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Differential mutation spectrum and immune landscape in African Americans *versus* Whites: A possible determinant to health disparity in head and neck cancer

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Conceptualization and design, S.C., D.G., M.A.M. and S.K.B.; Data acquisition and analysis, S.C., V.D., K.G., S.S., P.A., R.C. and D.G.; writing and original draft preparation, S.C., K.G., D.G. and M.A.M.; critical review and editing, S.C., V.D., K.G., J.L.C., R.C., D.G., Z.S., D.T.J., A.K.G., S.K.B. and M.A.M.; supervision, S.C., M.A.M. and S.K.B; funding acquisition, S.K.B. All authors have seen the final draft of the manuscript before submission.

Declaration of competing interest

SKB is one of the co-founders of Sanguine Diagnostics and Therapeutics, Inc. The other authors declare no competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.canlet.2020.07.029>.

Abstract

African Americans (AA) with Head and Neck Squamous Cell Carcinoma (HNSCC) have a worse disease prognosis than White patients despite adjusting for socio-economic factors, suggesting the potential biological contribution. Therefore, we investigated the genomic and immunological components that drive the differential tumor biology among race. We utilized the cancer genome atlas and cancer digital archive of HNSCC patients (1992–2013) for our study. We found that AA patients with HNSCC had a higher frequency of mutation compared to Whites in the key driver genes—*P53*, *FAT1*, *CASP8* and *HRAS*. AA tumors also exhibited lower intratumoral infiltration of effector immune cells (CD8⁺, $\gamma\delta$ T, resting memory CD4⁺ and activated memory CD4⁺ T cells) with shorter survival than Whites. Unsupervised hierarchical clustering of differentially expressed genes demonstrated distinct gene clusters between AA and White patients with unique signaling pathway enrichments. Connectivity map analysis identified drugs (Neratinib and Selumetinib) that target aberrant PI3K/RAS/MEK signaling and may reduce racial disparity in therapy response.

Keywords

HNSCC; Mutation burden; Immune response; Neo-antigen; Molecular determinant; Disease surveillance

1. Introduction

Epidemiological evidence indicates the potential influence of race and ethnic disparities in differential cancer incidence and mortality rates. Like many other cancers [1–3], African American (AA) patients with Head and Neck Squamous Cell Carcinoma (HNSCC) have a disproportionally increased tumor burden and a five-year survival lower (29.3–31.0%) than Whites (54.7–59.0%) [4–7]. The survival disadvantage is attributed to late disease presentation [6], while a recent retrospective study also revealed decreased survival of AA with localized HNSCC tumors [8]. The racial disparity is caused by a complex interplay between lifestyle, environment and genetic factors of patients [9]. Other factors associated with HNSCC disparity include treatment inequalities, lifestyle, socioeconomic status (SES) and environmental factors [10]. However, after adjusting for these extrinsic factors [11–13], the survival inconsistencies persisted among races and thus suggest the potential role of inherent biological factors in driving disparate racial outcomes [10]. The SEER 18 data (Surveillance, Epidemiology, and End Results Program, 2010–2014) have shown AAs to have greater disease susceptibility (incidence/mortality rates) than Whites (<https://seer.cancer.gov/data/>). The national policies are aimed to reduce racial disparity in cancer screening, diagnosis, treatment and mortality (Pub Law) [14]. Therefore, comprehensive identification of genetic and immunological alterations that contribute to disparate HNSCC outcome will improve disease surveillance and therapeutic modalities. HNSCC constitutes about 3.6% of all malignancies in the United States with a five-year survived rate of 64.8%, while early diagnosed cases (30%) demonstrated a better five-year survival rate of 79% [15]. This warrants the identification of somatic mutations in key driver genes across races during disease progression to facilitate disease detection and surveillance, and thereby provide a better therapeutic window.

