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Tumor molecular differences associated with outcome disparities of Black patients with head and neck cancer

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Abstract

Background: Numerous studies of head and neck squamous cell carcinoma (HNSCC) have demonstrated disparate outcomes by race and ethnicity. Beyond known associations with socioeconomic variables, whether these are also associated with differences in tumor molecular composition has thus far been poorly explored.

Methods: We downloaded clinical and multi-platform molecular data from The Cancer Genome Atlas and other published studies. These were compared between non-Hispanic Black (n=43)

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Author contributions: HAJK, PYZ, AS, ACN, JWB, KP and PCB designed the study. HAJK, PYZ, PCB, KP, JWB, JSM, and ACN performed the analyses. All authors wrote and revised the manuscript.

Presentations: This work was previously presented at the 13th AACR Conference on the Science of Cancer Health Disparities in Racial/Ethnic Minorities and the Medically Underserved, and the 74th Annual Meeting of the Canadian Society of Otolaryngology-Head & Neck Surgery.

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and White (n=354) patients with non-HPV-related tumors, using multivariable models. Publicly available validation cohorts were used.

Results: Black patients had poorer progression-free survival than White patients. Black patient tumors had greater copy number aberrations, and increased *SFRP1* methylation and miRNA-mediated *PRG4* silencing associated with poor survival. PI3K/AkT/mTOR pathway proteins were differentially expressed.

Conclusions: There are molecular differences between tumors from Black and White patients that may partially account for differences in survival. These may inform targeted treatment decisions to achieve equitable outcomes.

Keywords

genomics; mutational status; racio-ethnic group; HNSCC; HPV-negative

Introduction

Recent social events and the COVID-19 pandemic have provided the healthcare community an opportunity to reflect on health inequities between racio-ethnic groups[1]. A large body of literature demonstrates marked disparities in health outcomes in patients of different racial and ethnic backgrounds[2]. Head and neck squamous cell carcinoma (HNSCC) is no exception. Case series and large population database studies of HNSCC, particularly those comparing outcomes of African American to Caucasian patients in the United States, have consistently demonstrated poorer overall survival outcomes for the former[3–5].

Race and ethnicity are two factors frequently investigated in literature as influencing health outcomes, but the two terms are often not defined in these publications and are used interchangeably[6]. The term racio-ethnic group (REG) is one that integrates aspects of both race and ethnicity. Although race and ethnicity are social constructs, one cannot deny their tangible effects across social systems including healthcare[7]. Patient REG can potentially influence care, behavioural risk factors, and socioeconomic status[8]. One source of these disparities can be healthcare providers' attitudes toward and false beliefs about different REGs, which can influence patient interactions and treatment selection[9]. Further, different REGs vary in their modifiable risk factors for cancer severity, which are influenced by socioeconomic status. For example, smoking accounts for a disproportionate burden of advanced-stage tobacco-related cancers in African American and Latinx people compared to Caucasians[10].

The present study in no way sets out to diminish the indisputable impacts of social determinants of health (SDH) on health outcome disparities[11]. However, differences in tumour biology likely also play a critical role in HNSCC outcomes of different REGs. One source of these differences is acquired mutations.[5]. For example, HPV-positive tumors are significantly more common in Caucasian patients and are molecularly distinct from more aggressive HPV-negative tumors[12,13]. While tobacco use is a potentially modifiable risk factor that imparts high tumor mutational burden, there is no difference in smoking status by racial identity between African Americans and Caucasians in the United States[14].

Independently, genetic ancestry can be objectively defined by whole-genome sequencing techniques[15]. Such studies reveal differing frequencies of germline cancer risk genes, most notoriously BRCA1/2 mutations that are disproportionately common in women of African ancestry and associated with poorer overall survival[16]. In HNSCC, a germline mutation in *JAK3* has been identified in African American patients[8]. Conceivably these acquired and inherited differences in tumor genomics could predict heterogeneity of treatment response.

Earlier studies of a Johns Hopkins University HNSCC cohort and pan-cancer TCGA cohorts have found differences between African American and Caucasian patients in somatic mutations, chromosomal instability, protein abundances, immune signatures, and DNA methylation[8,17–19]. The TCGA HNSCC study provided comprehensive multiplatform molecular data on 515 patient samples. We have curated racial and ethnic data into REGs to carry out a comparison of clinical characteristics and molecular features, using methods distinct from previously published literature.

Methods

Data Collection

Clinical and mutation data for the TCGA HNSCC cohort, including Level 3 DNA Mutation Packager Calls, non-normalized mRNA and miRNA sequencing calls, and Merged Clinical datasets, were downloaded from the Broad Institute's Firehose databases (version GRCh38). Copy number aberration data from Firehose GISTIC2 were used for CNA comparisons[20]. Hypoxia scores were retrieved from supplementary materials of Bhandari *et al.*[21]. HPV status was assigned based on Bratman *et al.*'s identification of viral transcripts[22]. There were 442 HPV-negative and 73 HPV-positive primary HNSCC samples in TCGA, of which 431 and 67 had complete DNA mutation data respectively. The TCGA clinical data file provides a column with self-reported racial data categorized as White, Black/African American (Black), Asian, or American Indian/Alaskan Native (Native). We appreciate that these are not politically correct terms. Self-reported ethnicity was classified as Hispanic/Latino or Non-Hispanic/Latino. We combined the race and ethnicity definitinons into racio-ethnic groups (REG) to stratify Hispanic patients separately irrespective of race. As sample sizes for Hispanic (n=24), Asian (n=11), and Native (n=1) patients were small, they were excluded from analysis.

