Loss of epitranscriptomic control of selenocysteine utilization engages senescence and mitochondrial reprogramming

Thomas J. Begley
*University at Albany, State University of New York*

Andrea Leonardi
*University at Albany, State University of New York*

May Y. Lee
*SUNY Polytechnic Institute*

J. Andres Melendez
*SUNY Polytechnic Institute*

The University at Albany community has made this article openly available. Please share how this access benefits you.

Follow this and additional works at: [https://scholarsarchive.library.albany.edu/biology_fac_scholar](https://scholarsarchive.library.albany.edu/biology_fac_scholar)

**Recommended Citation**
Begley, Thomas J.; Leonardi, Andrea; Lee, May Y.; and Melendez, J. Andres, "Loss of epitranscriptomic control of selenocysteine utilization engages senescence and mitochondrial reprogramming" (2020). *Biological Sciences Faculty Scholarship*. 28.
[https://scholarsarchive.library.albany.edu/biology_fac_scholar/28](https://scholarsarchive.library.albany.edu/biology_fac_scholar/28)

This work is licensed under a [Creative Commons Attribution 4.0 International License](https://creativecommons.org/licenses/by/4.0/). This Article is brought to you for free and open access by the Biological Sciences at Scholars Archive. It has been accepted for inclusion in Biological Sciences Faculty Scholarship by an authorized administrator of Scholars Archive. Please see [Terms of Use](https://scholarsarchive.library.albany.edu/terms_of_use). For more information, please contact [scholarsarchive@albany.edu](mailto:scholarsarchive@albany.edu).
Loss of epitranscriptomic control of selenocysteine utilization engages senescence and mitochondrial reprogramming

May Y. Lea, Andrea Leondrib, Thomas J. Begleya,b,c, J. Andrés Melendeza,c,∗

Keywords:
Epitranscriptome
Selenium
Senescence
Mitochondria
Uncoupling protein

ABSTRACT

Critically important to the maintenance of the glutathione (GSH) redox cycle are the activities of many selenocysteine-containing GSH metabolizing enzymes whose translation is controlled by the epitranscriptomic writer alkylation repair homolog 8 (ALKBH8). ALKBH8 is a tRNA methyltransferase that methylates the wobble uridine of specific tRNAs to regulate the synthesis of selenoproteins. Here we demonstrate that a deficiency in the writer ALKBH8 (Alkbh8−/−), alters selenoprotein levels and engages senescence, regulates stress response genes and promotes mitochondrial reprogramming. Alkbh8−/− mouse embryonic fibroblasts (MEFs) increase many hallmarks of senescence, including senescence associated β-galactosidase, heterochromatic foci, the cyclin dependent kinase inhibitor p16Ink4a, markers of mitochondrial dynamics as well as the senescence associated secretory phenotype (SASP). Alkbh8−/− cells also acquire a stress resistance phenotype that is accompanied by an increase in a number redox-modifying transcripts. In addition, Alkbh8−/− MEFs undergo a metabolic shift that is highlighted by a striking increase in the level of uncoupling protein 2 (UCP2) which enhances oxygen consumption and promotes a reliance on glycolytic metabolism. Finally, we have shown that the Alkbh8 deficiency can be exploited and corresponding MEFs are killed by glycolytic inhibition. Our work demonstrates that defects in an epitranscriptomic writer promote senescence and mitochondrial reprogramming and unveils a novel adaptive mechanism for coping with defects in selenocysteine utilization.

1. Introduction

The epitranscriptome can serve to regulate translation through post-transcriptional modification of RNAs and its dysregulation is associated with disease and toxicant susceptibility [1–6]. Alkylation repair homolog 8 (ALKBH8) is an epitranscriptomic writer with tRNA methyltransferase activity and it can methylate the wobble uridine on homolog 8 (ALKBH8) is an epitranscriptomic writer with tRNA methyltransferase that methylates the wobble uridine of specific tRNAs to regulate the synthesis of selenoproteins. Here we demonstrate that a deficiency in the writer ALKBH8 (Alkbh8−/−), alters selenoprotein levels and engages senescence, regulates stress response genes and promotes mitochondrial reprogramming. Alkbh8−/− mouse embryonic fibroblasts (MEFs) increase many hallmarks of senescence, including senescence associated β-galactosidase, heterochromatic foci, the cyclin dependent kinase inhibitor p16Ink4a, markers of mitochondrial dynamics as well as the senescence associated secretory phenotype (SASP). Alkbh8−/− cells also acquire a stress resistance phenotype that is accompanied by an increase in a number redox-modifying transcripts. In addition, Alkbh8−/− MEFs undergo a metabolic shift that is highlighted by a striking increase in the level of uncoupling protein 2 (UCP2) which enhances oxygen consumption and promotes a reliance on glycolytic metabolism. Finally, we have shown that the Alkbh8 deficiency can be exploited and corresponding MEFs are killed by glycolytic inhibition. Our work demonstrates that defects in an epitranscriptomic writer promote senescence and mitochondrial reprogramming and unveils a novel adaptive mechanism for coping with defects in selenocysteine utilization.