Variations in genomic landscape elicit differential immunometabolic alterations in HNSCC patients [10]. Evidence from clinical trials suggested that the response to immunotherapy in HNSCC [16,17] depends on availability of neo-antigens and a local immunosuppressive tumor microenvironment. While multiple studies have reported the influence of genetic changes, epigenetic modifications and mutations in specific protein domains [18–23] in HNSCC disparity, the influence of a global somatic mutational signature on the intricate association between immune response and metabolic alterations is still elusive. Hence, the current study aims to comprehensively investigate the key genetic and epigenetic variations that drive the differential immune phenotype among AA and Whites with a predicted contribution to intratumoral metabolic milieu, resulting in disparate patient outcome. In addition, we also aim to identify gene signatures to predict potential drug candidates for novel therapeutic intervention to alleviate racial disparity in therapy response of HNSCC patients.

2. Materials and methods

2.1. Data source, sample size and cohort characteristics

HNSCC processed data (gene expression, somatic mutation and DNA methylation) and patient clinical characteristics were downloaded from the NCI GDC data portal (<https://portal.gdc.cancer.gov>) (July, 2018). Our dataset contains 279 HNSCC samples [24]. Samples (except normal) without race, ethnicity or HPV annotations were discarded. Clinical and demographic information for the selected samples was retrieved from the GDC portal (Table 1). The samples were further categorized according to race/ethnicity and HPV status, where 66 patients were HPV^{-ve} White (64.07%), 28 were HPV^{+ve} White (27.18%), 9 were HPV^{-ve} AA (hereon will be referred to as 'AA') (8.73%).

2.2. Somatic mutation analysis

Mutational analysis was performed using the available, processed mutation datasets provided in the Genomic Data Commons (GDC) portal (<https://portal.gdc.cancer.gov>). We merged all the three datasets corresponding to four mutation callers, to identify the unique number of altered cases for each gene per sample cohort. Subsequently, Fisher's exact test was employed to identify genes that have significantly variable mutation rates across the cohorts.

2.3. Differential gene expression (DGE) analysis

The raw gene expression counts (processed by HTSeq) were downloaded from the GDC portal. R Bioconductor package '*limma*' was then used to fit a linear model [25] to perform the differential gene expression analysis between normal samples and each of the sample cohorts.

2.4. Methylation analysis

We performed the methylation analysis in R using the Illumina Human Methylation450 beta values of CpG sites as provided by the TCGA (<https://portal.gdc.cancer.gov/>). To identify the differentially methylated CpG sites between groups, the mean DNA methylation for each CpG site of each group was calculated. Here we focused on differences between the three

groups- Normal ν AA, Normal ν HPV^{-ve} White, and Normal ν HPV^{+ve} White. The fold change ratio of mean DNA methylation between AA and HPV^{+ve} White was calculated and represented as log2 of fold change (log2FoldChange). Differentially expressed CpG sites across the groups were tested with Mann-Whitney-Wilcoxon test.

Due to large number of CpG sites, the results were represented as volcano plots (fold change vs. statistical significance of each CpG site across the groups). We divided the groups into four categories: the 'SignificantFC' group contains all CpG sites with significant fold change ($p < 0.05$) and an absolute log2FoldChange > 1 ; the 'significant' group contains CpG sites with $p < 0.05$ and absolute log2FoldChange < 1 ; the 'FC' group contains CpG sites with absolute log2FoldChange > 1 and $p > 0.05$; the rest of insignificant CpG sites are considered as 'NA' group.

2.5. Correlation analysis of expression and methylation signatures

DEG with a fold-change greater or less than 2.5 with a false discovery rate (FDR) of < 0.05 was used for the correlation analysis. DNA (CpG) methylation causes gene silencing therefore a negative correlation exists between gene expression and methylation. Correlation plots were generated for the genes that show significant association between expression and methylation which are indicated by Pearson's correlation coefficient and p-value < 0.05 .

2.6. Identification of immune signature

To identify the immune signature of race with HNSCC we used the 'Newman Immune Signature' to estimate the diverse tumor associated leukocyte (TAL) subsets based on the gene expression signature (AA, HPV^{-ve} White and HPV^{+ve} White) (<https://cibersort.stanford.edu/index.php>) [26]. Wilcoxon test was used to compare the differences in leukocyte signature among the three groups.

We extracted hematoxylin and eosin (H&E) stained images of HNSCC patients with best and worst (five patients each) survival from the TCGA digital archive (<http://cancer.digitalarchive.net/>) and analyzed immune cell composition in- and around the tumor cells. The TCGA archived images were accessed and scored by an experienced pathologist.

