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ORIGINAL RESEARCH

Prognostic Significance and Biologic Associations of Senescence-Associated Secretory Phenotype Biomarkers in Heart Failure

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BACKGROUND: The role of cellular senescence in human heart failure (HF) remains unclear. The senescence-associated secretory phenotype (SASP) is composed of proteins released by senescent cells. We assessed the prognostic significance and biologic pathways associated with the SASP in human HF using a plasma proteomics approach.

METHODS AND RESULTS: We measured 25 known SASP proteins among 2248 PHFS (Penn HF Study) participants using the SOMAScan V4 assay. We extracted the common variance in these proteins to generate SASP factor scores and assessed the relationship between these SASP factor scores and (1) all-cause death and (2) the composite of death or HF hospital admission. We also assessed the relationship of each SASP factor to 4746 other proteins, correcting for multiple comparisons, followed by pathway analyses. Two SASP factors were identified. Both factors were associated with older age, lower estimated glomerular filtration rate, and more advanced New York Heart Association class, among other clinical variables. Both SASP factors exhibited a significant positive association with the risk of death independent of the Meta-Analysis of Global-Group in Chronic HF score and NT-proBNP (N-terminal pro-B-type natriuretic peptide) levels. The 2 identified SASP factors were associated with 1201 and 1554 proteins, respectively, belonging to various pathways including the coagulation system, complement system, acute phase response signaling, and retinoid X receptor–related pathways that regulate cell metabolism.

CONCLUSIONS: Increased SASP components are independently associated with adverse outcomes in HF. Biologic pathways associated with SASP are predominantly related to coagulation, inflammation, and cell metabolism.

Key Words: aging ■ cell senescence ■ heart failure ■ pathways ■ proteomics

Heart failure (HF) is a heterogeneous condition of increasing prevalence in our aging population. Aging is a known potent risk factor for coronary artery disease, left ventricular hypertrophy, and HF.[1] Similarly, comorbid conditions such as diabetes, hypertension, obesity, chronic kidney disease, and atrial

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CLINICAL PERSPECTIVE

What Is New?

- This study leveraged a proteomic approach utilizing 25 known senescence-associated secretory phenotype (SASP) biomarkers to assess the clinical, proteomic, and biologic correlates of cell senescence in human heart failure (HF).
- We identified two main senescence factors which were associated with adverse outcomes, important clinical characteristics, and canonical biologic pathways.

What are the Clinical Implications?

- SASP factors were associated with various clinical variables such as older age, lower renal function, more advanced symptoms, male sex, lower blood pressure, and lower body mass index. One of the SASP factors was associated with a lower left ventricular ejection fraction, a lower proportion of participants with HF with recovered ejection fraction, and a higher proportion of participants with HF with reduced ejection fraction. The proportion of participants with HF with preserved ejection fraction did not differ across tertiles of either SASP factor.
- Both SASP factors were associated with the risk of all-cause death,[1] and the composite of death or HF hospital admission.[2] Various canonical biologic pathways were found to be associated with the SASP factors, and were predominantly related to glucose and lipid metabolism, coagulation, and inflammation.
- Whether cell senescence represents a suitable therapeutic target in HF remains to be investigated.

Nonstandard Abbreviations and Acronyms

DHFA	death or heart failure hospital admission
FBLN-1	fibulin-1
FSTL1	follistatin-related protein-1
FXR	Farnesoid X receptor
HFpEF	heart failure with preserved ejection fraction
LAMA2	laminin subunit α-2
LXR	liver X receptor
MAGGIC	Meta-Analysis of Global-Group in Chronic HF
MFAP4	microfibril-associated glycoprotein-4
NOTCH3	neurogenic locus notch holomog protein-3
PHFS	Penn HF Study
PXDN	peroxidasin homolog
RXR	retinoid X receptor
SASP	senescence-associated secretory phenotype
VSMC	vascular smooth muscle cell

fibrillation (AF), which exhibit an increased incidence with age,[2,3] are highly prevalent in patients with HF, and often contribute to its pathogenesis, pathophysiology, and outcomes.[4]

Cellular senescence, a hallmark of cellular aging, is the process by which cells exit the cell cycle permanently in response to various microenvironmental stressors and enter a senescent state characterized by tissue dysfunction and the production of a senescence-associated secretory phenotype (SASP).[5] The SASP is a collection of inflammatory cytokines, chemokines, growth factors, and proteases that are excreted by senescent cells that have been implicated in various pathologic processes.[5] Previous studies suggest an association between HF pathophysiological mechanisms and cellular senescence.[6,7] However, the role of cell senescence in human HF, including its clinical and biologic correlates and association with adverse outcomes, remains incompletely understood.

In this study, we aimed to (1) assess the prognostic significance of the SASP in HF using 25 known senescent proteins[8,9] and (2) assess the biologic pathways and proteomic correlates of SASP-associated proteins using broad plasma proteomics approaches (≈5000 plasma proteins along with knowledge-based pathway analysis).

METHODS

Study Population

We studied participants enrolled in the PHFS (Penn HF Study; n=2248) with available plasma samples for proteomics analyses. The PHFS is a prospective cohort study that recruited patients referred to 3 US HF specialty centers: University of Pennsylvania, Case Western Reserve University, and University of Wisconsin. The study was approved by the institutional review board at each participating center, and each participant gave written informed consent. The criteria for inclusion were referral to an outpatient HF specialty clinic at the mentioned centers for workup and treatment of HF and lack of other extracardiac illness that leads to death within 6 months. The raw data

and analytical methods of this article are not publicly available for purposes of reproducing the results or replicating the procedures. These data might be available subject to the establishment of appropriate data-sharing agreements and regulatory approvals.

Protein Measurements

We used the SomaScan assay version 4 (Somalogic Operating Co., Boulder, CO), which is a multiplexed, modified aptamer-based binding-assay for PHFS assays. This assay includes 4979 modified aptamer reagents to 4776 unique protein targets. The SomaScan assay es slow-off-rate modified aptamer reagents, which are chemically modified nucleotides, to bind and quantify target proteins in relative fluorescent units directly proportional to the amount of target protein in the sample.

Bioinformatics Methods

Given that we used multiple protein indicators of senescence, we used factor analysis to discern the common variability underlying these indicators. Factor analysis is a theory-driven statistical data reduction technique to extract the covariance among a set of observed variables into a smaller number of underlying factors. We selected 25 known SASP proteins that (1) were found to be part of the SASP in human lung fibroblasts and renal cortical epithelial cells exposed to 3 different inducers of senescence (irradiation, inducible Ras overexpression, and atazanavir [HIV protease inhibitor that induces senescence]),[8] (2) were reported to be associated with chronologic age in a previous study by Tanaka et al,[9] and (3) were included in the SOMAScan. These proteins included the following: phosphoglycerate kinase-1, tissue inhibitor of metalloproteinases-1/2, cystatin C, fibronectin-1 (FN1), matrix metalloproteinase-1/2, plasma protease C1 inhibitor, gelsolin, cathepsin-B/D/Z, glutathione-S-transferase P1, heat shock 70 kDa protein 1A, heat shock cognate 71 kDa protein, insulin-like growth factor binding protein-2/7, biglycan, 14-3-3 protein theta, stanniocalcin-1, activated leukocyte cell adhesion molecule, coactosin-like protein, periostin, Parkinson disease protein 7, growth/differentiation factor 15. We performed factor analysis with Varimax rotation to extract the underlying factors (hereby referred to as SASP factors) that explain at least 60% of the covariance among our selected proteins. Factors with eigenvalues >1.0 were retained.