Statistics:

Multiple testing correction: All p values from multiple testing for each analysis were adjusted for false discovery rate (FDR) using the Benjamini-Hochberg method, and the FDR significance threshold was set at 0.1 as in our previous studies[23,24].

Clincal features: All analyses were done in the R statistical environment (version 3.6.1). Fisher, χ^2 Goodness of fit, and Mann-Whitney U tests were performed to compare clinical variables of Black patients against White patients in the HPV-negative group. Smoking history was defined as never smokers, light smokers (<20 pack year history), or heavy smokers (>20 pack year history) as in our previous research[23,24].

Survival analyses—Survival analyses were conducted using the survival package (v 3.1.8)[25]. Proportional hazards assumptions were tested and verified for all Cox regressions throughout the study. Overall and progression-free survival outcomes were compared using a log-rank test between REGs in the HPV-negative cohort, and Kaplan-Meier curves constructed with risk tables. Cox models were used for multivariable regression of clinical covariates including sex, age, anatomical site, smoking history, tumor size and extent (T classification), nodal metastases (N classification), overall TNM stage, and treatment with adjuvant radiotherapy. Forward stepwise selection starting with an empty model was employed to devise the best multivariable model separately for overall and progression-free survival.

Multivariable modeling of molecular characteristics—Molecular data represented by continuous variables were normalized using the Yeo-Johnson variant of Box-Cox transformation that allows for non-positive values[26]. These were then compared between REGs within a multivariable linear regression model. Covariates of sex, age, smoking history, anatomical site, and T classification were selected for all analyses. The covariates smoking history, anatomical site, and T classification were dichotomized into “Heavy smoker vs Never/light smoker”, “Larynx vs Other”, and “T3-T4 vs T0-T2”.

Exome sequence mutations—TCGA exome sequencing data were compared using the maftools package (version 2.0.10)[27] within the Bioconductor framework. Fisher’s Exact test was used to compare mutation frequency of each gene between REGs. Synonymous mutation variants were excluded, and the *TTN* gene was also excluded because it is a large gene known to have a high frequency of passenger mutations[28]. Only genes that were mutated in at least five tumors of at least one comparison arm were evaluated.

Copy number aberrations—CNA frequencies were compared between REGs with respect to shallow deletions and gains, as determined by GISTIC2 analysis of the TCGA HNSCC cohort. Shallow deletions (GISTIC2 value: −1) were defined as heterozygous losses. Gains were copy number changes assigned GISTIC2 value +1. Fisher’s Exact tests were used to compare differences of CNA frequencies between REGs, and then to compare frequency of 3p arm loss (>97% vs <1% deletion of chromosomal material from one 3p arm) between REGs.

Tumor mutation burden and percent genome altered—Tumor mutation burden (TMB) was defined as the total number of SNV mutations across the genome. Percent genome altered (PGA) was defined as length of genome with a CNA as defined by GISTIC2 divided by total length of the genome multiplied by 100, as was done in previous studies[29]. Multivariable modeling comparing TMB and PGA between REGs was done as previously described.

Tumor microevironment—The cellular infiltration of each tumor’s microenvironment was estimated by inputting TCGA mRNA sequencing data into the immunedeconv package (v 2.0.0), which outputted scores calculated by the Microenvironment Cell Populations-counter (MCP-counter) method[30]. These scores are based on transcriptomic markers known to characterize and be proportional to the specific immune population within the TME, and

have demonstrated high accuracy in inter-sample comparisons[31]. Multivariable modeling comparing abundances of T cells, natural killer (NK) cells, B cells, monocytes, myeloid dendritic cells (DCs), neutrophils, endothelial cells, and cancer-associated fibroblasts (CAFs) between REGs was done as previously described.

Hypoxia scores characterized by eight different investigators were downloaded from Bhandari *et al.*'s supplementary materials[21]. These scores were generated by assigning +1 and −1 to the top 50% and bottom 50% of patients, respectively, based on mRNA abundance of each gene in the validated signature. Multivariable modeling comparing hypoxia scores between REGs was done as previously described.

Reverse phase protein array—Level 3 processed normalized RPPA data were downloaded from TCPA[32]. Multivariable modeling comparing protein abundance levels between REGs was done as previously described. We then associated our differentially abundant proteins with their mRNA abundance values using Spearman's rank correlation.

RNA sequencing—TCGA HNSCC mRNA and miRNA sequencing counts were normalized and compared using the DESeq2 package (version 1.24.0)[33] within the Bioconductor framework. The miRNA targets were searched using the multiMiR package (version 1.8.0)[34]. We then validated our differentially expressed miRNA with target mRNA abundance values using Spearman's rank correlation. Cox modeling was performed for each significant miRNA discovery to compare survival of patients who had tumors with miRNA transcript levels over and below the median.

Methylation—Level 3 processed TCGA HNSCC methylation data normalized using maximum negative correlation with mRNA abundance was analyzed. Multivariable modeling comparing beta values between REGs was done as previously described. Cox modeling was performed for the most significant methylated gene discovery to compare survival of patients who had tumors with methylation levels of the gene over and below the median.