2.7. Connectivity Map analysis to identify race specific genes

Connectivity Map (CMAP) analysis was performed in *limma* derived differentially expressed genes for each group. Each of the top 150 upregulated and downregulated genes was run against the updated CMAP (<https://clue.io/>) database to assess positive, negative and neutral connections [27]. The common negatively connected drugs were then assessed using a Venn diagram and the drugs specific to each group or common across groups were identified.

3. Results

3.1. African American HNSCC patients exhibit poor prognosis

The schematic workflow used in our analysis is provided in Fig. 1A. In order to understand the extent survival disparity among race, we analyzed the Center for Disease Control and Prevention data (<https://gis.cdc.gov/Cancer/USCS/DataViz>). We found that AA patients with HNSCC had a worse five-year relative survival than Whites (AA ν White: oral cavity and pharynx cancer- 44.6 ν 62%; larynx- 51.7 ν 60.1%) (Fig. 1B). When AA and White patients were segregated based on sex, we found that the difference in five-year relative survival between male and female exists within and between the race [(male ν female: oral cavity and pharynx- 60.2 ν 63.8%; larynx: 59.8 ν 56.7% (all races) and race (male ν female: oral cavity and pharynx- 61.9 ν 64.1% (White) and 41.4 ν 55.7% (AA); larynx: 61 ν 57.4% (White) and 51.8 ν 51.6% (AA)]. We next analyzed The Cancer Genome Atlas (TCGA) HNSCC dataset and observed a decreased trend in the survival probability of AA patients although it was not statistically significant ($p = 0.07$) (Fig. 1C). Survival disparity among AA and Whites also exists across various other malignancies; however, the difference was quite evident in case of Melanomas of the Skin, and Oral Cavity and Pharynx where AA population demonstrated a lower five-year survival than Whites by 17% or more (Fig. 1B).

3.2. African American with HNSCC tumors harbor higher somatic mutation frequency than whites

Mutations are acquired in response to various environmental stressors causing random DNA replication and DNA repair errors [28], thereby leading to the development of disease subtypes by clonal expansion of cells with a survival advantage [29]. HNSCC patients of different race showed a distinct mutation spectrum [22]. These gene mutations along with HPV infection are established to further contribute to inter- and intra-tumoral heterogeneity [18,20–23]. Therefore, in order to understand the molecular alterations in the genome of HNSCC tumors between races, we profiled somatic mutations and their impact on disease prognosis based on four callers used in the TCGA. Since HPV is one of the major contributor in HNSCC development in Whites, we segregated the patients into two groups based on HPV status. Our analysis demonstrated a significant difference ($p = 0.039$) in mutations per mega base between races (AA ν HPV^{-ve} White ν HPV^{+ve} White: 0.05 ν 0.04 ν 0.02) (Fig. 2A). The mutation frequency of numerous genes was higher in AA compared to White HNSCC patients (Fig. 2B, Supplementary Table S1). Interestingly, we found AA patients harbor a unique mutation spectrum in contrast to White (HPV^{-ve}) suggesting the existence of race-specific effect. When genes with somatic mutation were classified based on their molecular functions (Fig. 2B), we found the highly mutated genes in AA to cluster in various cancer-associated pathways- DNA repair, cell cycle and apoptosis, Wnt signaling, stem cell regulation, JAK/STAT signaling pathway, immune response system and metabolism. Our result showed that *TP53* mutation was most prevalent (0.89) in AA than White patients (HPV^{-ve} White, 0.85; HPV^{+ve} Whites, 0.04). We also observed certain known 'driver' genes (*TP53*, *FAT1*, *CASPS*, *HRAS* and *NOTCH1*) with high mutation frequency in AA, except for *NOTCH1*, which was high in HPV^{-ve} White patients (AA ν HPV^{-ve} White ν HPV^{+ve} White: 11 % ν 21 % ν 7%). To investigate race- or cancer-specific effect of identified mutations we analyzed the mutation frequencies of driver genes in AA