We performed association analyses to assess the biologic correlates of SASP factors. First, we regressed each of the factors against all other proteins in the SomaScan using linear regression. We corrected the α level for multiple comparisons using the number of principal components underlying >95% of the variability of all measured proteins, as previously described.[10–13] Associations between factors and individual proteins that were significant with a corrected $P<0.05$ were then used to perform knowledge-based pathway analyses using Ingenuity Pathway Analysis software (Qiagen; Hilden, Germany; www.qiagen.com/ingenuity). Proteins were identified according to their UniProt identification annotation. The totality of proteins included in the SomaScan assay was used as the reference set, and both direct and indirect experimentally confirmed relationships from all species were included. When proteins are represented by multiple aptamers, the aptamer that exhibited the highest absolute value of effect size was used for downstream analyses. The analysis calculates a *P* value (Fisher exact test and right-tailed) quantifying the overlap, and a *Z* score quantifying the likelihood and direction (upregulated or downregulated), between the plasma proteomics pattern and known canonical pathways.

Statistical Analysis

Participant characteristics were summarized using mean (SD) for continuous variables with symmetric distribution and median (interquartile range) for continuous variables with a skewed distribution. Categorical variables are expressed as counts (percentages). To compare continuous variables, we used ANOVA or the Kruskal–Wallis test as appropriate, whereas categorical variables were compared using the χ^2 or Fisher exact test, as appropriate.

To characterize the clinical correlates of SASP factors, we compared key clinical characteristics across tertiles of each SASP factor score. For survival analyses, we assessed the relationship between SASP factor scores and (1) the composite of death and heart failure hospital admission (DHFA) and (2) all-cause death. We plotted Kaplan–Meier survival curves for tertiles of the SASP factor scores and compared them using the log-rank test. We further assessed the relationship using Cox regression models in models that (1) did not adjust for clinical covariates and (2) adjusted for both the Meta-Analysis of Global-Group in Chronic HF (MAGGIC) risk score and NT-proBNP (N-terminal pro-B-type natriuretic peptide) levels. To perform unit-independent analyses that can be compared easily between factors and other predictors, we expressed hazard ratios (HRs) per SD increase (ie, 1-point increase in the *Z* score). Box-Cox transformation was applied to improve the normality of data distribution as appropriate. The assumptions of proportional hazards models were tested systematically, as presented in detail in Data S1 and Figure S1.

RESULTS

Baseline Characteristics

Table S1 shows a comparison of baseline clinical characteristics of study participants with (n=2248) versus those without (n=131) proteomics data. Participants with proteomics data tended to be younger, with a higher proportion of Black and White participants, as opposed to Asian participants and other racial groups. They also exhibited a slightly lower left ventricular ejection fraction and a lower proportion of participants on calcium channel blockers.

Factor Analysis of SASP Proteins

Figure 1A shows a heat map of the correlation matrix of the SASP biomarkers, organized in hierarchical clusters. Eight factors were found to represent >60% of the variability in all 25 SASP indicators. Figure S2 shows a scree plot for the eigenvalues of all derived factors. Based on the scree plot and the factor eigenvalues, we initially retained factors 1 through 3, which exhibited eigenvalues >1. Figure 1B shows a factor loadings map of these 3 SASP factors. Factors 1 and 2 loaded prominently on multiple proteins, consistent with their representation of common variability of SASP proteins. In contrast, factor 3 loaded on 3 aptamers for a single protein (fibronectin-1), indicating that it represented fibronectin-1 concentrations rather than common variance with other SASP proteins. Consequently, we only used factors 1 and 2 for downstream analyses.

Clinical Correlates of Senescence

A comparison of key clinical characteristics across SASP factor score 1 tertiles is shown in Table 1. Higher values of SASP factor score 1 were associated with older age, male sex, slightly lower blood pressure, lower body mass index, lower left ventricular ejection fraction, lower proportion of participants with HF with recovered ejection fraction with a higher proportion of participants with with reduced ejection fraction. Notably the proportion of participants with HF with preserved ejection fraction (HFpEF) did not differ significantly across SASP factor score 1 tertiles. Higher values of factor 1 were also associated with higher NT-proBNP, MAGGIC risk scores, worse New York Heart Association functional class, lower prevalence of angiotensin-converting enzyme inhibitor or angiotensin II receptor blocker use, aldosterone antagonist use, smoking, and higher prevalence of diabetes, AF or flutter, history of coronary artery bypass graft surgery, and warfarin use.

Similar to factor 1, higher tertiles of factor 2 (Table 2) exhibited similar associations with age, sex, systolic blood pressure, diastolic blood pressure, body mass index, diabetes, AF or atrial flutter, history of coronary artery bypass graft surgery, New York Heart Association functional class, angiotensin-converting enzyme inhibitor/angiotensin II receptor blocker use, warfarin use, NT-proBNP, and MAGGIC risk score. However, higher factor 2 tertiles were additionally associated with a higher proportion of White