Senescence is a form of replicative arrest which is triggered by a variety of stressors including but not limited to DNA damage, telomere erosion, and oxidative stress [12]. While senescence is beneficial as an innate tumor-suppressive mechanism responsible for inducing permanent replicative arrest in cells at risk of malignant transformation, it has been established that the accumulation of senescent cells with increasing age is deleterious in tissue microenvironments in vivo [13]. Upon senescent transformation, cells adopt a modulated secretome termed the senescence-associated secretory phenotype (SASP), increasing secretion of inflammatory cytokines, matrix metalloproteinases (MMPs), chemokines, and growth factors into the surrounding tissue microenvironment [14]. Through its ability to evoke responses from cells in a paracrine fashion, SASP has been linked to numerous age-associated disease pathologies including tumor invasion, cardiovascular dysfunction, neuroinflammation, osteoarthritis and renal disease [15–18].

While there is wealth of information linking senescence to many degenerative disease processes [17–22] the role of selenium in the
regulation of the senescence program has only recently been unveiled. Se supplementation can extend the replicative lifespan of cells in culture [23] and Se deprivation or supplementation can accelerate or delay the production of senescence associated markers, respectively [23–25]. While serum selenium levels are predictors of longevity and healthy aging [26–28] selenium deprivation has also been shown to promote longevity [29]. Selenium is functionally utilized by small repertoire of enzymes as Sec, which is incorporated translationally through the use of UGA stop codon recoding and under epitranscriptomic control. However, little is known with respect to the specific contribution of epitranscriptomic writers that control Sec utilization in regulating senescence. In this study, we show that deficiency in ALKBH8 engages transcriptomic writers that control Sec utilization in regulating senescence, mitochondrial reprogramming and confers a survival advantage to cells with a limited capacity to incorporate selenocysteine. Increases in selenocysteine containing proteins are often associated with senescence and under epitranscriptomic control. How- ever, enzymes as Sec, which is incorporated translationally through the use of UGA stop codon recoding and under epitranscriptomic control. However, little is known with respect to the specific contribution of epitranscriptomic writers that control Sec utilization in regulating senescence. In this study, we show that deficiency in ALKBH8 engages transcriptomic writers that control Sec utilization in regulating senescence, mitochondrial reprogramming and confers a survival advantage to cells with a limited capacity to incorporate selenocysteine. Increases in selenocysteine containing proteins are often associated with senescence and under epitranscriptomic control. However, little is known with respect to the specific contribution of epitranscriptomic writers that control Sec utilization in regulating senescence.

2. Materials and methods

2.1. Cell culture

Both wild type (WT) Mouse Embryonic Fibroblasts (MEFs) and Alkbh8 deficient (Alkbh8del or Def) MEFs were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) (Corning Inc., Corning, NY) supplemented with 10% fetal bovine serum (Corning Inc., Corning, NY), 10% non-essential amino acids (Corning Inc., Corning, NY), 100 Units/mL penicillin and 100 μg/ml streptomycin, at 21% oxygen tension at 37 °C and 5% CO2. These cells were lifted in 0.25% trypsin/EDTA and supplemented with 10% fetal bovine serum and incubated at 21% oxygen tension at 37 °C and 5% CO2. These cells were lifted in 0.25% trypsin/EDTA (Corning Inc., Corning, NY) and serially passaged at a dilution of 1:6.

Primary human fetal lung fibroblasts (IMR90) were cultured in Minimum Essential Medium (MEM) (Corning Inc., Corning, NY) supplemented with 10% fetal bovine serum and incubated at 21% oxygen tension at 37 °C with 5% CO2. These cells were lifted in 0.25% trypsin/EDTA and serially passaged at a dilution of 1:4.

To analyze cell viability in different oxygen concentrations, cells were cultured in 21% and 3% O2 for 5 days and counted every 24 h.

2.2. Cytotoxicity and colony formation

10,000 cells per well were seeded in 96 well plates (Celltreat, Pepperell, MA) and were treated for 24 h with 2 deoxy-glucose (2DG) (Cayman, Ann Arbor, MI) at indicated concentrations to measure cytotoxicity. Cells were then stained with crystal violet, followed by solubilizing with 10% acetic acid and absorbance was measured with Flex Station 3 plate reader (Molecular Devices, San Jose, CA) at a wavelength of 570 nm. 500 cells per well were seeded in 24 well plates with 0.31 mM 2DG. Cells were grown for 5 days and colonies formed were stained using crystal violet. Colonies of 15 or more cells were counted for quantification and surviving fraction was calculated as (number of colonies formed/500 cells plated).

2.3. Quantitative RT-PCR

In brief, total mRNA was extracted from respective cell lines using TRIzol reagents (Life Technologies, Carlsbad, CA) as described by manufacturer. cDNA was synthesized from 500 ng total mRNA using Maxima H Minus First strand kits (Thermo Fisher Scientific, Waltham, MA). Real-time quantitative RT-PCR was performed in triplicates on a 7500 PCR system (Applied Biosystems, Förster city, MA) and the sequences were described in Supplementary Table 1. Additional oxidative stress and antioxidant defense regulated genes were also analyzed using RT [2] profiler PCR array (Qiagen, Hilden, Germany).