and White patients across various cancer types (TCGA). Our pan-cancer analyses indicated increased mutation frequencies for certain driver genes in AAs (breast invasive carcinoma: AA ν White, TP53– 0.41 ν 0.29, HRAS– 0.01 ν 0.004; glioblastoma multiforme: AA ν White, TP53– 0.23 ν 0.17, *NOTCH1* - 0.02 ν 0.002, *CDKN2A*- 0.04 ν 0.007; lung adenocarcinoma: AA ν White, TP53–0.69 ν 0.48, *FAT1*-0.17 ν 0.09, HRAS– 0.01 ν 0.003, *CDKN2A*- 0.07 ν 0.03), while not all mutations observed in HNSCC were detected in all cancer types (Supplementary Table S2). These results show that the increased mutational frequency in driver genes were race-specific, however certain mutations were specific to HNSCC cancer type. We also observed a rare *APC* mutation in HNSCC which was 10-fold higher in AA patients than Whites (Fig. 2B). To determine the pathological implications of most prevalent mutations among AA and White patients a Fisher's exact test was performed. Not all differences observed in somatic mutations among races were clinically associated with survival outcome suggesting that most of these mutations were 'passenger' mutation. Although mutation frequency of *TP53* was high in AA and HPV^{-ve} Whites, we did not observe any significant effect on the survival outcome ($p = 0.15$) across races. Only genes such as *FAT1*, *APC*, *ATRX*, *HEXA* and *TLR6* with higher mutation frequencies in AA as compared to HPV^{±ve} Whites were significantly associated with survival disparity of patients (Fig. 2C). This data indicates a disparity in certain functionally relevant mutations that may explain the varied survival of HNSCC patients among races.

3.3. Gene expression and methylation signatures associated with race

To investigate the molecular pathways perturbed in HNSCC patients, a pairwise differential gene expression (DGE) analysis was performed. Unsupervised hierarchical clustering of DGE demonstrated a distinct gene cluster between HPV^{±ve} White and AA patients (Fig. 3A). Our analysis also identified 77 genes with similar expression in AA and HPV^{-ve} Whites and 47 and 68 mutually exclusive genes, respectively. However, when DGEs of HPV^{+ve} White patients were compared with AA and HPV^{-ve} Whites, few genes (26 genes, HPV^{+ve} White ν AA; 5 genes, HPV^{+ve} ν HPV^{-ve} White) converged while 119 genes appeared exclusively in HPV^{+ve} White patients (Fig. 3B), suggesting the differential effect of both race and viral infection on gene expression. We next performed gene ontology based pathway analysis of DGEs and found various terms enriched in races (Fig. 3C). In AA patients with HNSCC, cytokine mediated signaling pathway and cytoskeleton organization were the most enriched terms.

Gene expression in part is regulated by epigenetic modifications, particularly at the transcription level. To understand the effect of differential methylation in gene expression among races, we performed a correlation analysis between expression and p values (methylation level of CpG islands) of genes. Methylation analysis was performed in 103 patients, and each patient had 485,578 CpG islands in their genome. The differential p values between the races are represented as a volcano plot (Fig. 4A). Our analysis showed that hyper-methylated (*AIM2*, *CDO1*, *RPS6KA6*, *PHYHD1* and *GIPC2*) and hypo-methylated genes (*HOXD10*, *HOXC11* and *HOXC6*) correlated with gene expression in Whites (HPV^{-ve}). In AA patients, hyper-methylated genes *GIPC2*, *SELENBP1*, and *PPP1R3C* correlated with expression, and in HPV^{+ve} White patients, *ZNF544*, *TAF7L*, and *TCPI1* were hyper-methylated which correlated with expression (Fig. 4B). Among

the correlated genes, higher expression of *PHYHD1* was associated with shorter survival among HPV^{-ve} Whites, while higher expression of *TAF7L* correlated with longer survival in HPV^{+ve} Whites (Fig. S1).