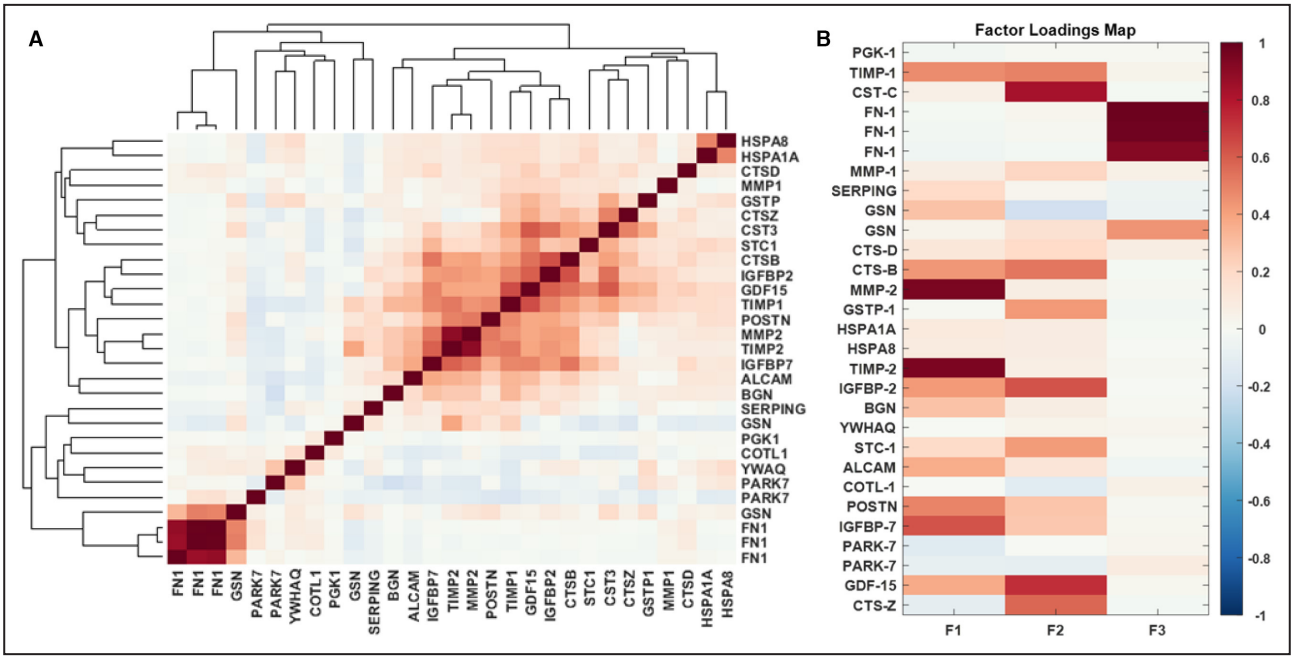

Figure 1. Heat map correlation matrix of senescence-associated secretory phenotype (SASP) biomarkers, organized into hierarchical clusters (A) and factor loadings map showing the association of each factor with different SASP biomarkers (B).

Table 1. Association of Clinical Characteristics With Tertiles of Senescence Factor Scores for Senescence Factor 1

Baseline characteristics	Lower tertile	Middle tertile	Upper tertile	*P* value
Demographic factors				
Age, y	53.1 (52–54.2)	54 (52.9–55.2)	56.5 (55.3–57.7)	<0.0001
Male sex	449 (60.76)	511 (68.22)	523 (70.01)	0.0003
Race				
White	561 (74.90)	552 (73.60)	528 (70.49)	0.2967
Black	142 (18.96)	155 (20.67)	174 (23.23)	
Asian	9 (1.20)	8 (1.07)	4 (0.53)	
Other	37 (4.94)	35 (4.67)	43 (5.74)	
Physical exam/laboratory tests				
Systolic blood pressure, mm Hg	113 (112–115)	115 (114–117)	111 (109–112)	<0.0001
Diastolic blood pressure, mm Hg	69.2 (68.3–70)	69.5 (68.7–70.4)	67.4 (66.6–68.3)	0.0011
Body mass index, kg/m^2	31 (30.5–31.5)	29.3 (28.8–29.8)	27.9 (27.5–28.4)	<0.0001
eGFR, mL/min per 1.73 m^2	50.8 (49–52.6)	55.4 (53.4–57.3)	51.1 (49.3–52.9)	0.0005
NT-proBNP	673 (625–720)	828 (769–887)	1297 (1206–1389)	<0.0001
MAGGIC risk score	14.1 (13.6– 14.7)	15.1 (14.5–15.6)	18.9 (18.1–19.6)	<0.0001
Medical history				
Diabetes	210 (28.04)	191 (25.47)	241 (32.18)	0.0149
History of stenting	144 (19.23)	175 (23.33)	179 (23.90)	0.0593
History of CABG	109 (14.55)	147 (19.60)	161 (21.50)	0.0017
History of atrial fibrillation/flutter	213 (28.44)	274 (36.53)	334 (44.59)	<0.0001
Smoker	83 (11.08)	71 (9.47)	49 (6.54)	0.008
Medications				
ACEI/ARB	655 (87.45)	640 (85.33)	608 (81.17)	0.0028
Aldosterone antagonist	273 (36.45)	226 (30.13)	274 (36.58)	0.011
Aspirin	425 (56.74)	450 (60.00)	412 (55.01)	0.1397
β Blocker	659 (87.98)	662 (88.27)	652 (87.05)	0.7533
Calcium channel blocker	62 (8.28)	75 (10.00)	68 (9.08)	0.5108
Hydralazine	54 (7.21)	62 (8.27)	75 (10.01)	0.1449
Nitrate	112 (14.95)	110 (14.67)	135 (18.02)	0.1433
Statin	399 (53.27)	393 (52.40)	377 (50.33)	0.5051
Warfarin	231 (30.84)	275 (36.67)	345 (46.06)	<0.0001
Insulin	96 (12.82)	85 (11.33)	103 (13.75)	0.3642
Heart failure type				
Left ventricular ejection fraction	31.3 (30, 32.5)	29.1 (27.9, 30.2)	26 (25, 27.1)	<0.0001
HFrecEF	99 (13.49)	62 (8.32)	45 (6.07)	<0.0001
HFpEF	86 (11.72)	81 (10.87)	86 (11.61)	0.8567
HFrEF	549 (74.80)	602 (80.81)	610 (82.32)	0.0008
NYHA functional class				
1	298 (20.41)	298 (20.05)	172 (11.53)	<0.0001
2	718 (49.18)	692 (46.57)	574 (38.47)	
3	390 (26.71)	444 (29.88)	562 (37.67)	
4	54 (3.70)	52 (3.50)	184 (12.33)	

Numbers represent median (interquartile range), or count (%).

ACEI/ARB indicates angiotensin-converting enzyme inhibitor/angiotensin II receptor blocker; CABG, coronary artery bypass graft; eGFR, estimated glomerular filtration rate; HFpEF, heart failure with preserved ejection fraction; HFrecEF, heart failure with recovered ejection fraction; HFrEF, heart failure with reduced ejection fraction; MAGGIC, Meta-Analysis Global Group in Chronic Heart Failure; NTproBNP, N-terminal pro-B-type natriuretic peptide; and NYHA, New York Heart Association.

participants, lower proportion of Black participants, markedly lower estimated glomerular filtration rate, and lower proportion of participants on β blockers. Higher factor 2 scores were also positively associated with higher proportions of participants receiving aspirin, hydralazine, nitrate, statin, and insulin. It

Table 2. Association of Clinical Characteristics With Tertiles of Senescence Factor Scores for Senescence Factor 2