2.4. Western blot

Cells were washed twice with cold phosphate-buffered saline (PBS) and lysed with radiomunoprecipitation (RIPA) lysis buffer. The lysates were centrifuged for 15 min at full speed at 4 °C. For sample preparation, 15 μg of whole cell lysates were used and 6X SDS sample buffer with 10% of 0.5 M DTT added to each sample prior to boiling for 15 min. The samples were then separated on an 8% SDA-polyarylamide gel (Thermo Fisher Scientific, Waltham, MA) and transferred onto nitrocellulose membrane (Thermo Fisher Scientific, Waltham, MA). The membrane was blocked with 5% dry milk in 0.1% Tris-buffere saline-Tween 20 for 1 h and incubated overnight at 4 °C with primary antibodies, UCP2 (1:400) (Santa Cruz Biotechnology, Dallas, TX), Mitofusin 1 (1:500) (Cell Signaling Technology, Danvers, MA), Mitofusin 2 (1:500) (Cell Signaling Technology, Danvers, MA), Drp1 (1:500) (Cell Signaling Technology, Danvers, MA), GAPDH (1:10000) (Santa Cruz Biotechnology, Dallas, TX), Alkbh8 (1:1000) (ABclonal, Woburn, MA), GPX1 (1:1000) (R&D Systems, Minneapolis, MN), and TRXR2 (1:1000) (ABCAM, Cambridge, MA) followed by incubation with respective secondary antibodies, either anti-mouse (1:10000) (Cell Signaling Technology, Danvers, MA), anti-goat(1:10000) (ABCAM, Cambridge, MA) or anti-rabbit (1:10000) (Cell Signaling Technology, Danvers, MA) for 1 h. The membranes were developed by Supersignal West Femto Maximum sensitivity substrate kit (Thermo Fisher Scientific, Waltham, MA) and imaged with ChemiDoc™ imaging system (BIO-RAD, Hercules, CA).

2.5. SA-β-gal staining

Senescence Associated β-Galactosidase (SA-β-Gal) staining was performed using senescence-galactosidase staining kit (Cell Signaling Technology, Danvers, MA) as per manufacturer’s protocol. Briefly, cells were seeded in 6-well tissue culture plates overnight and washed once with PBS. 1 ml of 1x fixative solution was added and incubated for 15 min at room temperature. The plates were washed twice with PBS and stained with 1 ml of β-Gal staining solution for overnight at 37 °C in a dry non-CO2 incubator. Images were taken in nine random fields using light microscopy and β-gal-positive cells are presented as intensity normalized to WT MEFs using ImageJ software.

2.6. Heterochromatin foci staining

Cells were seeded on coverslips in 6-well tissue culture plates overnight, followed by fixation of the cells with cold 4% formaldehyde solution for 15 min at room temperature. The plates were washed once with cold PBS and the coverslips were mounted on glass slides with Prolong™ Gold Antifade Mount containing DAPI (Thermo Fisher Scientific, Waltham, MA). The slides were left overnight to dry in the dark and sealed with clear nail polish. Images were taken in three random fields using florescent microscope and heterochromatin foci positive nuclei are presented as percentage of total number of counted nuclei.

2.7. Mitochondrial oxygen consumption rate

Oxygen Consumption rate (OCR), and extracellular acidification rate (ECAR) were measured at 37 °C as per manufacturer’s protocol for the seahorse XF24 extracellular flux analyzer (Agilent Technologies, Santa Clara, CA). Briefly, cells were seeded in 24-well tissue culture plate overnight, allowing the cells to adhere. Due to differences in proliferative capacity between cell types, cells were seeded at differing densities so that cell numbers were equivalent at time of metabolic flux analysis. On the day of analysis, Cells were changed to conditional serum-free medium in order to induce mitochondrial respiration. The plates were washed with PBS and 1 μM sodium pyruvate. Cells were incubated in non-CO2 incubator at 37 °C for 1 h and respiration was measured before and after injection of

M.Y. Lee, et al. Redox Biology 28 (2020) 101375
three compounds: oligomycin (1 μM), carbonyl cyanide 4-(tri-fluoromethoxy) phenylhydrazone (FCCP) (3 μM), and rotenone (1 μM). Immediately after the run, cells were lysed in RIPA buffer and the protein concentration was measured using BCA assay. The results were normalized with the protein OD value of corresponding wells and OCR, ECAR and cell energy phenotype were automatically calculated by Wave software (Agilent Technologies, Santa Clara, CA).

2.8. Mitotracker green staining

Cells were seeded on cover-slips for 24 h to reach desired confluency. The cells were then stained with 200 nM MitoTracker Green (Invitrogen, Carlsbad, CA) and incubated for 15 min at 37 °C. After incubation period, cells were washed once with PBS and fixed with 4% formaldehyde solution for 15 min at room temperature. The plates were then washed once with cold PBS and the coverslips mounted on glass slides with Prolong Gold Antifade Mount. The slides are immediately washed once with cold PBS and the coverslips mounted on glass.

2.9. 2′,7′-dichlorodihydrofluorescein diacetate (H2DCFDA) staining

Cells were seeded in 96-well black/clear bottom plate with or without 20 μM genipin treatment overnight. On the day of assay, the cells were washed once with PBS, and treated with 300 μM H2O2 for 30 min at 37 °C. The cells were then washed once with PBS and stained with 30 μM cell-permeant oxidation-sensitive dye H2DCFDA (Invitrogen, Carlsbad, CA) for 30 min at 37 °C, followed by fluorescence analysis (485 nm excitation, 535 nm emission) with Flex Station 3 plate reader. The oxidation-insensitive dye 5(6)-carboxyl-2′,7′-dichlorodihydrofluorescein diacetate (DCFDA) (Invitrogen, Carlsbad, CA) was used as control so that any fluorescence changes between groups with H2DCFDA is directly attributed to changes in the dye oxidation. Mean fluorescent values for H2DCFDA-treatment cells were normalized to values of the control DCFDA-treated cells.