3.4. Intra-tumoral immune infiltrates among races define differential immune response in races

A complex molecular symbiosis between the tumor cells and their local milieu of immune cells modulates cancer progression and also determines the response to immunotherapy [10]. Therefore, to determine the intratumoral immune variations among races, we used CIBERSORT (<https://cibersort.stanford.edu/>) [26], a method used for characterizing cellular composition in tumor cells based on gene expression signature. Based on the leukocyte gene signature matrix (LM22), we observed that AA patients with HNSCC harbored lower intra-tumoral immune cells overall as compared to Whites (Fig. 5A). The variation in immune infiltration between race was significant when White patients with HNSCC were stratified by HPV status (AA v White HPV^{+ve}: CD8, $p = 0.01$; resting memory CD4 T cells, $p = 0.01$; Tregs, $p = 0.01$; $\gamma\delta$ T cells, $p = 0.009$; Tfh cells, $p = 0.01$; resting NK cells, $p = 0.01$; activated NK cells, $p = 0.01$; neutrophils, $p = 0.29$). The type of immune infiltration was also significantly different amongst HPV^{-ve} and HPV^{+ve} Whites: CD8 T cells, $p = 0.001$; resting memory CD4 cells, $p = 0.005$, activated memory CD4, $p = 0.0007$; $\gamma\delta$ T cells, $p = 0.0008$; Tfh cells, $p = 0.001$; resting NK cells, $p = 0.004$; activated NK cells, $p = 0.003$. Furthermore, we also investigated if the variation in overall immune infiltration correlated with survival. To address this question we retrieved the curated histological images (H&E) of HNSCC tumors from the Cancer digital Slide Archive (<http://cancer.digitalslidearchive.net/>) (Fig. 5B) and examined the immune cell infiltration in AA and White HNSCC patients with longest and shortest survival (five each). H&E images of tumors indicated lower immune infiltration in patients with longer survival among AAs. However, this trend was reverse in White patients and higher immune infiltration correlated with better prognosis (Fig. 5C).

3.5. Connectivity Mapping (CMAP) analysis identifies a novel subgroup-specific chemotherapeutic drugs

Identification of small-molecule inhibitors can be used for therapeutic intervention in patients with the corresponding molecular profile. In order to identify novel drug candidates based on variations in tumor biology, we utilized the comprehensive cellular signatures from the Connectivity Map (CMAP) (<https://portals.broadinstitute.org/cmap/>) [27]. CMAP assists in identifying small-molecule inhibitors based on drug-gene signatures that associate with transcript perturbation in response to drug treatment. By using CMAP, we virtually identified a wide array of small-molecule inhibitors which were mostly unique for each race subgroup. However, we also identified four (L-690488, Neratinib, MT-21 and Selumetinib) novel inhibitors (Fig. 6A) common among races that can be potential chemotherapeutic molecule to decrease race-specific outcome.

4. Discussion

Racial disparities originate from inequality in patient care, health-related benefits, insurance coverage, treatment options, and other socio-economic factors that culminate to poor patient

outcomes in several cancers [10,12,30–36]. According to epidemiological studies, patients from African ancestry have higher disease susceptibility which is strongly associated with SES and behavioral practices. However, the poor prognosis in AA patients still existed even after adjustments for confounding factors [11–13], suggesting the presence of intrinsic factors in disparity. Hence, identification of race-related determinants of cancer susceptibility and disease prognosis may assist to address racial disparities in cancer outcomes. We therefore have systematically investigated the TCGA HNSCC dataset to characterize the differences in genomic, epigenomic, mutational and immune landscape among races. Further, based on DGE analysis, we employed CMAP to identify potential drug candidates to decrease race-specific disparity in therapy outcome.

