Adolescents and young adults with sickle cell disease exhibit accelerated aging with elevated T-cell p16^{INK4a} expression

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ABSTRACT

People living with sickle cell disease (SCD) experience complications indicative of an accelerated aging phenotype typified by early decline in physical function and increased risk for age-related conditions. Cellular senescence, measured by expression of p16^{INK4a} in peripheral T-lymphocytes, is recognized as one of the underlying contributors to organismal aging. To examine if cellular senescence is increased in SCD patients, we cross-sectionally measured and compared expression of p16 mRNA in peripheral blood T lymphocytes in 18 adolescents and young adults with SCD to 27 similarly aged individuals without SCD. Expression of p16 was dramatically higher in individuals with SCD vs. without SCD (10.1 vs. 8.7 log$_2$ p16 units, respectively, $p < 0.001$) — a gap of 43 years in biological age — consistent with accelerated aging in the SCD population. Race was not associated with the increased p16 expression in the SCD group. These initial results suggest that individuals with SCD have a significantly higher cellular senescence burden which may contribute to premature aging, physiological decline, and excess morbidities. Additional longitudinal assessment and consideration for trials of senolytic therapies among individuals living with SCD and high p16 expression are warranted to improve their health span.

INTRODUCTION

Life expectancy for individuals with sickle cell disease (SCD) is significantly lower than that of the general population; however, with advances in medical care, life expectancy has markedly improved over the past three decades in resource-rich countries [1]. Despite these gains, adolescents and adults with SCD still experience higher rates of aging-related morbidity and early mortality [2–4]. Individuals with SCD disproportionately experience functional decline, frailty, and increased rates of premature end-organ damage and

Table 1. Baseline characteristics of the study population.

	SCD (*n* = 18)	Non-SCD Comparators (*n* = 27)
Age (years), median (range)	22 (15–27)	20 (17–29)
Gender, *n* (%)		
Female	11 (61%)	20 (74%)
Male	7 (39%)	7 (26%)
Genotype, *n* (%)		
HbSS	16 (89%)	
HbSβ^0-thalassemia	2 (11%)	
WBC count (10^9/L), median (IQR)	11.3 (8.2–13.8)	
Hemoglobin (g/dL), median (IQR)	9.1 (8.7–9.6)	
Platelet count (10^9/L), median (IQR)	356 (282–477)	
Absolute reticulocytes (10^9/L), median (IQR)	223 (172–281)	
Absolute neutrophils (10^9/L), median (IQR)	6.5 (4.6–8.2)	
Absolute lymphocytes (10^9/L), median (IQR)	3.1 (1.9–3.5)	
On hydroxyurea, *n* (%)	10 (56%)	
Hydroxyurea dose[*] (mg/kg)	19 (16–21)	
Time on hydroxyurea[*] (months)	119 (64–140)	
On chronic transfusions, *n* (%)	8 (44%)	
Time on chronic transfusions[*] (months), median (IQR)	59 (6–120)	

[*]Includes only participants receiving this therapy.

malignancies [4–8]. While causes of these aging-related complications have not been elucidated, the presence of these complications suggests that many individuals with SCD experience accelerated aging, particularly in the bone marrow, possibly in response to chronic hypoxia, hemolysis and inflammation associated with a lifetime of ineffective erythropoiesis [3, 9, 10]. Earlier identification of individuals with SCD at greater risk for such age-related (and potentially life-threatening complications) is critical in improving long term outcomes and life expectancy in SCD.

Cellular senescence, a hallmark of biological aging, has been identified as a key contributor to functional decline and onset of aging-related diseases [11, 12]. Cells that undergo senescence are permanently cell-cycle arrested but remain metabolically active, secreting pro-inflammatory signals. Cellular senescence in the immune system has been shown to promote cellular senescence throughout organs and tissues and cause functional decline [13]. Expression of p16^{INK4a} (p16) is a reliable biomarker of cellular senescence [14]. In humans, increased expression of p16 mRNA in peripheral blood T lymphocytes (PBTL) is associated with well-established pro-aging exposures such as tobacco use, physical inactivity, chemotherapy, and radiation, and genetic alterations in the p16 locus have been associated with aging-related conditions in humans such as frailty, cardiovascular disease and type 2 diabetes [14, 15]. In adolescent and young adult

survivors of cancer, PBTL p16 expression is markedly increased in a treatment dose-dependent manner and higher expression is associated with frailty and sarcopenia [16]. Taken together, prior work suggests that increased cellular senescence, as measured by PBTL p16 expression, identifies accelerated biological aging [12, 14, 17]. Given SCD is associated with an accelerated aging phenotype [6], we hypothesized that p16 expression would be elevated in people with SCD.