2.10. Statistical methods

Data were analyzed using GraphPad Prism version 8 software. All statistical analyses were performed with the GraphPad Prism 8.0 statistical software package. Statistical significance was assessed using the two-tailed Student’s t-test or one-way ANOVA with Tukey-Kramer post-test. P values of < 0.05 were considered significant.

3. Results

3.1. Alkbh8 deficient cells engage cellular senescence

We have established that Alkbh8 deficiency limits selenoprotein synthesis, elevating cellular ROS levels [11] (Supplementary Figure 1), and that increases in steady state H2O2 production drive SASP [14]. We first performed a focused array using Qiagen’s Oxidative Stress and Antioxidant Defense RT [3] Profiler PCR Array to evaluate potential adaptive oxidant metabolizing genes that aid in the survival of cells deficient in Alkbh8 and the absence of many functional key selenocysteine containing proteins. Supplementary Table 2 summarizes our findings indicating that there is a robust increase in the levels of number of key oxidant-metabolizing genes, including Superoxide dismutase 2 (Sod2), neutrophil cytosol factor 1 (Ncf1), Uncoupling protein 2 and 3 (Ucp2 and Ucp3) and glutathione peroxidase 3 and 6 (Gpx3 and Gpx6). Indicating that selenoprotein deficiency is accompanied by adaptive increases in the transcripts of oxidant-metabolizing genes. We next evaluated whether the increased oxidizing capacity of Alkbh8def cells activate the senescent program. We first characterized the proliferative capacity of Alkbh8def cells as cellular senescence is accompanied by growth arrest. As shown in Fig. 1A, Alkbh8def MEFs display a significant proliferative defect, compared to their WT controls, that is reversed by growth in low oxygen (3% O2). The tumor suppressor p16Ink4a has been well established as a senescence marker and its expression was significantly upregulated in Alkbh8def MEFs (Fig. 1B) while the cell cycle regulator p21 was not. We next set out to determine if replicative senescence, which is observed in primary human diploid cells, is accompanied by alterations in the levels of Alkbh8. Transcript levels of Alkbh8 were decreased in senescent IMR-90 human diploid fibroblasts, relative to pre-senescent cells, which was reversed by culturing cells in 3% O2 (Fig. 1C). These, observations suggest that Alkbh8 depletion is associated with proliferative defects linked to the senescent program.

Cellular senescence is also associated with the formation of heterochromatin foci (HCF), which are enriched in chromatin modifications. HCF can be monitored using high magnification fluorescence microscopy in combination with DAPI staining as in Fig. 2A, demonstrating significant enrichment of HCF in Alkbh8def MEFs. Senescence associated beta-galactosidase (SA-β-gal) is also a key indicator of senescence engagement and can be detected at pH 6 in single cells using X-Gal staining [32]. Alkbh8def cells display significant increases in SA-β-gal staining compared to WT (Fig. 2B). The senescence associated secretory phenotype is common to all forms of senescence and is causal to age-related decline in organ function [33]. We have established that the senescence associated secretory phenotype is under redox-control [14] and it is likely that oxidative stress that accompanies the impairment in selenoprotein exacerbates SASP. As shown in Fig. 3, the levels of many of the most prominent SASP markers are dramatically upregulated...
when selenocysteine utilization is impaired by Alkbh8 deficiency. Further, Alkbh8 rescue negated any increases in SASP and HCF observed in the Alkbh8def cells (Supplementary Figure 2). Thus, Alkbh8 deficiency is clearly responsible for the increase in senescent marks.

3.2. Alkbh8 deficiency engages mitochondrial reprogramming

Mitochondrial fusion has also been shown to play an important role in adapting to stress and increased fusion events are correlated with senescence [34,35]. To determine if a deficiency in the epitranscriptomic writer ALKBH8 alters mitochondrial morphology, we performed mitochondrial staining using mitotracker green in combination with Fiji-ImageJ. Alkbh8def cells display a significant increase in mean mitochondrial length and mean summed branch length when compared to WT, while other mitochondrial network parameters remain the same. (Fig. 4A, Supplementary Fig. 3). Fusion serves a protective function in response to cell stress [36] and is controlled by key fusion (MFN1, MFN2 and OPA1) and fission associated proteins (FIS1 and DRP1). Analysis of a subset these fusion and fission proteins revealed no change in the levels of the fusion proteins MFN1 and MFN2 but a striking decrease in the levels of the fission protein DRP1 (Fig. 4B) in Alkbh8def cells. Decreases in the levels of fusion related proteins may in part explain the elongated mitochondrial structure in the Alkbh8 deficient MEFs. Overall, the above findings support the idea that mitochondria play an important role in adapting to epitranscriptomic defects.