EGFR, *PIK3C*, *NOTCH*, and *TP53* are the most frequently altered genes involved in HNSCC pathogenesis [37]. Previous studies on whole exome sequencing depicted comprehensive alterations in somatic mutation in HNSCC tumors with a distinct mutation pattern in HPV infected patients [24]. However, the variations in somatic mutation among races in HNSCC was not well defined. Our results demonstrate a higher mutation burden in AA HNSCC patients than Whites with increased frequency of well-known driver genes (*TP53*, *FAT1* and *CASP8*) [38]. Elevation in *TP53* mutation levels is very well illustrated in AA patients in various cancers [39–42]. Also, a previous analysis of 44 clinically relevant somatic mutations among high-risk HNSCC patients showed 100% of the cases to harbor *TP53* mutation along with mutation in other genes (*APC*, *ATM*, *ERBB4*, *GNAQ*, *KIT*, *RBI* and *ABL1*) [43]. In our study, AA HNSCC tumors harbored higher *TP53* mutation frequency than Whites which is consistent with previous retrospective study [22], however we did not observe any difference in survival outcome. Similar elevated frequency of *TP53* mutation was observed in AA HNSCC tumors with no survival difference [44]. However, there was a significant survival association in AA tumors when *TP53* mutation was combined with *PAX5* methylation pattern. The recent retrospective study by Wu et al. (2016) in 214 HNSCC patients (98 non-hispanic Whites, 72 Hispanic Whites and 44 AAs) identified tumor-related mutations in 10 genes (*CDKN2A*, *EGFR*, *FGFR3*, *HRAS*, *KRAS*, *MET*, *NOTCH1*, *PIK3CA*, *STK11* and *TP53*) [22]. Our mutation analysis in addition to consistently detecting 80% of these genes (except *KRAS* and *STK11*) also identified several novel genes with elevated mutation frequencies (>10%) in AA tumors (*FAT1*, *RIOK3*, *HEXA*, *ATRX*, *ATM*, *RECQ3*, *C3orf24*, *RAD50*, *RAD26L*, *ABL1*, *CCND3*, *MCM4*, *ITPR1*, *DDX43*, *IRF3*, *CCL4*, *CXCR5*, *MGAT5B*, *SGK223*, *HYAL4*, *APC*, *FOSI1*, *PRKCG*, *ROCK2*, *WIF1*, *EIF4B*, *IRS1*, *NPRL3*, *PRKCG*, *STRADB*, *TT11*, *REST1*, *IL13*, *IL13RA2*, *SOC55*, *TSLP*). However, the exact role of these genes in AA tumors are yet to be investigated. Interestingly, AA patients harbored a 10-fold higher *APC* mutation, a rare mutation in HNSCC patients compared to Whites. Albeit considering the elevated levels of mutation load in AA tumors it may possible that these tumors will be more susceptible to immune check-point blockade [10,45].

NOTCH signaling pathway is altered (mostly *NOTCH1* -inactivating mutation) in approximately 10–15% of HNSCC tumors which suggests its tumor suppressive role in HNSCC [46]. Typically HNSCC tumors with *NOTCH1* mutations have a worse disease prognosis compared to wild-type tumors but there are studies to suggest its bimodal function (tumor suppressor and oncogene) as *NOTCH* family members and their pathway genes

are upregulated during tumorigenesis [46]. We found that AA HNSCC harbored *NOTCH1* mutation at a higher frequency than Whites (HPV⁺) although it is unclear whether the mutation leads to its activation. However, the presence of activation mutation in *NOTCH1* in Asian cohort with HNSCC is well documented [46]. White patients harbor the highest proportion of HPV16, 18+/p16+ in oropharyngeal cancer compared to AAs [47]. As a result the HPV-related tumors represent with different mutational makeup in HNSCC [48]. Typically HPV⁻ HNSCC tumors harbor numerous mutations and chromosomal alternations [49]. This was also evident in our analysis where numerous genes with higher frequency were mutated in HPV⁻ tumors compared to HPV⁺. Such frequent mutation in these subsets of tumors may create neoantigens applicable for effective immunotherapies [49].

Immunogenicity of a tumor determines the response to immunotherapy [50,51]. Previous studies have showed overall higher immune infiltration in HPV⁺ patients and a differential spatial distribution of immune infiltrate types in HPV⁻ cases [10]. However, no studies yet define intratumoral immune infiltration among different races, which can be a beneficial predictive tool for response to immunotherapy. We observed that AA HNSCC patients harbor higher tumor mutational burden than White patients that may imply higher availability of neoantigens, leading to an ‘immune-hot’ phenotype. Interestingly, we observed that the AA HNSCC population harbors a significantly lower immune response as indicated by fewer intra-tumoral immune cells and lower immune-effector gene expression. A possible reason behind the ‘immune-cold’ condition in AA patient tumors despite their heightened mutational burden might be due to immunosuppressive microenvironment inside AA patient tumors, which either prevents immune infiltration or promote exhausted immune phenotype [10]. In general, proliferating tumor cells take up greater amounts of glucose than normal cells and produce lactate as a byproduct, in a process known as the Warburg effect. Consequently, the excess lactate turns the local microenvironment immunosuppressive. Our mutational analysis indicated that the highly mutated genes in AAs were associated with carbohydrate metabolism, which may further explain the high mutational burden but low tumor immune response in AA patients. Recent studies have shown that mutations in specific immune system genes or in specific domains of certain genes can decide the immune phenotype in various cancers [19]. The mutated protein domains in the cancer cells and expansion of the mutant cell by clonal proliferation eventually can result in an immunosuppressive state where the tumor-infiltrating lymphocytes are disarmed of their antitumor effects. Thus, deciphering the correlation between mutational status and immunometabolic phenotype in the HNSCC tumors of different races may facilitate patient stratification for future immunotherapy clinical trials.