The primary objective of this study was to measure p16 expression in adolescents and young adults (AYAs) living with SCD and compare them to a pre-existing sample of similarly aged individuals without SCD. Our hypothesis was that individuals with SCD would have higher p16 expression compared to the non-SCD comparators.

RESULTS

Participant characteristics

Twenty-one AYAs with SCD were consented for this study, with three participants excluded from the analysis due to failed sample quality control for the p16 assay. Eighteen participants and 27 comparators were included in the final analysis. Participant and available comparator characteristics are summarized in Table 1, and additional SCD patient characteristics are detailed in Supplementary Table 1. Due to the need to protect

the privacy of blood donors, there was no available demographic data beyond what is detailed in Table 1.

Expression of p16

Participants with SCD had significantly higher p16 expression vs. comparators −10.1 vs. 8.7 $\log_2$ p16 units. Based on prior analysis of the regression of p16 expression by chronological age, this mean difference of 1.3 $\log_2$ units (95% CI 0.79–1.9, $p < 0.001$) between the SCD and comparator groups may be as much as 43 years (95% CI 23–63 years) of increased biological aging (Figure 1A) [17]. These results were similar when stratifying by chronic transfusion therapy (CTT), a therapy for severe SCD complications such as stroke. The mean difference in p16 expression between the SCD participants on CTT and comparators was 1.3 $\log_2$ p16 units (10.0 vs. 8.7 $\log_2$ p16 units, $p = 0.002$), while the difference between the SCD participants not on CTT and comparators was 1.4 (10.1 vs. 8.7 $\log_2$ p16 units, $p = 0.001$) (Figure 1B).

Exploratory analysis

Within the SCD group, we explored associations between laboratory measures, SCD complications, and PROs. In this small sample, there were no associations between any of these measures and p16 expression (Supplementary Table 2). In the SCD group, p16 expression was higher throughout, regardless of age (Figure 2).

DISCUSSION

In this first assessment of PBTL p16 expression in SCD, we demonstrate that p16 expression, a marker of cellular senescence, is strikingly elevated in AYAs with SCD compared to non-SCD age matched comparators. The magnitude of this difference was unexpected, signifying an accelerated aging of up to 43 years in people with SCD, and is even larger than the difference between the AYA cancer survivors treated with pro-aging exposures (cytotoxic chemotherapy) and the same comparator group used in this study [16]. Our youngest participant, a 15-year-old with SCD, had a higher p16 expression than all the comparators, underscoring the early rise of p16 expression in this population. This work complements growing reports on accelerated aging in SCD [6, 18].

The etiology of the marked elevation in p16 expression in SCD is not yet known. Stress and ineffective erythropoiesis due to the hemolysis and increased RBC turnover, tissue hypoxia, anemia and chronic inflammation are all potential stressors to the bone marrow in people with SCD, which may manifest in accumulation of senescent cells and lead to accelerated organismal aging [9, 19].