Baseline characteristics	Lower tertile	Middle tertile	Upper tertile	*P* value
Demographic factors				
Age, y	46.7 (45.9–47.6)	56.3 (55.2–57.4)	61.7 (60.5–62.8)	<0.0001
Male sex	448 (60.05)	510 (68.18)	525 (70.85)	<0.0001
Race				
White	502 (67.02)	583 (77.73)	556 (74.23)	<0.0001
Black	199 (26.57)	127 (16.93)	145 (19.36)	
Asian	11 (1.47)	8 (1.07)	2 (0.27)	
Other	37 (4.94)	32 (4.27)	46 (6.14)	
Physical exam/labs				
Systolic blood pressure (mmHg)	114 (113, 116)	113 (112, 115)	111 (110, 113)	0.0084
Diastolic blood pressure (mmHg)	71.4 (70.6, 72.3)	69.3 (68.5, 70.1)	65.5 (64.7, 66.3)	<0.0001
Body mass index (Kg/m^2)	29.6 (29.1, 30.1)	29.8 (29.3, 30.3)	28.8 (28.3, 29.2)	0.0075
eGFR (mL/min/1.73m^2)	69.7 (67.7, 71.7)	56.9 (55.3, 58.5)	36.1 (35.1, 37.2)	<0.0001
NTproBNP	797 (740, 855)	781 (725, 838)	1161 (1077, 1246)	<0.0001
MAGGIC risk score	12.2 (11.7, 12.6)	15.4 (14.9, 16)	21.6 (20.8, 22.3)	<0.0001
Medical history				
Diabetes mellitus	126 (16.82)	210 (28.00)	306 (40.85)	<0.0001
History of stenting	117 (15.62)	170 (22.67)	211 (28.17)	<0.0001
History of CABG	65 (8.68)	141 (18.80)	211 (28.17)	<0.0001
History of atrial fibrillation/flutter	201 (26.84)	259 (34.53)	361 (48.20)	<0.0001
Smoker	64 (8.54)	78 (10.40)	61 (8.14)	0.2666
Medications				
ACEI/ARB	666 (88.92)	664 (88.53)	573 (76.50)	<0.0001
Aldosterone antagonists	237 (31.64)	269 (35.87)	267 (35.65)	0.1529
Aspirin	379 (50.60)	452 (60.27)	456 (60.88)	<0.0001
Beta blocker	679 (90.65)	657 (87.60)	637 (85.05)	0.0041
Calcium channel blocker	66 (8.81)	65 (8.67)	74 (9.88)	0.6725
Hydralazine	35 (4.67)	48 (6.40)	108 (14.42)	<0.0001
Nitrate	79 (10.55)	100 (13.33)	178 (23.77)	<0.0001
Statin	348 (46.46)	405 (54.00)	416 (55.54)	0.0008
Warfarin	213 (28.44)	298 (39.73)	340 (45.39)	<0.0001
Insulin	54 (7.21)	91 (12.13)	139 (18.56)	<0.0001
Heart failure type				
Left ventricular ejection fraction	28.6 (27.4, 29.8)	29.3 (28.1, 30.5)	28.2 (27, 29.3)	0.4014
HFrecEF	74 (10.01)	76 (10.20)	56 (7.61)	0.1600
HFpEF	72 (9.74)	84 (11.28)	97 (13.18)	0.1148
HFrEF	593 (80.24)	585 (78.52)	583 (79.21)	0.7126
NYHA functional class				
1	354 (23.82)	312 (20.97)	102 (6.97)	<0.0001
2	768 (51.68)	642 (43.15)	574 (39.21)	
3	322 (21.67)	454 (30.51)	620 (42.35)	
4	42 (2.83)	80 (5.38)	168 (11.48)	

Numbers represent median (interquartile range), or count (%).

ACEI/ARB indicates angiotensin converting enzyme inhibitor/angiotensin receptor blocker; CABG, coronary artery bypass graft; eGFR, estimated glomerular filtration rate; MAGGIC, Meta-Analysis Global Group in Chronic Heart Failure; NTproBNP, N-terminal pro-B-type natriuretic peptide; and NYHA, New York Heart Association.

is worth noting that the proportion of patients with HFpEF did not significantly differ across factor tertiles for both factors.

Survival Analysis

We built Cox proportional hazards regression models to examine the relationship between both factors and

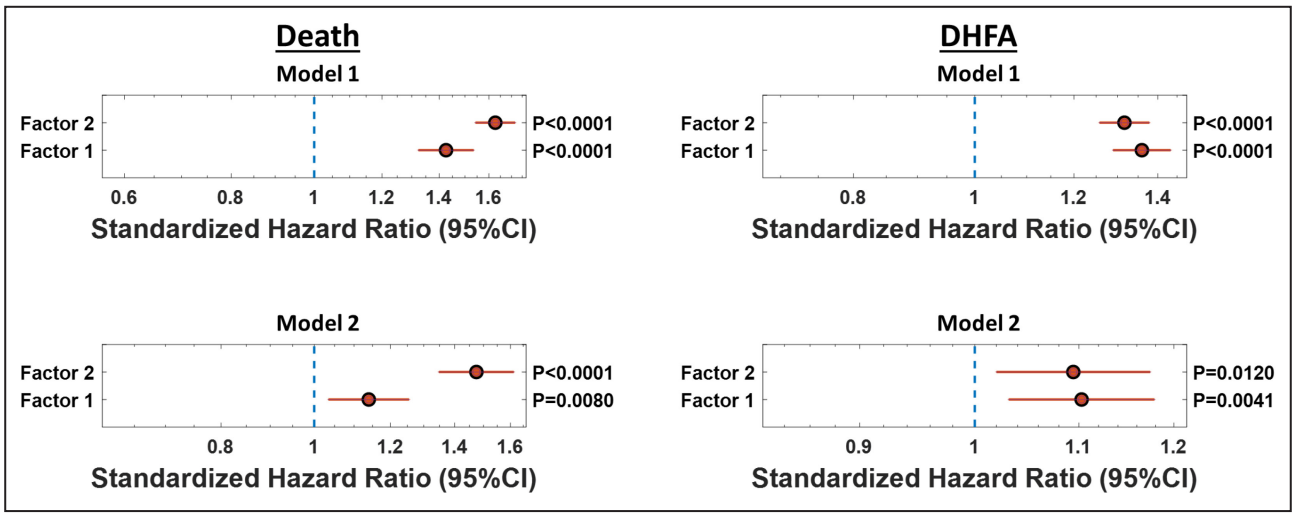

Figure 2. Box and whisker charts of Cox proportional hazards regression models for death and DHFA of (1) nonadjusted factors of senescence (model 1) and (2) factors of senescence adjusted for MAGGIC risk score and NT-proBNP (model 2).
In model 1, the association of each factor with risk of adverse outcomes was assessed separately; factors were not included within the same model. In model 2, adjustments were also done on each factor separately. DHFA indicates death or heart failure–related hospital admission; MAGGIC, Meta-Analysis Global Group in Chronic Heart Failure; and NT-proBNP, N-terminal pro-B-type natriuretic peptide.

the outcome measures of death and DHFA (Figure 2). In model 1, the association of each factor with the risk of adverse outcomes was assessed separately (ie, factors were not adjusted for one another). In model 2, adjustments were also done on each factor separately. Both factors were significantly associated with the risk of death (factor 1: HR, 1.42 [95% CI, 1.32–1.53]; *P*<0.0001; factor 2: HR, 1.63 [95% CI, 1.54–1.72]; *P*<0.0001) and DHFA (factor 1: HR, 1.36 [95% CI, 1.29–1.43]; *P*<0.0001; factor 2: HR, 1.32 [95% CI, 1.26–1.38]; *P*<0.0001) as seen in model 1. After adjustment for both the MAGGIC score and NTproBNP levels (model 2), both factors remained associated with death (factor 1: HR, 1.14 [95% CI, 1.03–1.25]; *P*=0.008; factor 2: HR,