3.3. Alkbh8 deficiency engages mitochondrial reprogramming

The resulting increase in mitochondrial length that accompanies Alkbh8 deficiency led us to investigate mitochondrial function. We monitored mitochondrial oxygen consumption and glycolytic flux in Alkbh8def MEFs using the XF24 Analyzer (Seahorse Biosciences). Due to differences in proliferative capacity between cell types (Fig. 1A) and the need for standard 24-h plate equilibration, cells were seeded at differing densities so that cell numbers were equivalent at time of metabolic flux analysis. Alkbh8def MEFs display significant increases in basal, maximal and non-mitochondrial respiration and extracellular acidification compared to WT MEFs (Fig. 5A and B). The cell energy phenotype indicates that Alkbh8def MEFs display energetic adaptation utilizing both aerobic and glycolytic metabolism in response to mitochondrial stress (Fig. 5C) which is also reversed by Alkbh8 rescue (Supplementary Fig. 4).

3.4. UCP2 plays a role in mitochondrial adaptation observed in Alkbh8def

Uncoupling protein 2 (Ucp2) was one of the most highly expressed genes in our focused array analyses and its expression has been reported to increase upon irradiation induced senescence [37], thus we tested whether its upregulation might explain the altered functional activity of mitochondria in the Alkbh8def cells. UCP2, is found in the inner mitochondrial membrane and plays a key role in uncoupling ATP synthase from electron transport chain as well as in limiting mitochondrial ROS.
production by decreasing electron leak [38]. We first confirmed its expression at both the transcript and protein levels (Fig. 6A). Interestingly, limiting metabolic oxygen exposure with low oxygen (3% O₂) tempered the elevations in Ucp2 expression that accompany Alkbh8 deficiency. The effects of elevated UCP2 on mitochondrial function vary and both inhibitory as well stimulatory activity have been observed [39].

We next measured mitochondrial oxygen consumption and extracellular acidification in Alkbh8-def MEFs but in the presence and absence of the UCP2 inhibitor genipin. As in Fig. 5, Alkbh8-def MEFs display significant increases in basal, maximal and non-mitochondrial respiration and extracellular acidification compared to WT MEFs, both of which are negated by treatment with UCP2 inhibitor, genipin (Fig. 6B). In addition, the cell energy phenotype indicates that Alkbh8-def MEFs display energetic adaptation utilizing both aerobic and glycolytic metabolism in response to mitochondrial stress and this adaptation is
not observed when cells were treated with genipin (Fig. 6C). These findings not only confirm that UCP2 is abundantly expressed in the Alkbh8def cells but that its expression is of functional significance.

3.5. Alkbh8Def cells rely on glycolysis for survival and are resistant to arsenic containing mitochondrial toxins

As the Alkbh8def cells display a glycolytic adaptation in their cell energy phenotype, we evaluated their sensitivity to the glycolytic inhibitor 2-deoxyglucose (2DG). Alkbh8def are significantly more sensitive to 2DG than their WT counterparts (Fig. 7A). In addition, while the Alkbh8def MEFs display a proliferative defect to growth in ambient air, they are unusually resistant to a number of arsenicals which have been shown modulate mitochondrial ROS levels [40] (Supplementary Figure 5) and display self-sufficiency (high clonogenic activity) relative to WT MEFs that is blocked in response to 2DG treatment (Fig. 7B) which is reversed by Alkbh8 rescue (Supplementary Figure 6).

4. Discussion

Epitranscriptomic writer deficient cells adapt by engaging senescence, reprogramming stress response systems and altering mitochondrial function.

Our previous work demonstrated cellular senescence increases H2O2 production and engages the senescence associated secretory phenotype (SASP) [41–44]. Our objective here was to determine whether defects in an epitranscriptomic writer linked to Sec utilization leads to a similar redox-based activation of the senescent program. Here we report that selenoprotein loss resulting from Alkbh8 deficiency limits H2O2 removal and engages the senescence program. Selenoprotein loss resulting from selenium restriction can also induce senescence which is reversed by selenium supplementation [24,45]. The Alkbh8def mice may therefore be a good model to mimic a dietary loss of selenium. As Alkbh8 deficiency increases DNA damage and enhances basal oxidant production [11], we first demonstrated that Alkbh8def MEFs display a proliferative defect, which is reversed when cells are cultured in a low oxygen condition. The ability of low oxygen to delay senescence associated proliferative defects was first reported by Ames and confirmed by others [46,47] and similar to these reports the reversal of the growth impediment of the Alkbh8def MEFs is likely attributed to reduction in metabolic H2O2.

While senescence engagement is only one mechanism by which cells adapt to oxidant burden, they often compensate by increasing...
antioxidant enzyme levels or limiting mitochondrial electron leak [48]. The compensatory increase in many redox modifying genes is likely, in part, responsible for the enhanced stress resistance of Alkbh8−/− cells. Interestingly many of these oxidant metabolizing systems are commonly upregulated during carcinogenesis, oncogenic transformation and metastatic disease [49].

Like senescent cells, Alkbh8−/− MEFs display increased mitochondrial mass, ROS production, oxygen consumption and decrease ATP production [50]. A number of ALKBH family members have also been linked to defects in mitochondrial function. ALKBH1 is involved in biogenesis of 5-formyl-2′-O-methylcytidine (f5Cm) at position 34 and loss Alkbh1 decreases mitochondrial translation and reduced respiratory complex activities [51]. ALKBH7 can trigger the collapse of mitochondrial membrane potential, energy depletion, and loss of mitochondrial function, which leads to oxidation induced programmed necrosis [52]. Thus, defects in the ALKBH family activity cause mitochondrial dysfunction with the potential to increase ROS production promote DNA damage and senescence growth arrest [53].