Validation cohorts—Patient samples from GSE107830 and GSE79556 validation cohorts were downloaded from Gene Expression Omnibus database[35–37]. After excluding samples listed as having failed quality control, GSE107830 contained 92 oral cancer patients without HPV status definition. It derived miRNA data from the Illumina NextSeq 500 platform, and reported prognosis based on disease-specific survival and recurrence-free interval as a binary variable. Using this cohort, we compared survival of patients who had tumors with hsa-miR-935 transcript levels above and below the median by Fisher's Exact test.

GSE79556 contained 83 oral tongue cancer patients without HPV status definition. It derived methylation data from the Illumina HumanMethylation450 BeadChip platform, and reported overall survival as a linear variable. Similarly to the pre-processing method used by Broad Institute for TCGA methylation data, we excluded probes with standard deviation of beta values below the median for the gene[38]. Using this cohort, we compared survival of patients who had tumors with *SFRP1* methylation levels above and below the median by Cox modeling.

Results

Differences in demographics, stage treatment and survival by racio-ethnic group

The TCGA cohort contained 422 White and 46 Black non-Hispanic patients. HPV-positive patients were almost exclusively White (68/71, p=0.03, Supplementary Table S1). Thus, we focused our analysis on solely the HPV-negative group. Within the HPV-negative cohort, there were 354 White and 43 Black patients, and we compared their clinical characteristics (Table 1). Compared to White patients, Black patients were younger, heavier smokers, more likely to present with higher T classification, overall TNM stage, and laryngeal primaries, and more likely to have positive surgical margins post-resection ($p<0.05$).

Black patients had significantly poorer overall survival compared to White patients in the entire cohort (logrank p=0.048, Figure 1). In the HPV-negative cohort, Black patients had significantly poorer progression-free survival compared to White patients on multivariable analysis (HR=2.1, 95%CI=1.1–3.8, p=0.021, Supplementary Figure 2a, Supplementary Table S2). There was no difference in overall survival (HR=1.4, 95%CI=0.75–2.5, p=0.31, Supplementary Figure 2b, Supplementary Table S3, Supplementary Figure 1).

Copy number aberrations, particularly shallow deletions, differed between tumors from Black and White patients.

No differences in TMB or frequency of SNVs in any genes were observed between Black and White patients (FDR=1, Supplementary Figure S3). However, tumors from Black patients had higher PGA compared to White patients, after controlling for age, sex, smoking history, anatomical site, and T classification (fold change (FC)=1.7, 95%CI=1.2–2.5, FDR=0.0076, Supplementary Figure S4, Supplementary Table S4). Indeed, there were 2,217 shallow deletions and 135 gains that occurred more frequently in Black patients compared to White patients (Supplementary Tables S5–6). When these CNA differences were grouped by the chromosomal arms on which they are located, we identified the 3p arm as most frequently altered (Figure 2). Subtotal 3p arm loss occurred more frequently in tumors of HPV-negative Black patients compared to White patients (93% vs 74%, p=0.0041).

Next, we investigated whether these numerous CNA events resulted in a corresponding change in mRNA transcript levels of the affected genes. We found, relatively, 33 genes up-regulated in tumors of Black patients and 112 genes up-regulated in tumors of White patients (FDR<0.01 and absolute fold change >2, Supplementary Figure S5, Supplementary Table S7). As such, 11 genes were more frequently deleted and down-regulated in tumors of Black patients compared to White patients (Supplementary Table S8).

Protein abundances differed between tumors from Black and White patients

We found 15 differentially abundant proteins and phosphoproteins between Black and White patients after controlling for sex, age, smoking history, anatomical site, and T classification (FDR<0.1, Table 2). Eight of these were more highly abundant in Black patients and seven were more highly abundant in White patients. Four and four of the more highly abundant

proteins in Black patients and White patients, respectively, had significant correlations to mRNA transcript abundance (FDR<0.1, Supplementary Table S9).

Downregulation of PRG4 by miRNA is more frequent in tumors from Black patients and predicts poor overall survival

Micro-RNAs exert a variety of post-transcriptional effects on mRNA transcripts, although they are classically thought of as repressors[39]. We found 40 significantly differentially abundant miRNAs between tumors from Black and White patients (FDR<0.1), and three of these had validated mRNA targets (Supplementary Table S10). Hsa-miR-935, more highly expressed in tumors of Black patients, had an mRNA target, *PRG4*, that also had significantly lower transcript level (log2 fold change=−1.2, FDR=2.6×10^{-4}), and further analysis revealed that the miRNA and mRNA levels were correlated ($\rho=-0.22$, $p=1.3\times10^{-5}$).

In addition, survival analysis of each of the three miRNAs that were associated with changes in mRNA abundance revealed that higher hsa-miR-935 abundance was associated with poorer overall survival within the TCGA cohort of HPV-negative Black and White patients (HR=1.4, 95%CI=1.0–1.9, FDR=0.097, Figure 3, Supplementary Table S10). This survival difference persisted when adding control for REG (HR=1.4, 95%CI=1.0–1.9, $p=0.036$). Higher hsa-miR-935 abundance trended toward poorer prognosis in our validation cohort for oral cavity tumors (OR=2.0, 95%CI=0.83–5, $p=0.14$).