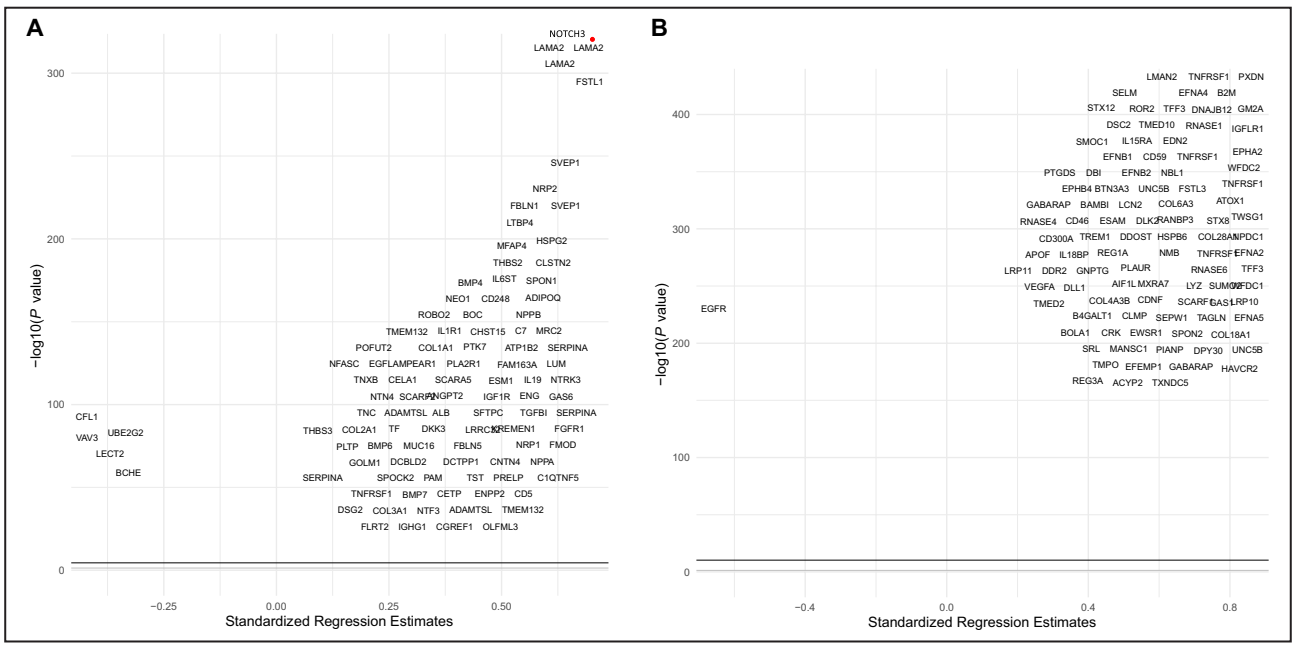

Figure 3. Volcano plots showing proteins that are significantly associated with (A) first factor of senescence (B) second factor of senescence.
Lower faint solid line represents an α level of 0.05. Upper solid line represents an αlevel corrected for multiple comparisons. Only the top 100 aptamers are shown.

1.47 [95% CI, 1.35–1.61]; *P*<0.0001) and DHFA (factor 1: HR, 1.10; [95% CI, 1.03–1.18]; *P*=0.004; factor 2: HR, 1.09 [95% CI, 1.02–1.17]; *P*=0.012).

We also built Kaplan–Meier survival curves to examine the probability of death and DHFA across tertiles of factors 1 and 2 (Figure S3). There was a significantly increased probability of death and DHFA in higher tertiles of both factors.

As shown in Table S2, the incorporation of the SASP factors into a base model assessing the association of the MAGGIC risk score and NT-proBNP with risk of death significantly increased the concordance index of the model. Similarly, an increase in the model concordance index was observed upon incorporating SASP factor 1 into a base model assessing the association of the MAGGIC risk score and NT-proBNP with the risk of DHFA. In contrast, there was no significant increase in the concordance index upon adding factor 2 to a base model aiming to predict risk of DHFA.

We conducted additional supplemental analyses that adjusted for age, sex, AF, estimated glomerular filtration rate, MAGGIC risk score, and NT-proBNP. The results of these analyses are shown in Figure S4. After including age, sex, history of AF, estimated glomerular filtration rate, and NT-proBNP in the survival models, both factors of senescence retained their significant association with both death and DHFA.

Association Analysis With Other Proteins and Pathway Analyses

Figure 3A shows a volcano plot demonstrating the correlation of SASP factor 1 with other measured proteins. Factor 1 was significantly associated with 2356 aptamer-based protein levels. The top 5 significant proteins were NOTCH3 (neurogenic locus notch holomog protein-3) (), laminin subunit alpha-2, FSTL1 (follistatin-related protein-1), FBLN-1 (fibulin-1), and MFAP4 (microfibril-associated glycoprotein-4). All mentioned proteins exhibited a significant positive correlation with factor 1. Figure 3B shows a volcano plot showing proteins significantly correlated with factor 2. Factor 2 was significantly associated with 3445 aptamer-based protein levels. The top 5 significant proteins were tumor necrosis factor receptor superfamily-1, β_2-microglobulin, peroxidasin homolog (PXDN), DNAJ homolog subfamily B member 12, and ganglioside GM2 activator. All mentioned proteins exhibited a significant positive correlation with factor 2.

Figure 4 shows the top canonical pathways for the proteins that are significantly associated with both factors. Proteins that correlated with factor 1 exhibited a significant association with pathways related to coagulation (coagulation system, glycoprotein-6 signaling, and intrinsic and extrinsic prothrombin pathway), glucose and lipid metabolism (liver X receptor [LXR]/ retinoid X receptor [RXR], Farnesoid X receptor [FXR]/RXR, and 24-dehydrocholesterol reductase signaling pathways), inflammation (phagosome formation and osteoarthritic pathway), and other pathways (axonal guidance signaling, rho-GDP-dissociation inhibitor signaling, sperm motility, and macropinocytosis, hepatic fibrosis, and integrin signaling). Among these, rho-GDP-dissociation inhibitor signaling exhibited a significant positive association with factor 1, whereas phagosome formation and integrin signaling exhibited a negative one. The rest of the pathways had a significant association with factor 1 with indeterminate directionality.

Factor 2 exhibited a significant association with pathways related to coagulation (coagulation system), glucose and lipid metabolism (LXR/RXR, FXR/RXR, and 24-dehydrocholesterol reductase signaling pathways), inflammation (role of Janus kinase 2 in hormone-like cytokine signaling, pathogen-induced cytokine storm signaling, and T-helper 1/2 activation pathways), and other pathways (actin-binding–rho-activating protein signaling, growth$_2$/mitosis DNA damage checkpoint regulation, and retinoate biosynthesis-2). Among these, the LXR/RXR activation pathway exhibited a significant negative correlation with SASP factor 2, whereas growth$_2$/mitosis DNA damage checkpoint regulation pathway exhibited a significant positive association with this factor. The rest of the pathways had a significant association with factor 1 with indeterminate directionality.