To protect the privacy of the blood donors, the core of the comparator group, self-identified race of these participants is not available. We explored whether the
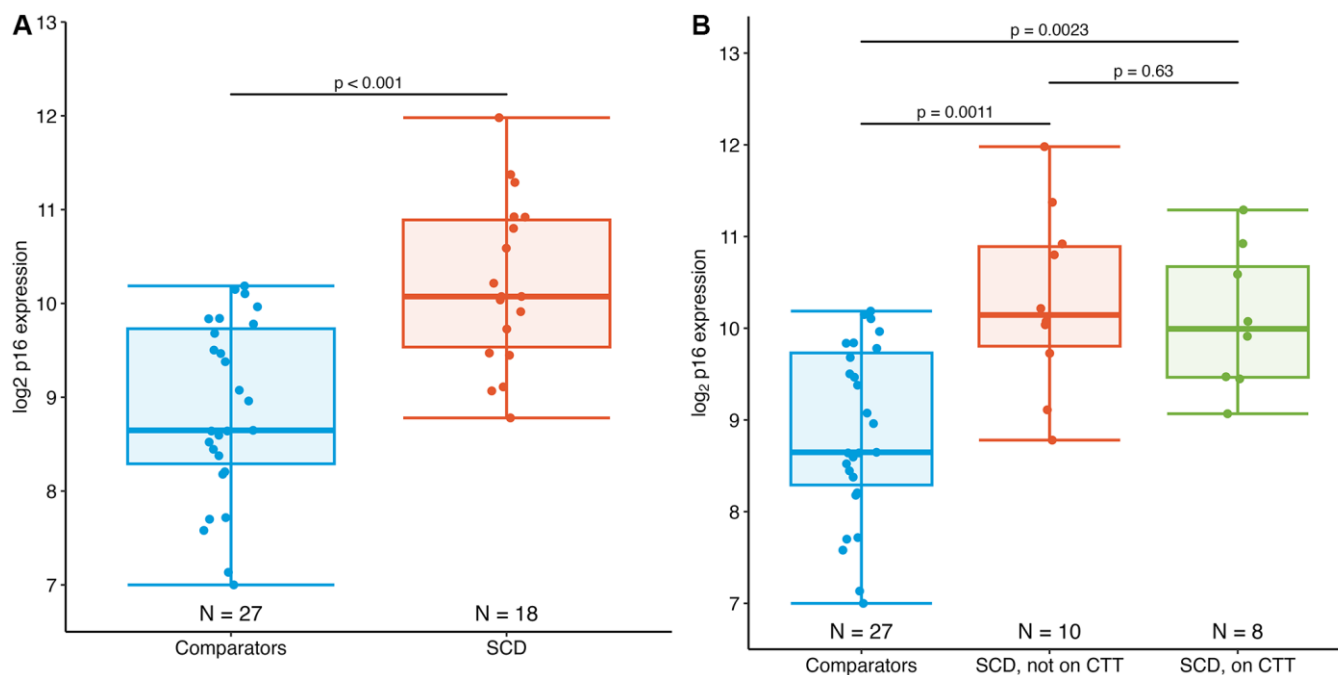

Figure 1. Expression of PBTL p16^{INK4a} in AYAs with SCD. (**A**) Mean p16 expression in AYAs with SCD (10.1 $\log_2$ p16 units) is significantly elevated compared to those without SCD (8.7 $\log_2$ p16 units), $p < 0.001$. (**B**) Expression of p16 in individuals with SCD did not differ by chronic transfusion therapy (CTT) status.

marked difference in p16 expression observed in this study between the two groups could be partially explained by racial differences, as >90% of individuals with SCD identify as Black/African-American. There is no convincing evidence that p16 expression varies by race. Prior studies have observed no difference in p16 expression between Black/African-American and Caucasian populations [15]. To further explore, we compared p16 expression in the SCD cohort to nine similarly-aged individuals who self-identified as Black/African-American from the previous AYA cancer survivor p16 study, all of whom had received prior chemotherapy [20]. This comparison showed higher mean p16 expression in the SCD cohort (10.1 vs. 8.8 $\log_2$ units, $p = 0.2$), albeit the difference was not statistically significant likely due to the small sample (see Supplementary Figure 1). Subgroup comparisons within the AYA cancer survivor cohort also showed no differences by race. We also observed no overall differences in p16 expression by race after combining participants from multiple datasets from prior p16 studies (see Supplementary Figure 2). Altogether, these findings support that race alone does not account for the surprisingly large differences in p16 expression observed in this study.

Lastly, we explored any association between p16 expression and clinical/PRO measures in SCD participants in this small SCD patient sample to yield insight into potential drivers and clinical significance. As shown in Supplementary Table 2, we did not detect an association between p16 expression and any of the assessed clinical and PRO measures. Additionally, there was no association between disease-modifying therapies such as hydroxyurea, chronic transfusion therapy and p16 expression. There are several potential explanations for these findings: (1) This study was underpowered to detect these associations, (2) the p16 expression observed is a predictive marker of future morbidity and thereby may be more suitable for a longitudinal cohort, or most likely (3) the widespread impact and burden of SCD on cellular senescence itself may be greater than these various markers of clinical severity, which often does not capture disease severity in SCD, which is poorly defined [21]. We anticipate that a minimum sample size of at least 80–90 SCD participants is necessary to have 80% power to detect these associations.