Comparison of tumor microenvironments

Deconvolution of mRNA abundance data revealed no differences in the immune cell populations in the TME of Black and White patients after controlling for sex, age, smoking history, anatomical site, and T classification (Supplementary Figure S6, Supplementary Table S11). There were also no differences in hypoxia in the TME of Black and White patients after controlling for sex, age, smoking history, anatomical site, and T classification (Supplementary Figure S7, Supplementary Table S12).

Methylation of SFRP1 in Black patients predicts poor overall survival

Modification of DNA by methylation can result in decreased transcription of genes[40]. Among the 145 genes with differential mRNA abundance between Black and White tumors, six demonstrated markedly differential methylation after controlling for sex, age, smoking history, anatomical site, and T classification (FDR<0.01). *SFRP1* had markedly higher methylation in tumors of Black patients (FC=2.0, 95%CI=1.3–2.9, FDR=0.091, Supplementary Table S13).

Survival analysis of the top hit revealed that increased *SFRP1* methylation was associated with poorer overall survival within the TCGA cohort of HPV-negative Black and White patients (HR=1.4, 95%CI=1.0–1.9, $p=0.040$, Figure 4). When controlling for REG in the survival analysis, there remained a trend toward poorer overall survival (HR=1.3, 95%CI=0.99–1.8, $p=0.060$). On the mRNA level, lower *SFRP1* transcript abundance was associated with poorer overall survival (HR=1.4, 95%CI=1.0–1.9, p=0.025). Higher methylation levels of *SFRP1* in our validation cohort was also associated with poorer overall survival (HR=2.0, 95%CI=1.2–3.1, FDR=0.081, Supplementary Table S14).

Discussion

Social determinants of health are likely the largest driver of outcome disparities between REGs, however our detailed multiplatform analysis of tumors from Black and White HNSCC patients using TCGA data suggest that molecular differences play a significant role. Consistent with other reports, we identified poorer survival for Black patients compared to White patients in the entire cohort as well as the HPV-negative cohort. Furthermore, there were important molecular differences on the CNA, mRNA, protein, miRNA, and methylation levels. These findings and their implications described below warrant further validation.

SFRP1 methylation was greater in tumors from Black patients compared to White patients, and associated with poorer overall survival in both our TCGA cohort and an external cohort of oral tongue cancer patients[37]. Tumor suppressor *SFRP1* encodes secreted Frizzled-related protein 1, which inhibits Wnt signalling, and is downregulated in many cancers via DNA methylation[41]. A previous pan-cancer study supports our findings that *SFRP1* had decreased expression in HNSCC, and that these low levels were associated with poorer overall survival[41]. Further, high *SFRP1* methylation has been implicated in resistance to chemotherapeutic agents including taxanes and platinum agents, both of which are used routinely for HNSCC[42,43]. Demethylating agents such as 5-azacytadine have been shown to restore *SFRP1* expression and resensitize tumors to radiotherapy, including in HNSCC[42].

Hsa-miR-935 was more abundant in Black patients compared to White patients, and associated with poorer survival in our TCGA cohort. Hsa-miR-935 is known to downregulate proteoglycan 4 (PRG4), which was indeed downregulated in Black patients. In breast cancer, PRG4 has been shown suppress cancer cell invasion by preventing cell adhesion and inducing tumor-suppressive transforming growth factor beta[44]. In contrast with our findings, overexpression of hsa-miR-935 was recently reported to inhibit oral cancer cell lines by inducing inositol polyphosphate-4-phosphatase type I A[45]. On the other hand, contradictory roles of hsa-miR-935 as a tumor-promoting and suppressing miRNA have both been reported in non-small cell lung cancer, and the same may be true in HNSCC[46,47].

There were a large number of shallow deletions and gains on multiple chromosome arms occurring at higher frequencies in Black patients compared to White patients. Although demographic surveys suggest no difference in smoking between Black and White patients[14], the differences in smoking history observed in TCGA may partially account for these differences in CNAs through tobacco-related increased chromosomal instability, previously suggested to lead to higher levels of aneuploidy in tumors of Black patients[17,48]. The most common chromosomal event was 3p arm loss, an early initiating event in HNSCC progression associated with poor outcome[49].

Our RPPA analysis demonstrated differences in protein abundance between tumors of Black and White patients suggesting differential activation of the PI3K/Akt/mTOR pathway. This pathway is deregulated in around 80–90% of HNSCC and has many nodes that can be targeted with kinase inhibitors[50]. One study previously suggested that this pathway may be less active in patients of African American ancestry based on TCPA RPPA data[17].

Indeed in our study, Akt, with an activating phosphorylation site on S473[51], was found at higher levels in White patients than Black patients, as were several proteins we identified downstream of the PI3K/Akt/mTOR pathway. Akt activates the mTORC1 complex, and this activation is consistently correlated with improved clinical outcome in HNSCC[50]. Patients with PI3K/Akt/mTOR pathway deregulation may derive increased benefit from targeted therapies such as rapamycin, which is well-tolerated and results in significant clinical responses over a brief treatment duration[52]. Interestingly, although there was no difference in hypoxia in the TME on multivariable analysis, the hypoxic signalling protein HSP70 was almost three times as highly expressed in tumors of Black patients compared to White patients. This poor prognostic indicator in tumors of Black patients may be related to the high stage at presentation, and could potentially be targeted by the antifungal nimorazole, shown to improve radiotherapy outcomes in hypoxic tumors when given concurrently[53,54].