We conducted sensitivity analyses assessing the proteomic and biologic correlates of each of our factors of senescence after adjusting for age, sex, and estimated glomerular filtration rate. As illustrated in Figure S5, we found an association between senescence factors and pathways linked to glucose and lipid metabolism (such as LXR/RXR, glucose transporter type 4 translocation, and 24-dehydrocholesterol reductase), pathways associated with coagulation (eg, coagulation system, glycoprotein 6 activation, prothrombin activation, fibrin clot formation), and inflammation (eg, phagosome formation, osteoarthritis, complement system activation, granulocyte adhesion, acute-phase response signaling), in line with our initial findings.

DISCUSSION

In this study, we assessed the prognostic significance of 25 known SASP biomarkers in HF using a proteomic approach (measurements of ≈5000 plasma proteins) to identify molecules and canonical pathways associated with senescence in HF. We identified 2 underlying factors that represented common variability of multiple plasma senescence–associated proteins. These factors were associated with multiple important clinical phenotypes and cardiovascular comorbidities,

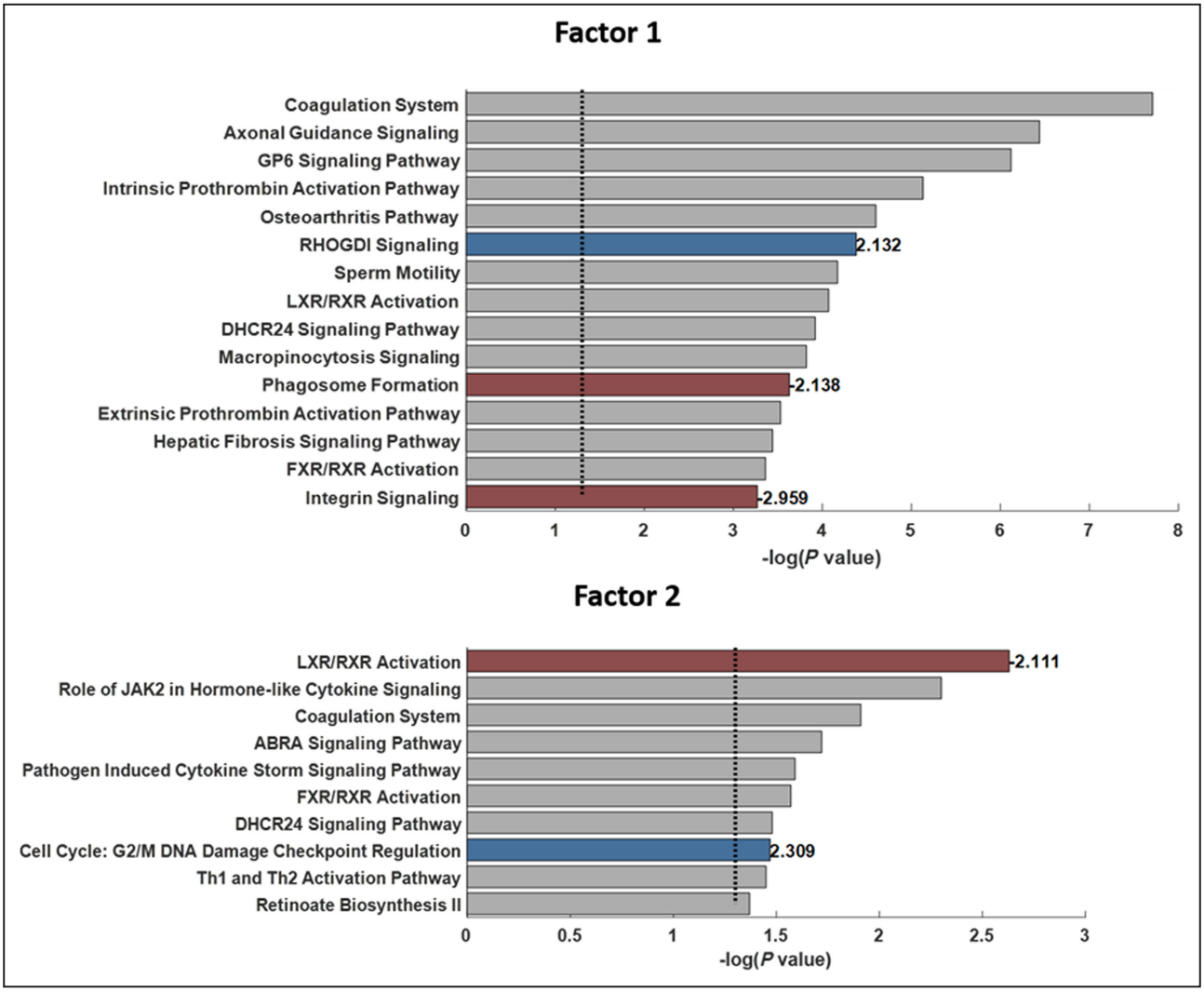

Figure 4. Pathways significantly correlated with (top) first factor of senescence factor scores and (bottom) second factor of senescence factor scores.

Pathways in gray indicate a significant association with indeterminate direction. Blue bars indicate a significant positive association. Red bars indicate a significant negative association. Numbers at the end of the bars indicate the *Z* score corresponding to direction and strength of association. ABRA indicates Actin-Binding Rho-activating Protein; DHCR24, 24-Dehydrocholesterol Reductase; FXR, Farnesoid X receptor; G2/M, growth$_2$/mitosis; GP6, glycoprotein 6; LXR, liver X receptor; RHOGDI, rho-GDP-dissociation inhibitor; RXR, retinoid X receptor; and Th1/2, T-helper cell 1/2.

including older age, male sex, lower blood pressure, lower body mass index, the presence of diabetes, AF/atrial flutter, and high New York Heart Association functional class, NT-proBNP, and MAGGIC risk scores. Interestingly, the proportion of HFpEF did not vary across senescence factor tertiles, but 1 of the senescence factors was associated with a lower proportion of participants with HF with recovered ejection fraction and a higher proportion of participants with HF with reduced ejection fraction. We also report that both senescence factors are significantly associated with adverse events in HF, independent of the MAGGIC risk score and NT-proBNP. Finally, we identified plasma proteins and biologic pathways associated with these factors of senescence in HF, mostly related to inflammation, immunity, coagulation, and lipid and glucose metabolism. Our findings add to the body of evidence demonstrating the relevance of cell senescence in human HF, and identify clinical and biologic correlates of senescence in this population.