These findings must be interpreted in the setting of study limitations. A cross-sectional design and limited sample size may not capture the relationship between
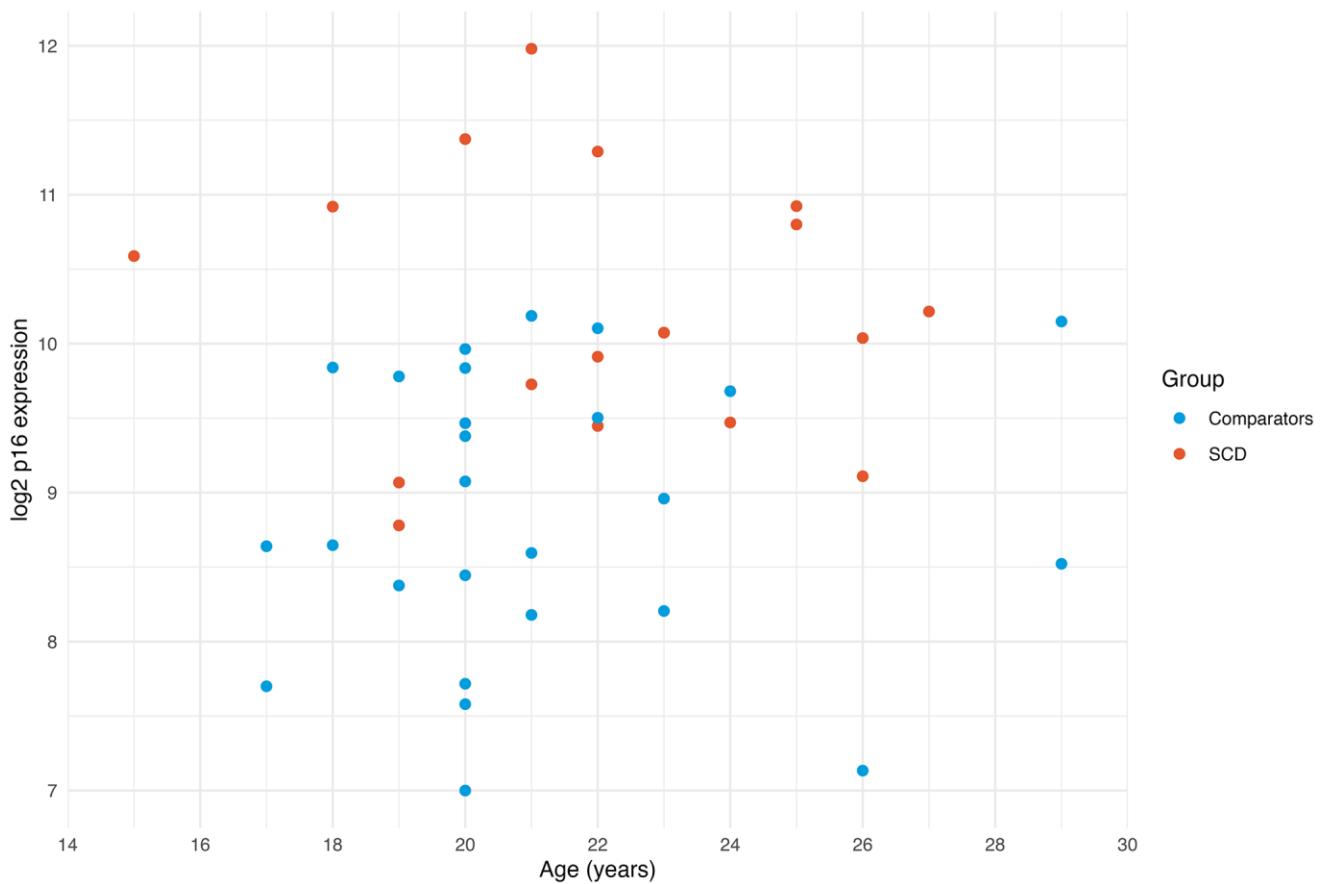

Figure 2. Expression of p16 by age. Scatterplot of p16 expression by age demonstrates that the SCD group generally has higher p16 expression across the entire age range. The youngest SCD participant has higher p16 expression than all the comparators.

p16 expression and treatments and clinical outcomes. A larger longitudinal study may be able to dissect causes of elevated p16 expression and may lead to interventional studies to address accelerated aging in SCD. Additionally, the bone marrow compartment in people living with SCD may be more prone to the accumulation of senescent cells, making PBTL p16 expression an incomplete reflection of organismal aging in this population. Thus, the age gap we found in this study may be an overestimate. Future studies should also examine the correlation of PBTL p16 expression with other senescence and immune markers from other body compartments in individuals with SCD.