Mitochondria also cope with stress by controlling fusion/fission processes [54] and mitochondrial elongation is a common phenotype in response to energy stress involved in tumor cell survival [55]. The length of mitochondria and its dynamics are determined by the balance between fusion (MFN1, MFN2 and OPA1), and fission proteins (FIS1 and DRP1) [56,57]. The ratio of fusion and fission proteins is critical in disease-related processes such as apoptosis, mitophagy and cell survival [58]. Mitochondrial fusion has also been shown to play an important role in adapting to stress and increase in fusion events are correlated with senescence [34]. Loss of UCP2 is associated with an increase in fragmentation and decrease in the ratio of fusion/fission related proteins which is reversed by UCP2 overexpression [59], indicating that UCP2 may assist in driving the fusion processes. Our findings indicate that although no change was detected in MFN1 and MFN2 levels, Alkbh8−/− MEFs display a significant decrease in the fission protein, DRP1, resulting in an increase of ratio of fusion and fission which likely promotes mitochondrial elongation. Whether this is a UCP2 mediated process remains to be determined.

Increased UCP2 has been reported to limit mitochondrial oxidant production and participate in many physiological processes including glucose and fatty acid utilization [60] and linked to type 2 diabetes and cancer [61–68]. Arsenic containing compounds are mitochondrial toxins that disrupt ATP production and are often lethal to normal cells [69,70]. Thus, increased UCP2 may promote survival of the Alkbh8−/− MEFs in response to arsenic by limiting ROS production. Resistance to arsenic containing compounds but high sensitivity to 2DG indicates that glycolytic adaptation is also important to the survival of Alkbh8−/− MEFs. While the proliferative capacity of Alkbh8−/− MEFs is also impaired they display a significant survival advantage when grown at low seeding density compare to the WT MEFs. High clonogenic activity, stress resistance and a reliance on glycolysis for survival are key features of tumorigenic cell lines [71] and it is possible that conditions which limit ALKBH8 activity may potentiate tumorigenic activity.

4.1. Epitranscriptomic defects and adaptive phenotypes can be exploited to kill senescent cells

It is clear from our findings that cells adapt dramatically to the loss of ALKBH8 and defects in selenoprotein levels. In addition to mcm [5]U and mcm [5]Um modifications by ALKBH8, tRNA\(^{Sec}\) contains 2 modified bases at the T-arm, 1-methyladenosine (m\(^1\)A) at position 58 and pseudouridine (Ψ) at position 55, and one modified base at anti-codon arm, N\(^{6}\)-isopentenyl-adenosine (i\(^6\)A) at position 37 [72]. m\(^1\)A modification is required for Ψ synthesis, which is essential for maintaining the tertiary structure of tRNA\(^{Sec}\) [73]. i\(^6\)A modification of tRNA\(^{Sec}\) catalyzed by tRNA isopentenyltransferase 1 (TRIT1) is required for decoding of UGA stop codon, as well as selenoproteins synthesis [74] and knock down of TRIT1 reduces selenoprotein expression [75]. Whether selenoprotein defects can engage cellular senescence and the relation between other epitranscriptomic modifications of tRNA\(^{Sec}\) and cellular senescence need to be further investigated.

Senescence has emerged as a key therapeutic target for many disease interventions as its abatement in distinct murine models limits age related decline in cognitive, cardiac and renal function, muscle atrophy,
catastrophes and delays tumor burden [19,76]. Senescent abatement has been shown to delay atherosclerosis, pulmonary fibrosis, neurodegenerative disease, heart failure and tumor onset [15,77–81]. We demonstrate that the UCP2 inhibitor, genipin, negated increases in mitochondrial OCR in Akbhhδ/MEFs, as well as their glycolytic behavior. Our study is an early example of how an epitranscriptomic defect can be metabolically exploited to limit cell growth and may have potential applications in killing tumors with similar genetic profiles.

We have previously reported that the SASP is redox-regulated through a mechanism that involves redox-dependent transcriptional activation and impeding inhibitory signaling phosphatases [14]. Selenium deprivation has been established to engage senescent programming and our findings further support the importance of selenoproteins in this process likely through a similar redox based signaling process. Our study further indicates that additional, adaptive responses are engaged in response to selenoprotein deficiency. Senescence engagement coupled with stress resistance would create a microenvironment that is ripe for disease development and that targeting the epitranscriptome in combination with selenium supplementation may prove useful as a therapeutic strategy for disease intervention (See Fig. 8).

Funding

Research was funded by grants from the National Institutes of Health (Thomas Begley - R01ES026856 and R01ES024615) and SUNY Polytechnic Institute COR Seed Grant (J. Andres Melendez - 1147718).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.redox.2019.101375.