The prior TCGA pan-cancer studies defined their cohorts by genetic ancestry rather than self-identification and did not stratify by HPV status[17–19]. Still, they reported findings consistent with our study, including differences in chromosomal instability and PI3K pathway. The study by Guerrero-Preston *et al.* compared self-identifying Black and non-Latinx White HNSCC patients treated at Johns Hopkins University[8]. In contrast with our study, they found higher tumor-infiltrating lymphocytes in Black compared to White patients, although their analysis did not control for important covariates such as anatomical site and stage. These have previously recognized associations with immune cell populations[24,55], and were indeed critical confounders in our study. Guerrero-Preston *et al.* also discovered significant differences in SNV frequencies between Black and White patients, including a germline *JAK3* mutation[8]. This may be in part because they limited their sequencing to 50 known cancer-related genes, and ran comparisons without excluding HPV-positive tumors[12]. Additionally, through an integrated analysis of DNA methylation and SDH, the authors identified a complex interplay between these factors influencing HNSCC disparities.

Our study uses self-reported definitions of race and ethnicity, which we recognize are social constructs distinct from genetic ancestry[56,57]. We were unable to obtain data on germline mutations in TCGA at the time of our study, which precluded the use of the latter. The self-reported REGs likely have a closer interplay with SDH that was notably missing from TCGA. Beyond germline mutations, high levels of biopsychosocial stress exposure associated with SDH and being a racial minority in the United States likely create molecular fingerprints that are inherited across generations[8,58,59]. In addition, the multifactorial challenges in accessing healthcare result in cancers presenting at higher stage[58]. One disconcerting finding in our study was the considerably higher rate of positive surgical margins after resection in Black patients, which will require further study to explain Lastly, studies like ours reporting on REG are limited in power by the lower number of non-White patients[56], and our future aims are to develop more balanced cohorts with better representation of other REGs to further delineate both germline and tumor molecular differenes. On a related note, the validation cohorts that we identified only had oral cavity cancers, which may not be representative of TCGA. This difficulty is unfortunately in part secondary to underreporting of race in sequencing studies[56].

Conclusion

This study demonstrated that tumors from Black and White HNSCC patients have molecular differences, some of which were associated with survival. These findings need to be taken in context with SDH, which largely underpin health disparities. As sequencing costs decline, developing prospective cohorts of patients with balanced heterogeneity of ancestry and SDH will be ideal to build on our work.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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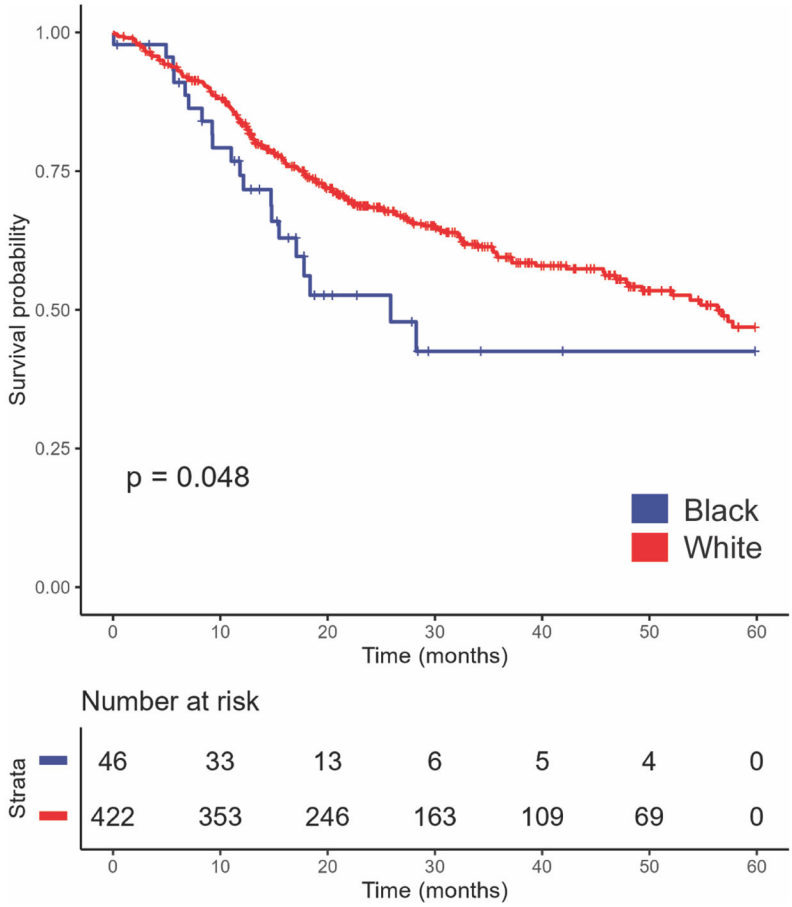

Figure 1. Overall survival of Black and White patients in the entire TCGA HNSCC cohort.

Kaplan-Meier curves were constructed for Black and White patients in the entire TCGA HNSCC cohort, and the log-rank *p* value is shown.