CONCLUSIONS

We demonstrate that AYAs with SCD exhibit early and accelerated biological aging with markedly increased p16 expression, a marker of cellular senescence. While further prospective research is needed to establish the clinical implications and predictive utility for elevated p16 expression in patients with SCD, these initial findings provide molecular evidence of biological aging beginning at a young age in individuals living with SCD.

METHODS

Study design and participants

The study cross-sectionally measured p16 expression in both individuals with and without SCD. The study protocol was approved by the Institutional Review Board at the University of North Carolina (UNC). AYAs with SCD (HbSS or HbSβ^0-thalassemia genotypes only) ages 15 to 29 years receiving care at UNC SCD clinics from 2021–2022 were enrolled following informed consent and assent as appropriate. A 10mL peripheral blood specimen was collected for p16 analysis and participants completed patient-reported outcome (PRO) measures validated for use among people with SCD. We used a convenient sample of AYAs without SCD comparators that was previously recruited from 2018–2020 for an AYA cancer survivor study of p16 expression [16]. These comparators were either recruited from the UNC Platelet Donation Center (healthy individuals) or from the UNC Children's Hematology Clinic (seen for non-malignant, non-SCD disorders such as iron deficiency anemia) and provided a blood sample similarly to individuals with SCD.

Measures

For all participants, p16 expression was measured as previously described [14–16, 22]. Briefly, CD3+ cells were purified from whole blood and used to isolate total RNA. Expression of p16 was measured by a TaqMan RT-PCR (Sapere Bio, Research Triangle Park, NC, USA) and reported as log$_2$ units. Collection of key patient and treatment data was performed by medical record abstraction. In non-SCD samples where p16 expression was below the limit of detection, the value of 7 log$_2$ p16 units (lower limit of assay detection) was assigned. None of the SCD samples had p16 expression below the limit of detection.

Statistical analysis

Expression of p16 in log$_2$ units was compared among groups using 2-sided Student's t-tests. Other measures were compared to p16 expression with linear regression. Analysis was performed with R (R Foundation for Statistical Computing, Vienna, Austria). Statistical significance was defined as p-value < 0.05 with 95% confidence intervals (CI) reported for all tests. See Supplementary Methods for additional details.

Data sharing statement

De-identified data from this study will be shared upon reasonable request via email to the corresponding author.

AUTHOR CONTRIBUTIONS

S.R.W contributed to study design and execution, data analysis and interpretation, and wrote and revised the manuscript. N.M. contributed to p16 expression analysis and assisted in manuscript preparation. V.A.M. contributed to study design and execution, enrollment, and assisted in manuscript revision. A.B.S. and M.A.C. supervised the project and contributed to study conception and design, data analysis and interpretation, and critically revised the manuscript.

CONFLICTS OF INTEREST

M.A.C. is currently an employee of Cogent Biosciences, which provided no funding for this work. N.M. is a founder and a shareholder of Sapere Bio. The remaining authors have no other relevant conflicts of interest to disclose.

ETHICAL STATEMENT AND CONSENT

This study was approved by the Institutional Review Board at UNC, the protocol number IRB# 20-2196. All participants provided informed consent and assent.