References

[1] Morena, F., Argentati, C., Bazzucchi, M., Emiliani, C. & Martino, S. Above the
[2] M.E., Senescence-associated secretory phenotype: insights into the pathophysiology of lung diseases, Mol. Biol. Chem. 288 (2013) 32149–32159.
[3] J. Dasgupta, et al., Reactive oxygen species control senescence-associated matrix
[4] J. Dasgupta, et al., Reactive oxygen species control senescence-associated matrix
[5] K.K. Nelson, et al., Elevated sod2 activity augments matrix metalloproteinase ex-
[6] D.A. McCarthy, R.R. Clark, T.R. Bartling, M. Trebak, J.A. Melendez, Redox control of the senescence regulator interleukin-1α and the secretory phenotype, J. Biol. Chem. 288 (2013) 32149–32159.
[7] D. Fu, et al., Human AlkB homolog ABH8 is a tRNA methyltransferase required for
[8] J. Campisi, Senescent cells, tumor suppression, and the senescent secretory phenotype: therapeutic opportunities, J. Clin. Investig. 123 (2013) 966–972.
[9] P.A. Pérez-Mancera, A.R.J. Young, M. Narita, Inside and out: the activities of se-
[10] R.M. Naylor, D.J. Baker, J.M. van Deursen, Senescent cells: a novel therapeutic
[11] L. Endres, et al., Alkbh8 regulates selenocysteine-protein expression to protect
[12] J. Campisi, Aging, cellular senescence, and cancer, Annu. Rev. Physiol. 75 (2013)
[13] J. Campisi, Aging, cellular senescence, and cancer, Annu. Rev. Physiol. 75 (2013)
[14] D.A. McCarthy, R.R. Clark, T.R. Bartling, M. Trebak, J.A. Melendez, Redox control of the senescence regulator interleukin-1α and the secretory phenotype, J. Biol. Chem. 288 (2013) 32149–32159.
[15] T. Kadota, et al., Emerging role of extracellular vesicles as a senescence-associated
[16] J.-P. Coppé, et al., Senescence-associated secretory phenotypes reveal cell-
[17] J.-P. Coppé, P.-Y. Desprez, A. Krotolica, J. Campisi, The senescence-associated-secretory phenotype: the dark side of tumor suppression, Annu. Rev. Pathol. 5 (2010) 99–118.
[18] T. Tchekonia, Y. Zhu, J. van Deursen, J. Campisi, J.L. Kirkland, Cellular senescence and the senescent secretory phenotype: therapeutic opportunities, J. Clin. Investig. 123 (2013) 966–972.
[19] P.A. Pérez-Mancera, A.R.J. Young, M. Narita, Inside and out: the activities of se-
[20] R.M. Naylor, D.J. Baker, J.M. van Deursen, Senescent cells: a novel therapeutic
[21] J. Campisi, Aging, cellular senescence, and cancer, Annu. Rev. Physiol. 75 (2013)
[22] L. Zhang, H. Zeng, W.-H. Cheng, Beneficial and paradoxical roles of selenium at nutritional levels of intake in healthypan and longevity, Free Radic. Med. Biol. 127 (2018) 3–13.
[23] J. Campisi, Aging, cellular senescence, and cancer, Annu. Rev. Physiol. 75 (2013)
[24] G. Hammad, et al., Interplay between selenium levels and replicative senescence in
[25] P.J. Hornsby, S.E. Harris, Oxidative damage to DNA and replicative lifespan in WI-38 human fibroblasts, J. Biol. Chem. 289 (2014) 6299–6310.
[26] G. Hammad, et al., Interplay between selenium levels and replicative senescence in WI-38 human fibroblasts: a proteomic approach, Antioxidants (Basel, Switzerland) 7 (2018).
[27] E. Mocchegiani, et al., Zinc, metallothioneins and longevity: interrelationships with niacin and selenium. Curr. Pharmaceut. Des. 14 (2008) 2719–2732.
[28] R.K. Dagda, M. Rice, R.A. Merrill, K.H. Flippo, S. Strack, Techniques to investigate mitochondrial function in neurons, Neuromethods 123 (2017) 1–27.
[29] A.J. Valente, L.A. Maddalena, E.L. Robb, F. Moradi, J.A. Stuart, A simple image
[30] R. Alis, et al., Trace elements levels in centenarian ‘dodgers’, J. Trace Elem. Med. Biol. 35 (2016) 103–106.
[31] L. Zhang, H. Zeng, W.-H. Cheng, Beneficial and paradoxical roles of selenium at nutritional levels of intake in healthypan and longevity, Free Radic. Med. Biol. 127 (2018) 3–13.
[32] G.P. Dimri, et al., A biomarker that identifies senescent human cells in culture and in aging skin in-vivo, Proc. Natl. Acad. Sci. U.S.A. 92 (1995) 9363–9367.
[33] D.J. Baker, et al., Naturally occurring pl6kn4a-positive cells shorten healthy life-
[34] Nature 530 (2016) 184–189.
[35] D.V. Ziegler, C.D. Wylie, M.C. Velarde, Mitochondrial effectors of cellular senes-
[36] T.A. Müller, S.L. Struble, K. Meek, R.P. Hausinger, Characterization of human AlkB
[37] A. Böck, et al., Selenocysteine: the 21st amino acid, Mol. Microbiol. 5 (1991)
[38] A. Böck, et al., Selenocysteine: the 21st amino acid, Mol. Microbiol. 5 (1991)
[39] M.K. Shigenaga, T.M. Hagen, B.N. Ames, Oxidative damage and mitochondrial
decay in aging, Proc. Natl. Acad. Sci. U.S.A. 91 (1994) 10771–10778.
[40] D. Fu, et al., Human AlkB homolog ABH8 is a tRNA methyltransferase required for
[41] A. Moret, F., Argentati, C., Bazzucchi, M., Emiliani, C. & Martino, S. Above the
[42] M. Esteller, P.P. Pandolfi, B. Israel, The epitranscriptome of non-coding RNAs in
[43] A.G. Org, E. Batle, L. Ribas de Pouplana, Role of tRNA modifications in human
diseases, Trends Mol. Med. 20 (2014) 306–314.
[44] S. Huber, A. Leonard, P. Dedon, T. Begley, The versatile roles of the RNA epitranscriptome during cellular responses to toxic exposures and environmental stress, Toxics 7 (2019) 17.
[45] L. Triul, A. Lusser, The dynamic RNA modification S-methylcysteine and its emerging role as an epitranscriptomic mark, Wiley Interdiscip. Rev. RNA 10 (2019)
[46] M.T. Bohnsack, K.E. Sloan, The mitochondrial epitranscriptome: the roles of RNA modifications in mitochondrial translation and human disease, Cell. Mol. Life Sci. 75 (2018) 241–261.
[47] D. Fu, et al., Human AlkB homolog ABH8 is a tRNA methyltransferase required for
[48] J. Campisi, Aging, cellular senescence, and cancer, Annu. Rev. Physiol. 75 (2013)
[49] G. Pani, T. Galeotti, P. Chiarugi, Metastasis: cancer cell’s escape from oxidative
[50] M.K. Shigenaga, T.M. Hagen, B.N. Ames, Oxidative damage and mitochondrial
decay in aging, Proc. Natl. Acad. Sci. U.S.A. 91 (1994) 10771–10778.
[51] T.A. Müller, S.L. Struble, K. Meek, R.P. Hausinger, Characterization of human AlkB
dysfunction in ALKBH1-deficient cells, Biochem. Biophys. Res. Commun. 495 (2018) 98–103.