Abbreviations: TCGA=The Cancer Genome Atlas, HNSCC=head and neck squamous cell carcinoma

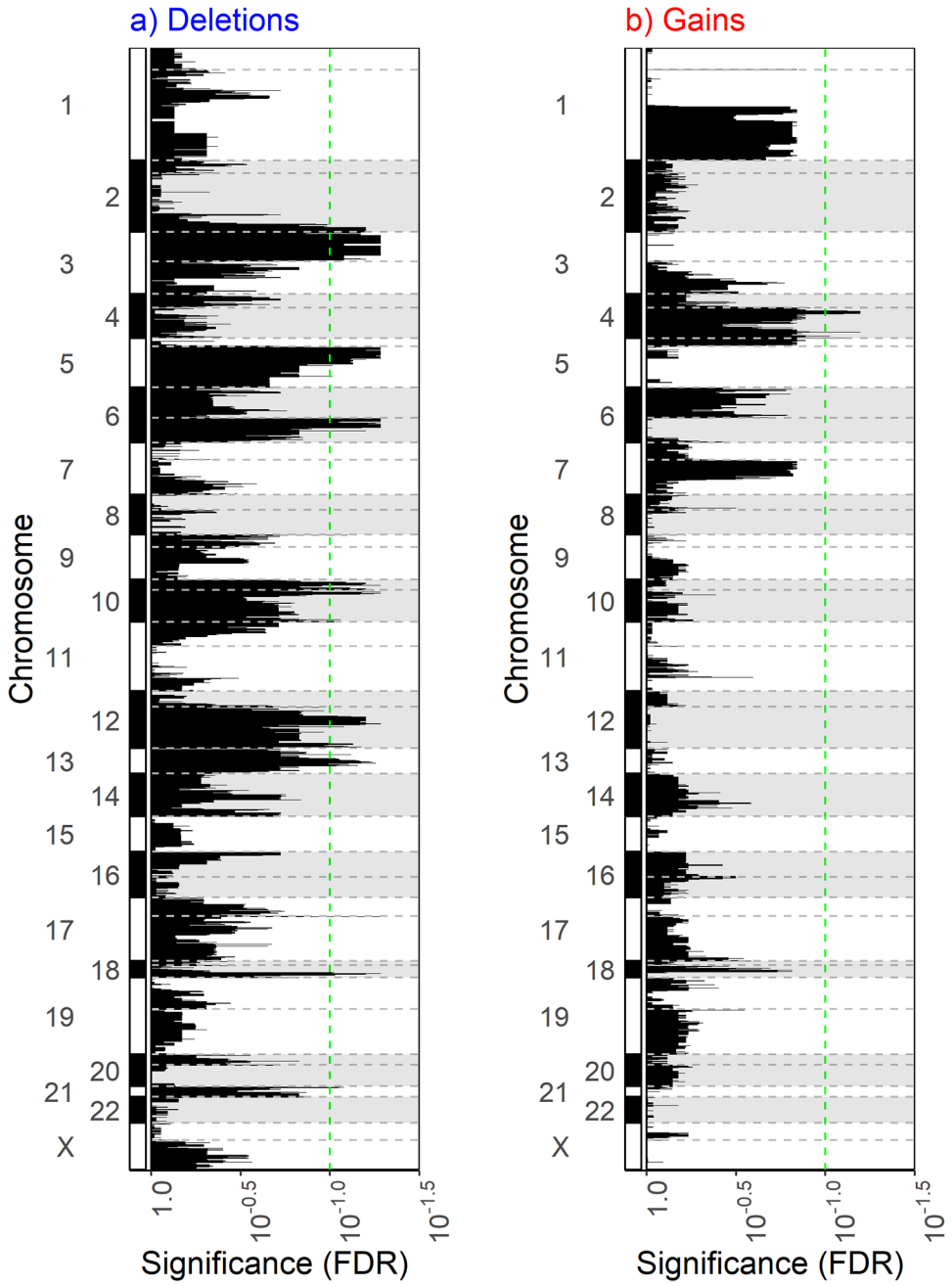

Figure 2. Chromosomal arm distribution of significant CNAs between HPV-negative Black and White patient samples.

A) Shallow deletion and B) gain frequency were compared between Black and White patients using Fisher's Exact test. The Benjamini-Hochberg-corrected FDR significance values were plotted by chromosomal mapping location. The green hatched line signifies the threshold FDR=0.1.

Abbreviations: HPV=human papillomavirus, CNA=copy number aberration, FDR=false discovery rate