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SUPPLEMENTARY MATERIALS

Supplementary Methods

Surveys

Following informed consent, participants aged 18 and over completed the (1) Adult Sickle Cell Quality of Life Measurement Information System (ASCQ-Me) surveys, including the Sickle Cell Medical History Checklist, Emotional Impact Computer Adaptive Test (CAT) v2.0, Pain Impact CAT v2.0, Social Functioning Impact CAT v2.0, Sleep Impact CAT v2.0, Stiffness Impact CAT v2.0, and Pain Episodes Frequency and Severity and (2) Patient-Reported Outcomes Measurement Information System® (PROMIS) 29 Profile v2.0. For participants under age 18, participants or guardians completed the PROMIS® Pediatric Profile v2.0 Profile-25, depending on patient or guardian preference. A peripheral blood specimen was then collected for p16 expression testing. Comparators were recruited from the UNC Platelet Donation Center and the UNC Children's Hematology Clinic (seen for non-malignant, non-SCD disorders such as iron deficiency anemia).

Measurement of p16 expression

p16 expression analysis was performed by Sapere Bio (Research Triangle Park, NC) using a previously described protocol [1–4]. Each run included positive and negative controls to monitor assay performance. Expression of p16 among age-matched comparators without SCD was collected in a prior study, as previously described [1]. As p16 expression is generally undetectable in healthy individuals under age 20 [5], comparator samples that had undetectable p16 expression were assigned the lower limit of detection of 7 $\log_2$ units for data visualization.

Other measures

SCD-related comorbidities, hydroxyurea dose, chronic transfusion therapy use, and laboratory measures were obtained through chart review from the electronic medical record. Laboratory measures such as white blood cell (WBC) count, hemoglobin, platelet count, absolute reticulocyte count (ARC), and absolute neutrophil count (ANC) were extracted from the most recent visit, usually the day of enrollment. SCD genotype was confirmed via chart review. Each of the ASCQ-Me and PROMIS surveys were scored per the corresponding manual, and the total score for each survey was used as a continuous measure. The standardized score for each of these surveys has a mean of 50 and a standard deviation of 10. For the

ASCQ-Me measures, higher scores suggest healthier or improved function, except for the Pain Episode Frequency and Severity measures in which higher scores signify increased pain burden [6]. For the PROMIS-29 profile, higher scores for Pain Interference, Depression, Fatigue, Anxiety, and Sleep Disturbance signify worse health, while higher scores for Physical Function and Ability to Participate in Social Roles and Activities correspond to improved function [7].
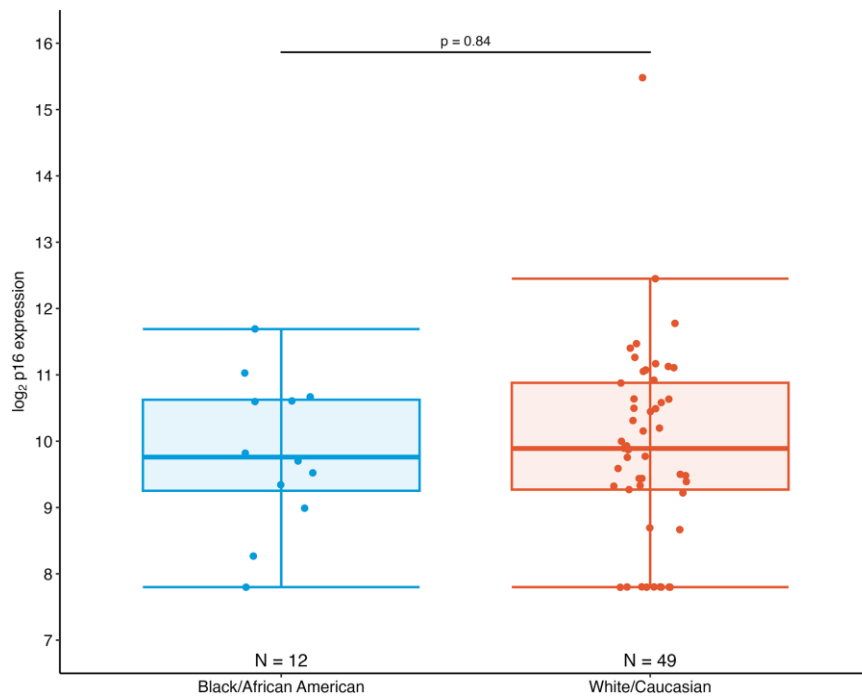
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Supplementary Figures
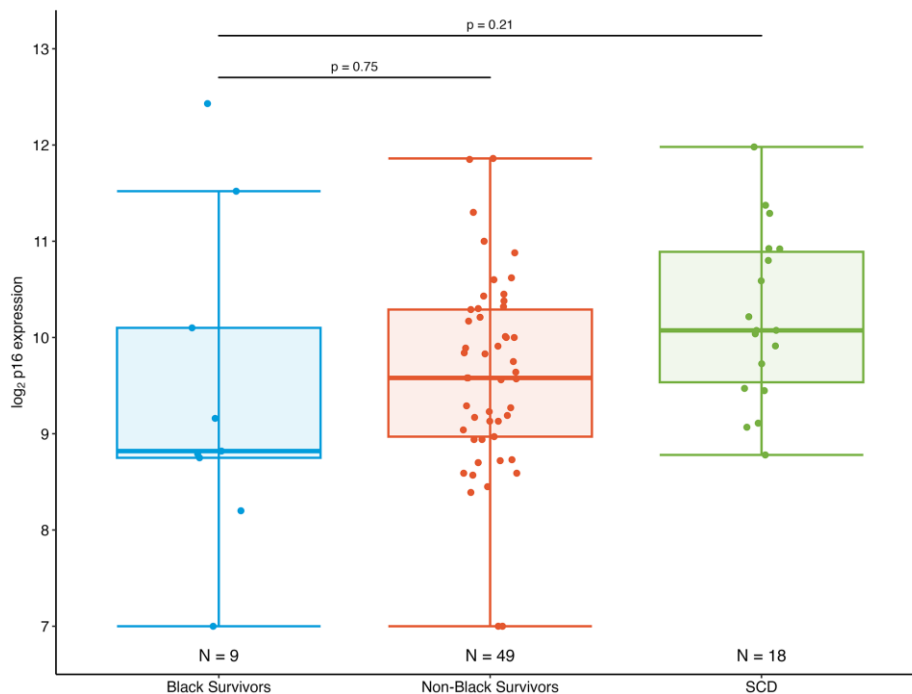
Supplementary Figure 1. Comparison of mean p16 expression in self-identified Black/African-American cancer survivors, non-Black survivors, and the sickle cell disease (SCD) groups show that individuals with SCD have a distinctively higher mean p16 expression (not significant). These results imply that race alone does not account for the higher mean p16 expression in SCD observed in this study.