Although cell senescence is a distinct cellular state, the molecular heterogeneity of the secretory proteome from senescent cells impedes reliance on individual SASP biomarkers.[5] Basisty et al demonstrated the high degree of complexity and variability of SASP in the context of different cell types and senescence inducers.[8] To obtain a more robust set of indicators of cell senescence, we therefore chose proteins that[1] were

reported to be components of the SASP in human lung fibroblasts and renal cortical epithelial cells exposed to 3 different inducers of senescence (irradiation, inducible Ras overexpression, and atazanavir [an HIV protease inhibitor known to induce cell senescence]), as reported by Basisty et al[8] and were found to be associated with chronological age in humans,[2] as measured in plasma with the same method used in our study (SomaScan platform).[9] We then used factor analysis to extract factors that underlie common variability in these senescence indicators. Our study is the first to assess the clinical, proteomic, and biologic correlates of senescence, as well as its prognostic significance, in human HF, using a comprehensive combination of SASP biomarkers.

There is a growing body of evidence highlighting the role of cellular senescence in the pathophysiology of age-related heart disease, including cardiac hypertrophy, fibrosis, and HF.[1,14–19] SASP components secreted from senescent cardiac cell types (such as senescent endothelial cells or cardiomyocytes) has been shown to cause fibroblastic activation and differentiation, cardiomyocyte apoptosis and hypertrophy, and endothelial dysfunction.[18,20] In addition, various studies have linked cellular senescence with well-known pathophysiological mechanisms involved in HF, such as mitochondrial dysfunction, renin–angiotensin–aldosterone pathway activation, and autophagic downregulation.[1] A prior study in participants with HFpEF showed a significant positive association of plasma insulin-like growth factor–binding protein-7, a single SASP component (which was also included as a senescence indicator in our study), with diastolic dysfunction and left atrial dilation.[14] Adamson et al, demonstrated a significant association of insulin-like growth factor–binding protein-7 with more advanced New York Heart Association functional class and lower quality of life in HF with reduced ejection fraction, as well as a significant positive association with the risk of HF hospitalization and cardiovascular death independent of NT-proBNP levels and other factors.[15] In senescent-accelerated murine models, endothelial dysfunction was apparent at 24 weeks and when given a high-salt and high-fat diet, these mice developed an HFpEF phenotype.[19]

In addition to establishing an association between cell senescence and the risk of adverse outcomes in our study population, we assessed its relationship with a broad set of plasma proteins and associated biologic pathways. Among the top proteins significantly associated with senescence, FSTL1, NOTCH3, FBLN1, MFAP4, and PXDN have all been associated with cardiovascular risk factors and myocardial and vascular remodeling. FSTL1 is a secreted glycoprotein that acts by neutralizing activins, which are part of transforming growth factor-β family and are involved in various biological processes such as inflammation, fibrosis, proliferation, and differentiation.[21] FSTL1 appears to play a protective role in HF.[21–23] For instance, in murine HFpEF models, cardiomyocyte-specific expression of FSTL1 attenuated myocardial hypertrophy and diastolic dysfunction.[22] In contrast with the murine models, in participants with HF with reduced ejection fraction, serum FSTL1 levels correlated with left ventricular hypertrophy.[23] We found a positive correlation between senescence and FSTL1 in our study, but the causal relationship between senescence and FSTL1 biology in HF remains unknown and requires further investigation.

NOTCH3 is involved in adaptive cardiovascular remodeling in response to pressure overload and has been found to act as a tumor suppressor by inducing senescence in human tumor cell lines.[24–27] NOTCH receptors and their ligands are a group of evolutionary conserved signaling molecules involved in various processes like cellular differentiation, proliferation, and apoptosis.[25] NOTCH3 is predominantly expressed in the vascular smooth muscle cells (VSMCs).[25] Its deletion in angiotensin II–induced hypertensive mouse models showed significantly increased rates of decompensated HF and death as well as reduced vascular medial hypertrophy, VSMC differentiation, and angiogenesis.[26] On the other hand, Jui et al demonstrated an increased expression of NOTCH3 in VSMCs driving aortic hypermuscularization in murine models in response to elastin deficiency.[27] However, whether age-related medial elastin degradation affects VSMC NOTCH3 expression and whether NOTCH3 affects aortic structure remains unclear. NOTCH3 gene mutations cause cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy, and NOTCH3 has been proposed to contribute to cognitive decline in aging and Alzheimer disease.[28] The significance of circulating NOTCH3 regarding organ-specific phenotypes in human HF requires further study.

FBLN1 exhibited a significant positive correlation with senescence. FBLN1 appears to play an important role in arterial stiffening, a key aging phenotype and a known mediator of adverse myocardial remodeling, diastolic dysfunction, and microvascular target organ damage.[29,30] FBLN1 is an extracellular matrix and secreted glycoprotein that associates with extracellular matrix components (like elastin, fibronectin, and basement membranes) and is expressed throughout the arterial wall; mainly in association with the external elastic lamina in the outermost layer of the tunica media.[30,31] Multiple studies have demonstrated a significant positive association of serum FBLN1 with markers of arterial stiffness; for example, Luo et al showed that serum FBLN1 exhibited a significant positive correlation with ankle–brachial pulse wave velocity independent of age and blood pressure.[29] Whether FBLN1 is causally involved in the association between cell senescence and aortic stiffness remains to be determined.

We reported that MFAP4 exhibited a significant positive correlation with cellular senescence. MFAP4 is a ubiquitous protein predominantly found in elastin-rich regions and blood vessel walls of elastin-rich organs like the lung, heart, and skin. It associates with elastin microfibrils and is involved in elastogenesis.[32] MFAP4 has been proposed to play a role in various age-related conditions, including renal fibrosis, left atrial fibrosis, lung aging, and skin photoaging.[32] The role of MFAP4 in myocardial disease remains unclear. Wang et al demonstrated a significantly higher expression of cardiac MFAP4 in murine pressure overload models as compared with non–pressure overload controls. Interestingly, Wang et al showed that MFAP4 deficient mice exhibited significantly better systolic function, lower interstitial myocardial fibrosis, and lower expression of multiple fibrotic markers after being subjected to pressure overload as compared with MFAP4 sufficient pressure overload controls.[33] In contrast, Dorn et al showed that MFAP4 deficient mice exhibited an increased maladaptive hypertrophic response in the setting of pressure overload as compared with MFAP4 sufficient pressure overloaded controls.[34] Notably, in both studies, MFAP4 deficiency did not have any effect on myocardial hypertrophy and fibrosis in non–pressure overload murine controls, indicating that MFAP4 might act specifically in the setting of increased left ventricular pressure and/or myocardial wall stress.