The last decade has seen minimal improvement in the development of novel therapeutics or immunotherapy-based clinical trials in HNSCC [52–55]. This can be partially explained by the inefficient patient selection criterion arising from the variation in tumor etiology of different races. While conventional monotherapy has not benefitted patient outcomes, combination regimens have showed substantial toxicity hazards. From our global gene expression analysis, we identified differentially regulated genes in AA and White HNSCC patients. By exploiting the gene expression profile from each patient subgroup, we aimed to identify druggable molecular targets. Using CMAP, we identified several drug candidates

specific for AA and White subgroups alongside four potential drugs (L-69488, Neratinib, MT-21 and Selumetinib) that are predicted to improve patient outcome irrespective of race. Selumetinib is a selective non-ATP-competitive MEK1/2 tyrosine kinase inhibitor which is currently in phase I trial ([NCT01362803](#)) to determine the maximum tolerated dose and pharmacokinetics for the treatment of plexiform neurofibromas [56]. Selumetinib is shown to have no adverse effect even after long term dose-adjusted treatment and may present advantage against off-target effect. Majority of HNSCC tumors (>60%) harbors genomic alternations in the key receptor tyrosine kinases such as *PI3KCA* and *HRAS* that converge onto PI3K and MEK signaling pathways [57]. Like selumetinib, neratinib is another irreversible pan-HER family (HER1, 2 and 4) and EGFR tyrosine kinase inhibitor that targets PI3K/AKT and MAPK signaling. Neratinib is in phase II trial for HER2-positive breast cancer ([NCT03289039](#)) and HER-2 positive patients with brain metastasis ([NCT01494662](#)). In addition, phase III trial with neratinib significantly increased the five-year invasive disease free survival in breast cancer patients who previously completed the trastuzumab-based adjuvant therapy [58–60]. The altered mutation frequencies in *HRAS* and *PI3KCA* in the tumors of different race may result in aberrant PI3K/RAS/MEK and downstream signaling. In this context, selumetinib or neratinib may present a high therapeutic prospect against such aberrant signaling to reduce tumor burden and disparity in survival outcome. Overall, the current integrated study comprehensively elucidate the genetic landscape and tumor biology in AA and White HNSCC patients (Fig. 6B).

4.1. Limitations and conclusions

The role of the genetic landscape in cancer susceptibility cannot be denied [11–13], although it is not well established in HNSCC racial disparity. Therefore, we analyzed the TCGA HNSCC dataset to study the possible genetic link between race and cancer susceptibility. To our knowledge, this is the largest dataset [24] that contains 48 patients of AA origin, however, only nine were curated for viral status. In spite of considering this low number of AA patients as the major limitation of the current study, it is tempting to believe that this study provides a comprehensive understanding of inherent factors that influence the survival disparity in HNSCC patients. This study details the importance of mutation landscape and immune milieu of HNSCC patients from a different race in determining disease aggressiveness and impaired therapy response. The observations from this study characterize the somatic mutations and immune cell composition that may help in understanding the disease etiology in AA and White patients. Moreover, this study forms a stepping-stone for future research in identifying the screening modalities and therapeutic regimens to decrease race-specific disparity and thereby improving the patient outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Schema of TCGA HNSCC disparity analysis and survival disparity in several cancers. A. Flow chart describing methodology for analyzing The Cancer Genome Atlas (TCGA) and Cancer Digital Slide Archive of Head and Cancer Squamous Cell Carcinoma (HNSCC) patients (<http://cancer.digitalslidearchive.net/>). B. Five-year Relative Survival in percentage by Cancer Type and Race (both sexes included) (<https://gis.cdc.gov/Cancer/USCS/DataViz.html>). Data compiled based on 39 registries from the National Program of Cancer Registries (NPCR). Estimates are based on cases reported by NPCR registries from 2001 to 2014 and follow-up of patients through 2014. C. Survival of TCGA head and neck patients in days stratified based on race. AA, African American; HPV^{-ve} White, human papilloma virus-negative White; HPV^{+ve} White, human papilloma virus-positive White.