[52] D. Fu, J.J. Jordan, L.D. Samson, Human ALKBH7 is required for alkylation and oxidation-induced programmed necrosis, Genes Dev. 27 (2013) 1089–1100.

[53] J. Kaplon, et al., A key role for mitochondrial gatekeeper pyruvate dehydrogenase in oncogene-induced senescence, Nature 498 (2013) 109–112.

[54] J. Suárez-Rivero, et al., Mitochondrial dynamics in mitochondrial diseases, Diseases 5 (2016) 1.

[55] J. Li, et al., Mitochondrial elongation-mediated glucose metabolism reprogramming is essential for tumour cell survival during energy stress, Oncogene 36 (2017) 4901–4912.

[56] B. Westermann, Mitochondrial fusion and fission in cell life and death, Nat. Rev. Mol. Cell Biol. 11 (2010) 872–884.

[57] L. Simula, F. Nazio, S. Campello, The mitochondrial dynamics in cancer and immune-surveillance, Semin. Cancer Biol. 47 (2017) 29–42.

[58] A.M. van der Bliek, Q. Shen, S. Kawajiri, Mechanisms of mitochondrial fission and fusion, Cold Spring Harb. Perspect. Biol. 5 (2013) a011072–a011072.

[59] Y. Shimasaki, et al., Uncoupling protein 2 impacts endothelial phenotype via p53-mediated control of mitochondrial dynamics, Circ. Res. 113 (2013) 891–901.

[60] C. Pecquer, C. Alves-Guerra, D. Ricquier, F. Bouillaud, UCP2, a metabolic sensor coupling glucose oxidation to mitochondrial metabolism? IUBMB Life 61 (2009) 762–767.

[61] J.S. Moon, et al., UCP2-induced fatty acid synthase promotes NLRP3 inflammasome activation during sepsis, J. Clin. Investig. 125 (2015) 665–680.

[62] V. Ayyasamy, et al., Cellular model of warburg effect identifies tumor promoting function of UCP2 in breast cancer and its suppression by genipin, PLoS One 6 (2011) e24792.

[63] J. Su, et al., Cytoprotective effect of the UCP2-SIRT3 signaling pathway by decreasing mitochondrial oxidative stress on cerebral ischemia–reperfusion injury, Int. J. Mol. Sci. 18 (2017).

[64] N. Tsukeyama-Kasada, K. Sano, C. Shozawa, T. Osaka, O. Ezaki, Studies of UCP2 transgenic and knockout mice reveal that liver UCP2 is not essential for the anti-obesity effects of fish oil, Am. J. Physiol. Endocrinol. Metab. 294 (2008) E600–E606.

[65] N. Li, M. Karaca, P. Machler, Upregulation of UCP2 in beta-cells confers partial protection against both oxidative stress and glucotoxicity, Redox Biol. 13 (2017) 541–549.

[66] M.A. Pitt, Overexpression of uncoupling protein-2 in cancer: metabolic and heat changes, inhibition and effects on drug resistance, Inflammopharmacology 23 (2015) 365–369.

[67] C.Y. Zhang, et al., Uncoupling protein-2 negatively regulates insulin secretion and is a major link between obesity, beta cell dysfunction, and type 2 diabetes, Cell 105 (2001) 745–755.

[68] H.Y. Lim, et al., Metabolic signatures of renal cell carcinoma, Biochem. Biophys. Res. Commun. 460 (2015) 938–943.

[69] C.H. Tseng, et al., Mammalian Trit1 is a tRNA [Ser]Sec -isopentenyl transferase required for full selenoprotein expression, J. Biol. Chem. 275 (2000) 28110–28119.

[70] N. Fradejas, et al., Mammalian Trit1 is a tRNA [Ser]Sec -isopentenyl transferase lacking isopentenyladenosine, J. Biol. Chem. 275 (2000) 28110–28119.