THE ROLE OF RIBOSOMAL COMPOSITION IN SELECTIVE TRANSLATION OF LONGEVITY GENES UNDER DIETARY RESTRICTION

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Dietary restriction influences longevity through changes in gene expression on the level of transcription and translation. Our lab is investigating the changes occurring under dietary restriction in C. elegans on the level of individual ribosomal proteins (RPs) and changes in RP phosphorylation and how they shape gene expression and longevity. To look at the impact of changes in RP expression we used RNAi knockdowns starting at adulthood and assayed lifespan and health span. Two RPs knockdowns that increased longevity, rpl-7A and rpl-22, were then subjected to transcriptome and translatome analysis using polysome profiling and mRNA-seq. Both knockdowns resulted in similar transcriptomic changes with an up-regulation of genes related to translational fidelity and mitochondrion organization. In contrast, the translatome analysis showed minimal changes upon loss of rpl-7A and rpl-22 suggesting that they are not responsible for increase in selective translation previously observed under DR. To look at impact of changes in phosphorylation of rps-6 on DR, we generated mutant lines incapable of being phosphorylated by TOR signaling. These mutants were assayed for changes in lifespan, health span and selective translation. While phenotypically similar to controls under well fed conditions, the mutants were longer lived under DR. Furthermore, preliminary results indicated that the selective translation of the anti-longevity microchondrial electron transport genes cytochrome c oxidase and ubiquinol-cytochrome c reductase binding protein was repressed when rps-6 is not phosphorylated. In summary, our preliminary results that ribosomal protein phosphorylation but not protein composition is responsible for guiding selective translation pf longevity genes under DR.

SENESCENCE-ASSOCIATED SECRETOME COMPONENTS AS BIOMARKERS FOR AGING

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Cellular senescence is a state of stable growth arrest in response to stress, which is a fundamental process of biological aging. They secrete products, the Senescence-Associated Secretory Phenotype (SASP), which consists of inflammatory cytokines, chemokines, growth factors and matrix remodeling proteins. Senescent cells accumulate with advancing age and partial elimination of senescent cells can reverse age-related dysfunction and increase mean lifespan in mice. However, it is not clear whether components of the SASP can be measured in human plasma and serve as aging biomarkers. Here we generated a candidate panel of senescence markers based on a multiplexed bead-based assay of proteins secreted by senescent preadipocytes, endothelial cells, fibroblasts, myoblasts, preadipocytes, and epithelial cells compared to non-senescent controls. The SASP undoubtedly varies by cell type; however, we observed that multiple components of the SASP are conserved. We then assessed circulating SASP components in human plasma samples from Mayo Clinic Biobank participants (n=280, 20 male and 20 female per decade, age 20-90) using the same method. We confirmed that components of the SASP can be quantified in human plasma with the multiplexed bead-based assay and observed several SASP components robustly increase with chronological age in humans. Our study illustrates that senescence-associated secretome components are detectable in human plasma and could potentially serve as biomarkers of systemic aging and senescent cell burden.

SENESCENCE SECRETOME: BIOMARKERS OF BIOLOGICAL AGING AND POSTOPERATIVE OUTCOMES

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Senescent cells drive age-related tissue dysfunction through their potent secretome, termed the senescence associated secretory phenotype (SASP). Circulating concentrations of SASP factors may reflect biological age and serve as clinically useful biomarkers of surgical risk and ultimately, surrogate endpoints in clinical trials. However, they remain largely uncharacterized. We tested associations between circulating concentrations of SASP proteins and biological age, as determined by the accumulation of age-related health deficits, and/or postoperative outcomes in a sample of residents in Olmstead County, MN, age 60-90 years (n = 115) and cohorts of older adults undergoing surgery for severe aortic stenosis (prospective; n = 97) or ovarian cancer (case-control; n = 36). Circulating concentrations of SASP factors were associated with biological age and adverse postoperative outcomes, including risk of any adverse event or rehospitalization within the year following surgery (aortic stenosis group) or admission to an intensive care unit within 30 days of hospital discharge (ovarian cancer group). Gradient boosting machine modeling revealed a panel of SASP factors that predicted adverse outcomes across both surgical groups better than biological age or chronological age and sex. This suggests that the circulating SASP is a robust indicator of age-related health status and may help guide clinical decision making. Furthermore, circulating SASP factors may be harnessed as a readily quantifiable biomarkers in senescence-targeting interventional human studies.

INCREASED HSP25 DRIVES THE TRANSITION FROM PROTEASOME TO AUTOPHAGY-MEDIATED DEGRADATION UNDER PROTEOTOXIC STRESS

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Accumulation of protein aggregates are a common pathology in many neurodegenerative disorders. This accumulation may be due to a function decline in the protein homeostasis network known to occur during the aging process. Small heat shock proteins are a class of molecular chaperones that assist in protein folding and ameliorates the degradation activity of the proteasome and autolysosome

thereby decreasing disease-associated aggregates. Prior work in rodents and C. elegans has shown expression levels of the small heat shock protein 25 (HSP25) correlates with maximum lifespan potential. Increased levels of HSP25 extends lifespan in a transgenic C. elegans model. This lifespan extension is dependent on skn-1 with evidence suggesting an enrichment in several skn-1-related pathways, such as lysosomal genes. Concomitantly, proteasome activity declines while autolysosome activity increases. This observation might suggest a switch from proteasome degradation to autophagy as the main driver of protein degradation in C. elegans in this transgenic model. To investigate if a reduction of proteasome function and elevated lysosomal gene activation during aging and under proteotoxic stress are modulated by HSP25 we have crossed our HSP25-transgenic worm with an aggregating and non-aggregating tau worm model. This work will elucidate a possible mechanism that explains the change in the protein degradation response pathways potentially modulated by HSP25 during increased protein misfolding.