We reported that PXDN, which was shown to be involved in endothelial cell senescent transformation in diabetic rats,[35] exhibited a significant positive correlation with senescence in our study. PXDN is a peroxidase that uses H_2O_2 as a substrate to form sulfilimine crosslinks in uncrosslinked collagen type IV within the basement membrane of the extracellular matrix. It is expressed across multiple cell types, especially in cardiovascular tissue.[36] Interestingly, a recent study by Chan et al showed that PXDN inhibited macroautophagy in insulin-resistant cardiomyocytes and exhibited an increased expression in those that were apoptotic.[37] Macroautophagy is a highly conserved evolutionary mechanism that is involved in protein and organelle quality control,[38] which plays a protective role against maladaptive cardiac remodeling, deterioration of cardiac function, and myocardial fibrosis in the setting of pressure overload, cardiac aging, and diabetic cardiomyopathy.[38] Notably, macroautophagy has been shown to be downregulated with normal aging[38] but the exact role of PXDN in this setting remains unclear. PXDN has been also shown to be implicated in VSMC proliferation and vascular remodeling in pressure-overload murine models, as well as myocardial hypertrophy.[36,39]

We found multiple biologic pathways related to senescence in HF. Among the top pathways that exhibited a significant correlation with senescence and were concordant across both factors were pathways related to glucose and lipid metabolism (LXR/RXR, FXR/RXR, and 24-dehydrocholesterol reductase pathways), coagulation, and inflammation. LXR/RXR is a heterodimeric complex that, upon oxysterol binding, attaches to regulatory regions of target genes related to lipid and glucose metabolism, cholesterol homeostasis, and inflammation.[40] Its activation has been shown to have multifaceted favorable effects on various HF-related comorbidities like hypertension, diabetes, and chronic kidney disease,[40] diastolic function and myocardial remodeling/fibrosis, cardiac inflammation, and metabolism.[41] LXR signaling has been also implicated in cellular senescence. Hayashi et al demonstrated that LXR signaling prevented human endothelial cell high-glucose–induced senescence in vitro.[42] Similarly, Dai et al showed that LXR agonism inhibited amyloid-induced inflammatory and senescent responses through inhibition of nuclear factor κ-light-chain enhancer of activated B cells, a family of conserved transcription factors involved in expression of a wide range of proinflammatory genes,[43] in cultured human retinal pigment epithelial cells.[44] Hence, LXR signaling, which we found to be negatively correlated with senescence, may play a role in inhibiting cellular senescent transition and associated inflammatory responses as well as mediating favorable cardiovascular effects. This potential causal effect in human HF remains to be assessed in future studies.

Similar to LXR/RXR, FXR/RXR is also a heterodimeric nuclear receptor and it binds to nuclear bile acids thereby influencing expression of genes related to glucose and lipid metabolism, bile acid homeostasis, and inflammation.[45,46] Although the role of FXR signaling in glucose tolerance and insulin sensitivity is conflicting and dependent on the specific model used, multiple studies have demonstrated better glycemic control and insulin sensitivity in several diabetic and obese models upon FXR activation.[45] FXR signaling has been also associated with atherogenesis across multiple murine studies; however, its effects seem to be sex specific with mixed results.[45] On the other hand, its role in hypertension is less controversial. FXR signaling has been shown to lower blood pressure through upregulation of endothelial nitric oxide synthase expression and downregulation of endothelin-1.[46] Moreover, FXR signaling has been found to increase nitric oxide bioavailability by upregulating the enzymatic degradation of asymmetric dimethylarginine, an inhibitor of endothelial nitric oxide synthase–mediated nitric oxide production, which increases oxidative stress and has been shown to be associated with cardiovascular risk.[46] FXR signaling has also been found to inhibit cellular senescent transition. More studies are required to further characterize the role of FXR in cardiovascular senescence and HF pathogenesis.

Interestingly, we found growth$_2$/mitosis DNA damage checkpoint regulation pathway to exhibit a significant positive correlation with senescence. Although senescence has been classically known to induce cell cycle arrest at growth$_1$/synthesis, recent evidence indicates that the cellular senescent transformation also occurs at the growth$_2$/mitosis checkpoint.[47] However, it should be noted that plasma proteomic signatures may not adequately reflect intracellular pathways such as DNA damage checkpoint regulation, and our findings should be interpreted with caution.

Finally, senescence was also found to be associated with multiple inflammatory pathways and the coagulation system. Although there is considerable variability among SASP components across different cell types and inducers of senescence, inflammatory mediators are a known common denominator to SASPs that facilitate its biological function and perpetuate its pathogenic effects.[5]

An important issue is whether specific SASP proteins could be therapeutically targeted, and if doing so would be beneficial. Multiple pharmacologic interventions that preferentially induce apoptosis in senescent cells have been developed and tested in murine models as well as early-phase clinical trials.[48] These include agents that target members of the B-cell lymphoma 2 family, a family of antiapoptotic proteins upregulated in senescent cells.[48] In aged murine models, navitoclax eliminated senescent cardiomyocytes which subsequently attenuated fibrotic and hypertrophic myocardial remodeling.[18] Moreover, Zhu et al demonstrated that the combination of dasatinib, a tyrosine kinase inhibitor, and quercetin, an antioxidative agent, decreases the number of senescent cells and improves cardiac and vascular endothelial function in aged murine models.[49] This combination has been shown to be tolerated and to significantly improve functional parameters in a pilot study among participants with idiopathic pulmonary fibrosis.[50] Another pilot study suggested that dasatinib and quercetin reduce senescent cell burden in adipocytic tissue among participants with diabetic kidney disease.[51] Whether targeting cell senescence can exert beneficial clinical effects in human HF remains to be determined.

STRENGTHS AND LIMITATIONS

Our study should be interpreted in the context of its strengths and limitations. Strengths of our study include the assessment of well-characterized HF cohorts with rigorous adjudication of incident events, the use of biomarkers with a continuous nature, enhancing the power to detect associations with clinical factors and other proteins, the use of a broad proteomics platform to assess ≈5000 plasma protein measurements, and the use of factor analysis to extract the common variability of SASPs. Our comprehensive multimarker approach using the common variability of 25 known senescence biomarkers, which is a more likely to represent cell senescence as a biologic process, is also a strength of our study. Our study also has a number of significant limitations. Our findings are observational and do not assess causality. In an effort to assess senescence-associated proteins commonly elicited across both mesenchymal and epithelial cell types as well as various inducers of senescence, we used plasma proteomic signatures identified in vitro from both epithelial cells and lung fibroblasts exposed to various inducers of senescence, using the same proteomic platform used in our study. However, we acknowledge that assessment of senescence via plasma proteomics may lack organ specificity and should be interpreted cautiously. Finally, our study aimed to assess the clinical relevance of cell senescence in human heart failure by assessing its relationship with clinically important outcomes, as well as to identify potential related biologic pathways. However, further mechanistic studies are required to characterize pathways through which cell senescence may impact outcomes in heart failure, as well to assess the potential clinical impact of targeting senescence with therapeutic interventions.

CONCLUSIONS

Cellular senescence, assessed using a representative combination of senescence-associated proteins, exhibits a significant positive association with the risk of adverse events in human heart failure. Cellular senescence was significantly associated with lower blood pressure, lower use of antihypertensive medications, lower body mass index, worse functional status, and increased prevalence of AF, among other clinical correlates. It was also found to be associated with proteins related to myocardial and vascular remodeling, as well as pathways related to glucose and lipid metabolism, coagulation and inflammation. Further studies are needed to assess whether cell senescence or individual SAPS components represent suitable therapeutic targets in HF.

ARTICLE INFORMATION

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Supplemental Material

Data S1

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