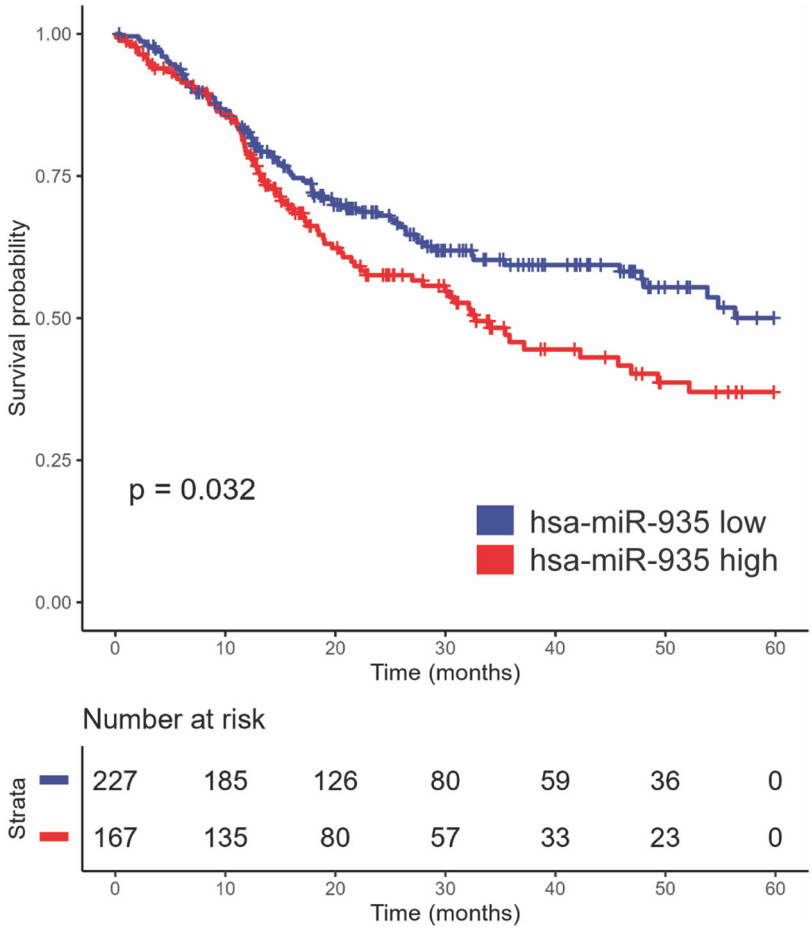

Figure 3. Overall survival difference by hsa-miR-935 abundance level in combined HPV-negative Black and White cohort.

The combined cohort of HPV-negative Black and White patients was dichotomized by whether hsa-miR-935 abundance level was above or below the median. Log-ranked *p* value is shown.

Abbreviations: HPV=human papillomavirus

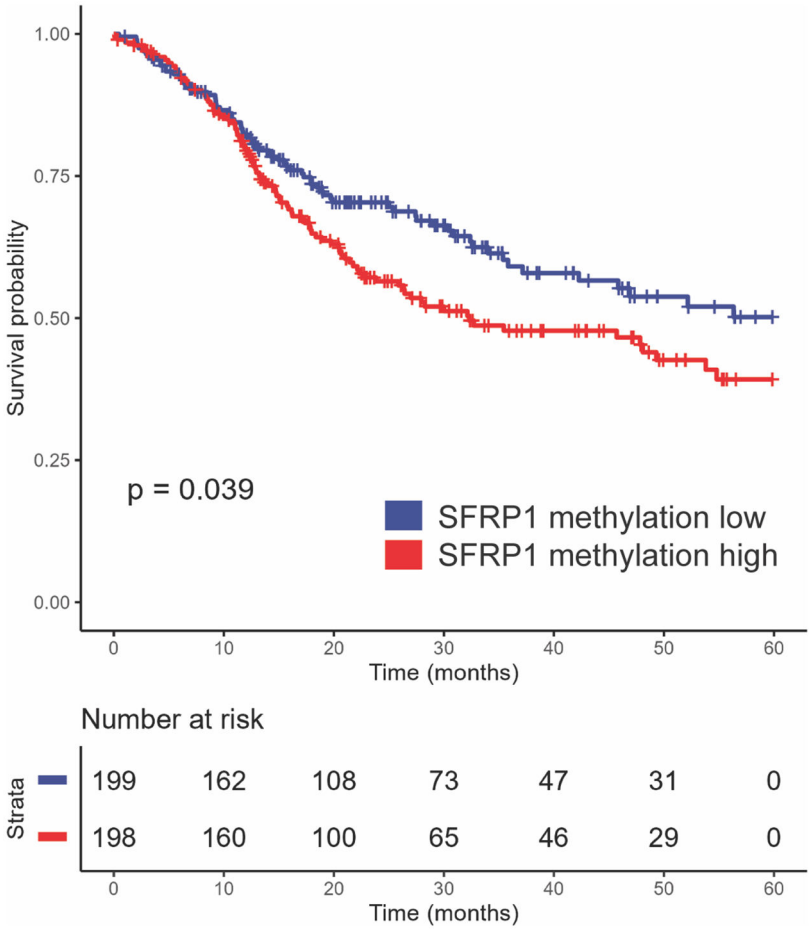

Figure 4. Overall survival difference by *SFRP1* methylation level in combined HPV-negative Black and White cohort.

The combined cohort of HPV-negative Black and White patients was dichotomized by whether *SFRP1* methylation level was above or below the median. Log-ranked *p* value is shown.

Abbreviations: HPV=human papillomavirus

Table 1.
Demographic differences by REG in HPV-negative samples.

P values represent the results of Fisher's Exact test, $\chi 2$ Goodness of fit, and Mann-Whitney U tests. Bolded *p* values indicate statistically significant results.