Supplementary Figure 2. Cross-sectional compilation of similarly aged cohort either with cancer prior to therapy or healthy comparators demonstrate no difference in p16 expression between self-identified Black/African-American and White/Caucasians.

Supplementary Tables

Supplementary Table 1. Extended SCD participant characteristics.

Characteristic	Overall, N = 18	Not on CTT (N = 10)	On CTT (N = 8)
Age (years), median (range)	22 (15–27)	22 (18–27)	22 (15–25)
Gender, n (%)			
Female	11 (61%)	6 (60%)	5 (62%)
Male	7 (39%)	4 (40%)	3 (38%)
Genotype, n (%)			
HbSS	16 (89%)	9 (90%)	7 (88%)
HbSβ^0-thalassemia	2 (11%)	1 (10%)	1 (12%)
WBC count (10^9/L), median (IQR)	11.3 (8.2–13.8)	10.9 (8.1–13.3)	11.3 (10.2–13.8)
Hemoglobin (g/dL), median (IQR)	9.1 (8.7–9.6)	9.1 (8.5–9.2)	9.4 (8.9–10.0)
Platelet count (10^9/L), median (IQR)	356 (282–477)	306 (181–350)	472 (435–554)
Absolute reticulocytes (10^9/L), median (IQR)	223 (172–281)	199 (164–244)	259 (175–290)
Absolute neutrophils (10^9/L), median (IQR)	6.5 (4.6–8.2)	5.3 (3.6–7.1)	8.1 (6.2–8.2)
Absolute lymphocytes (10^9/L), median (IQR)	3.05 (1.9–3.5)	3.25 (2.8–3.6)	2.2 (1.8–3.4)
On hydroxyurea, n (%)	10 (56%)	8 (80%)	2 (25%)
Hydroxyurea dose[*] (mg/kg), median (IQR)	19 (16–21)	19 (18–22)	16 (14–18)
Time on hydroxyurea[*] (months), median, (IQR)	119 (64–140)	119 (50–135)	126 (105–147)
Time on CTT (months)[*], median (IQR)	59 (6–120)		59 (6–120)
History of acute chest syndrome, n (%)	15 (83%)	8 (80%)	7 (88%)
History of stroke, n (%)	4 (22%)	0 (0%)	4 (50%)
History of priapism, n (% of males)	4 (67%)	3 (75%)	1 (50%)
History of splenic sequestration, n (%)	9 (53%)	5 (56%)	4 (50%)
ASCQ-Me Measures, median (IQR)			
Emotional Impact	57 (50–64)	58 (56–66)	54 (49–59)
Pain Impact	59 (44–67)	67 (46–70)	48 (44–61)
Social Functioning Impact	61 (50–69)	63 (51–69)	61 (47–65)
Sleep Impact	55 (50–58)	55 (50–64)	56 (42–58)
Stiffness Impact	63 (49–66)	66 (64–70)	54 (47–59)
Pain Episode Frequency	52 (40–56)	54 (45–56)	44 (34–58)
Pain Episode Severity	45 (43–52)	45 (43–53)	50 (44–52)
PROMIS-29 Profile, median (IQR)			
Pain Interference	42 (42–61)	42 (42–56)	52 (42–63)
Depression/Sadness	49 (41–55)	41 (41–52)	51 (50–56)
Physical Function	48 (40–57)	57 (46–57)	43 (40–52)
Ability to Participate in Social Roles and Activities	52 (52–64)	55 (52–64)	52 (47–64)
Fatigue	49 (42–57)	45 (34–56)	49 (47–60)
Anxiety/Fear	51 (40–56)	40 (40–56)	52 (50–56)
Sleep Disturbance	53 (46–58)	52 (46–54)	53 (46–63)