INDICES OF RESILIENCY IN CELLS FROM UM-HET3 MICE MAY CORRELATE WITH INDIVIDUAL FUTURE HEALTH OUTCOMES

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The ability of an organism to respond to physical stresses and return to homeostasis (i.e. resilience) has been suggested to correlate with longevity. Here, we investigated whether this extends to resilience at a cellular level using primary fibroblasts isolated from tail skin of genetically heterogeneous young adult UM-HET3 mice. Cells isolated from each individual mouse (cell line) were tested in their response to concentrations of agents or conditions predicted to induce a cellular challenge, including paraquat, hydrogen peroxide, antimycin a, cadmium chloride, mdivi-1, thapsigargin, and nutrient starvation. Cell viability was monitored in real-time using an incucyte S3 live cell analysis system and we addressed the response following challenge as a marker of resilience. Cellular uptake of ethidium homodimer-1 was used to determine the loss of viability. Cellular bioenergetics were assessed using a seahorse XF24. We found that cell lines that were resistant to paraquat were also resistant to antimycin a, and hydrogen peroxide. Cell lines that were resistant to nutrient starvation were also resistant to mdivi-1. Indices of cellular bioenergetics status including ATP production rate and cell respiratory control ratio, revealed potential relationships with resiliency. Taken together, our data indicate that skin fibroblasts retain individual physiological programs that may in part explain the patterns of resiliency or sensitivity to a stressor at the organismal level. Since the cell lines tested in this study were obtained from living mice, future work will investigate whether these patterns of resiliency change with age and elucidate their utility in predicting future health outcome.

THE ENDOPLASMIC RETICULUM PROTEIN QUALITY CONTROL ADAPTATION IN A LONG-LIVED C. ELEGANS PROTEASOMAL MUTANT

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Protein degradation mechanisms are integral to protein homeostasis. Their reduced efficiency during aging leads to accumulation of misfolded and aggregated proteins which potentiate proteotoxic disorders. Paradoxically, our lab reported that the Caenorhabditis elegans rpn-10(ok1865) proteasome mutant possesses enhanced proteostasis and extended lifespan. RPN-10/PSMD4 is a ubiquitin receptor of the 26S proteasome that targets polyubiquitinated substrates to its catalytic core for degradation. Proteasome dysfunction of the rpn-10 mutant is characterized by reduced, not inhibited, ubiquitin fusion degradation. We ascertained that upregulated autophagy and SKN-1/Nrf-mediated responses partially contribute to the robust rpn-10 mutant phenotype. Further investigation of its underlying mechanism revealed that several ERQC genes are transcriptionally upregulated in the rpn-10 mutant. Thus, we hypothesized that the rpn-10 mutant exhibits improved ER proteostasis which mediates its elevated cellular stress resistance. Accordingly, the rpn-10 mutant shows increased ER stress resistance and altered ER homeostasis. Complementarily, attenuated expression of the aggregation-prone α-1 antitrypsin (ATZ) reporter proves that ER proteostasis is ameliorated in the rpn-10 mutant. Via a genetic screen for suppressors of decreased ATZ aggregation in the rpn-10 mutant, we identified novel player H04D03.3, which is a homolog of the proteasome adaptor ECM29. This suggests that assembly of the rpn-10 mutant proteasome itself critically regulates its ER proteostasis. Moreover, we observed that cytosolic proteostasis and longevity depend on ER master chaperone hsp-3/-4(BiP) and ER ATPase cdc-48.2(p97/VCP), further highlighting ERQC significance in the rpn-10 mutant. Altogether, it appears that mild proteasomal dysfunction induces ERQC adaptation that underlies proteostasis and longevity benefits of the rpn-10 mutant.

THE LONGEVITY ASSOCIATED ALLELE OF FOXO3 PROTECTS AGAINST TELOMERE ATTRITION DURING AGING

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Telomere attrition in proliferative tissues is a hallmark feature of human aging. To date, the genetic influence on the rate of telomere attrition is poorly understood. Previously we discovered a variant of the FOXO3 gene that is strongly associated with human longevity, an observation that has been now reproduced in over a dozen independent studies. In the present study, we sought to assess the effect of the longevity associated variant of FOXO3 (rs2802292 - G allele) on the rate of telomere attrition during aging. The results from a cohort of Okinawan-Japanese (N=121), ranging in age from 25 – 94 years, demonstrates carriers of 1 or 2 copies of the longevity-associated G allele of FOXO3 showed markedly reduced rates of telomere loss in peripheral blood leucocytes