postulate that the activated form of Akt is not present in the nucleus of senescent cells, similar to the recent reports that the phosphorylated form of ERK does not translocate to the nucleus of senescent cells (37, 38). This lack of ERK translocation may underlie the decreased fos expression observed in senescent cells (39). In the case of Akt, a decrease in the nuclear form of the activated form may partially account for the increased collagenase expression that occurs during replicative senescence. A model describing the possible regulation of the collagenase gene in senescent cells is provided in Fig. 5.

Several of the Fox transcription factors are negatively regulated following phosphorylation by Akt including FKHR, AFX, and FKHRL1 (40). Our results indicate that FKHRL1 and perhaps other forkhead family members are involved in the regulation of gene expression during replicative senescence. Specifically, the results obtained in this study suggest that the collagenase promoter is activated, at least in part, by an increase in the activity of a forkhead protein during replicative senescence. This interpretation is supported by data presented in Fig. 2A. If the increased activity of the collagenase promoter in senescent cells is the result of an increase in the activity of forkhead transcription factors, then the introduction of forkhead or Akt should have a less dramatic effect on the activity of the collagenase promoter in the senescent cells than in the early passage cells. As seen in Fig. 2A, the introduction of the FKHRL1-TM into senescent cells has much less effect on the wild type collagenase promoter in the senescent cells than in the early passage cells. These results suggest that the presence of active forkhead transcription factors in the senescent cells dampens the effects of the exogenous FKHRL1 on the collagenase promoter.

It seems likely that the increased activity of FKHRL1 or other forkhead transcription factors mediates increases in the expression of several genes during replicative senescence and thus may contribute to the altered pattern of gene expression that occurs during replicative senescence. It is very possible that only specific forkhead transcription factors are activated during replicative senescence. For example, the introduction of FRHK into early passage WI-38 cells had little effect on the collagenase promoter (data not shown). This indicates that not all forkhead transcription factors can regulate the collagenase promoter and that the increased expression of collagenase may result from the selective activation of one or more forkhead proteins during replicative senescence. Whether forkhead proteins bind directly to the collagenase promoter or regulate this promoter via protein-protein interactions is not clear. The region of the collagenase promoter deleted in the pGL3470Δ construct is centered around bp −3100 and contains an ATAACG sequence. This provides a potential forkhead binding site based on the forkhead binding site consensus sequence.
ATAAAC, leaving open the possibility that FKHRL1 or another forkhead protein binds directly to the collagenase promoter to regulate transcription.

In summary, our results indicate that increased activity of forkhead transcription factors plays a role in replicative senescence and mediates increased collagenase expression during senescence.

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FIG. 5. Loss of nuclear translocation of Akt signaling in senescent cells. A schematic representation of Akt signaling as it relates to forkhead transcription factors is presented. The early passage cells are represented in panel A. The activation of Akt leads to the phosphorylation of both cytoplasmic and nuclear targets. The cytoplasmic targets include components of the apoptotic machinery such as BAD and caspases 3 and 9. The nuclear targets of Akt include the forkhead transcription factors FKHRL1, AFX, and FHR in addition to other transcription factors such as CREB. The phosphorylation of forkhead transcription factors leads to a repression of their activity and thus negatively regulates collagenase gene expression. In senescent cells a relative decrease in the nuclear form of phospho-Akt may be the result of either a decrease in nuclear translocation or increased phosphatase activity. In either case this would lead to a relative increase in the activity of forkhead transcription factor activity resulting in increased collagenase expression.
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