		HPV-Negative Samples, No. (%)		
Variables		**White (n=354)**	**Black (n=43)**	***P* value**
Age	Median (range)	62 (19–90)	59 (41–84)	**0.0042**
Sex	Female	112 (32)	7 (16)	0.051
	Male	242 (68)	36 (84)	
Anatomical site	Oral cavity	242 (68)	20 (47)	**0.039**
	Oropharynx	20 (6)	4 (9)	
	Hypopharynx	7 (2)	1 (2)	
	Larynx	85 (24)	18 (42)	
Smoking history	Never smoker	73 (26)	2 (7)	**0.033**
	Light smoker	35 (12)	7 (24)	
	Heavy smoker	173 (62)	20 (69)	
T classification	T0-T2/TX	142 (42)	11 (26)	**0.048**
	T3-T4	200 (58)	32 (74)	
N classification	N0-N2a, NX	227 (67)	22 (51)	0.061
	N2b-N3	114 (33)	21 (49)	
Overall TNM stage	I-III	130 (41)	8 (22)	**0.031**
	IV	189 (59)	29 (78)	
Adjuvant radiotherapy	No	116 (38)	10 (26)	0.21
	Yes	193 (62)	28 (74)	
Surgical margins	Negative	299 (91)	24 (71)	**0.0019**
	Positive	31 (9)	10 (29)	

Abbreviations: HPV=human papillomavirus, REG=racio-ethnic group

Table 2.
Multivariable analysis of RPPA differences between Black and White patients.

Multiple linear regression models including REG, sex, age, smoking history, anatomical site, and T classification were used to compare Yeo-Johnson-normalized protein levels in the RPPA data between Black and White patients. FDR correction of the p values for the REG coefficient was done using Benjamini-Hochberg method.

Protein	REG=Black FC [95%CI]	Sex=Male FC [95%CI]	Age per 1y FC [95%CI]	Smoking=Yes FC [95% CI]	Anatomical site=Larynx FC [95%CI]	T classification=High FC [95%CI]	REG FDR
HSP70	2.61 [1.59,4.29]	1.15 [0.84,1.57]	1 [0.99,1.01]	0.65 [0.48,0.87]	0.97 [0.69,1.36]	0.85 [0.63,1.14]	0.0428
NCADHERIN	2.54 [1.53,4.21]	1.03 [0.75,1.42]	1 [0.98,1.01]	0.79 [0.58,1.07]	0.73 [0.52,1.03]	0.88 [0.65,1.19]	0.0428
ASNS	2.27 [1.4,3.68]	1.17 [0.86,1.58]	1 [0.99,1.02]	1.12 [0.84,1.5]	1.52 [1.09,2.12]	1.02 [0.77,1.36]	0.0464
PDCD4	0.41 [0.25,0.69]	0.82 [0.6,1.14]	0.99 [0.98,1]	0.81 [0.6,1.11]	1.24 [0.87,1.77]	0.92 [0.67,1.25]	0.0464
EZH2	3.23 [1.64,6.36]	1.18 [0.78,1.78]	1.01 [0.99,1.02]	1.03 [0.69,1.53]	0.87 [0.53,1.41]	1.08 [0.72,1.62]	0.0464
SRC_pY527	0.43 [0.26,0.71]	0.84 [0.61,1.16]	1 [0.99,1.01]	0.93 [0.68,1.26]	0.88 [0.62,1.25]	0.7 [0.52,0.95]	0.0471
AKT_pS473	0.45 [0.27,0.74]	1.02 [0.75,1.39]	1 [0.99,1.01]	1.2 [0.89,1.61]	0.88 [0.62,1.23]	0.81 [0.6,1.09]	0.0633
XRCC1	2.25 [1.33,3.79]	1 [0.72,1.38]	1.01 [0.99,1.02]	1.16 [0.85,1.59]	1.41 [0.99,2.02]	0.79 [0.58,1.08]	0.0770
FOXO3A	2.11 [1.25,3.56]	1.13 [0.81,1.56]	1 [0.99,1.01]	0.86 [0.63,1.18]	1.09 [0.77,1.57]	0.92 [0.67,1.25]	0.0997
PDK1_pS241	0.5 [0.31,0.81]	0.99 [0.73,1.34]	1 [0.99,1.01]	1 [0.74,1.33]	1.1 [0.79,1.54]	1.44 [1.08,1.93]	0.0997
BAD_pS112	0.48 [0.28,0.8]	0.66 [0.48,0.91]	0.99 [0.97,1]	0.98 [0.72,1.34]	0.87 [0.61,1.25]	0.93 [0.68,1.26]	0.0997
NDRG1_pT346	0.46 [0.27,0.78]	1.14 [0.82,1.58]	0.99 [0.98,1.01]	0.89 [0.65,1.22]	0.94 [0.65,1.34]	0.97 [0.71,1.33]	0.0997
RICTOR	0.47 [0.27,0.8]	1 [0.71,1.41]	1 [0.99,1.02]	1.17 [0.85,1.62]	1.12 [0.77,1.62]	1.21 [0.88,1.68]	0.0997
1433BETA	2.05 [1.23,3.42]	0.88 [0.64,1.21]	1 [0.99,1.01]	1 [0.73,1.36]	0.79 [0.55,1.12]	1 [0.74,1.37]	0.0997
JAB1	2.07 [1.24,3.45]	1.19 [0.86,1.64]	1 [0.99,1.02]	1.08 [0.79,1.46]	1.19 [0.84,1.69]	1.01 [0.74,1.37]	0.0997

Abbreviations: RPPA=reverse phase protein assay, REG=racio-ethnic group, FDR=false discovery rate, FC=fold change, CI=confidence interval.