[*]Includes only participants receiving this therapy. ASCQ-Me: Adult Sickle Cell Quality of Life Measurement Information System; PROMIS: Patient-Reported Outcomes Measurement Information System; CTT: chronic transfusion therapy.

Supplementary Table 2. Association between p16 and participant characteristics in the SCD group.

Characteristic	*N*	Beta	95% CI	*p*-value
Age	18	−0.03	−0.17–0.12	0.7
WBC count	18	0.07	−0.04–0.18	0.2
Hemoglobin	18	0.07	−0.54–0.68	0.8
Platelet count	18	0.00	0.00–0.00	0.5
Absolute Reticulocytes	17	0.00	0.00–0.01	0.6
Absolute Neutrophil count	18	0.11	−0.04–0.25	0.14
Absolute Lymphocyte count	18	0.02	−0.35–0.39	>0.9
Total Bilirubin	18	0.12	−0.07–0.30	0.2
Creatinine	18	−0.24	−1.3–0.82	0.6
On Hydroxyurea	18	0.25	−0.66–1.2	0.6
Hydroxyurea dose	10	−0.02	−0.12–0.08	0.7
Time on hydroxyurea therapy (months)	10	0.00	−0.02–0.01	0.6
History of acute chest syndrome	18	0.61	−0.56–1.8	0.3
History of stroke	18	−0.48	−1.5–0.58	0.4
ASCQ-Me Emotional Impact	17	−0.01	−0.05–0.04	0.8
ASCQ-Me Pain Impact	17	0.00	−0.03–0.04	0.8
ASCQ-Me Social Functioning Impact	17	−0.02	−0.05–0.02	0.4
ASCQ-Me Sleep Impact	17	0.01	−0.03–0.05	0.6
ASCQ-Me Stiffness Impact	17	0.00	−0.05–0.05	>0.9
ASCQ-Me Pain Episode Frequency	17	0.03	−0.02–0.07	0.2
ASCQ-Me Pain Episode Severity	17	0.00	−0.05–0.05	>0.9
PROMIS-29 Pain Interference	17	0.00	−0.04–0.05	0.9
PROMIS-29 Depression/Sadness	17	0.02	−0.03–0.07	0.3
PROMIS-29 Physical Function	17	−0.02	−0.08–0.04	0.4
PROMIS-29 Ability to Participate in Social Roles/Activities	17	−0.02	−0.08–0.03	0.4
PROMIS-29 Fatigue	17	0.00	−0.03–0.04	0.8
PROMIS-29 Anxiety	17	−0.01	−0.07–0.04	0.5
PROMIS-29 Sleep Disturbance	17	0.00	−0.04–0.04	0.9