Fig. 2.

Somatic mutation spectrum across race. A. Mutations per mega base observed in TCGA HNSCC patients of different races. B. Differential mutation spectrum across races categorized based on molecular function. C. Patient survival based on mutation presence or absence in specific genes across races. AA, African American; HPV^{-ve} White, human papilloma virus-negative White; HPV^{+ve} White, human papilloma virus-positive White, HNSCC, Head and Neck Squamous Cell Carcinoma.

Fig. 3.

Differential gene expression and pathway enrichment across race. A. Heat map with hierarchical clustering of differentially expressed genes (DGEs) (top 100 up and down, 200 genes in total) in AA and White HNSCC patients. B. Venn diagram of DGEs in patient subgroups. C. Pathway analysis of DGEs based on Gene Ontology (GO). The negative log₁₀ of p values of various pathways identified using ConsensusPathDB (<http://cpdb.molgen.mpg.de>) was plotted using GraphPad Prism 5.00. AA, African American; HPV^{-ve} White, human papilloma virus-negative White; HPV^{+ve} White, human papilloma virus-positive White.

Fig. 4. Differential methylation spectrum across race and its correlation with gene expression. A. Beta values of differentially methylated genes normalized with values from normal group were represented as volcano plots. B. Correlation plots (Pearson's correlation) between methylation and gene expression. AA, African American; HPV^{-ve} White, human papilloma virus-negative White; HPV^{+ve} White, human papilloma virus-positive White.

Fig. 5.

Differential expression of immune response genes and immune infiltration across race.

A. Differential expression of immune response genes identified based on the 'Newman immune signature' using CIBERSORT (<https://cibersort.stanford.edu>). B. Hematoxylin and eosin images (extracted from Cancer Digital Slide Archive) depicting differential immune infiltration across HNSCC tumors stratified according to race. C. Patient survival based on immune cell infiltration. Immune infiltration scores for five longest and shortest survivors are plotted across race to determine association with patient survival. AA, African American; HPV^{-ve} White, human papilloma virus-negative White; HPV^{+ve} White, human papilloma virus-positive White. * < 0.05.

Fig. 6.

Connectivity Map analysis based on differential gene perturbations across race. A. Venn diagram depicting drugs identified for each racial group. Four common drugs identified across race. AA, African American; HPV⁻ve White, Human papilloma virus-negative White; HPV⁺ve White, human papilloma virus-positive White. B. Molecular determinants such as overall mutation load, mutational frequency in key driver genes, expression and methylation status of genes, and variation in immune response were correlated with survival disparities among AA and White HNSCC patients. Differential gene expression-based CMAP was exploited to identify drugs or small molecule inhibitors for precision medicine which are projected to reduce racial disparity in therapeutic outcome of HNSCC patients.

Table 1

Clinicopathological characteristics of HNSCC patients from TCGA data set.

	AA	HPV ^{-ve} White	HPV ^{+ve} White	Total
Total, n (%)	9 (8.73)	66 (64.07)	28 (27.18)	103
Sex, n (%)				
Male	7 (77.77)	50 (75.75)	25 (89.28)	82 (79.61)
Female	2 (22.22)	16 (24.24)	3 (10.71)	21 (20.38)
Age (Median in years)	59	60	56.5	
<40 years	0	5 (7.57)	0 (0)	5 (4.85)
40–55 years	3	17 (25.75)	10 (35.71)	30 (2.91)
>55 years	6	44 (66.66)	18 (64.28)	68 (66.01)
Stage, n (%)				
I	0 (0)	4 (6)	2 (7)	6 (5.82)
II	0 (0)	11 (16.66)	5 (17.85)	16 (15.53)
III	1 (11.11)	13 (19.69)	3 (10.7)	17 (16.50)
IVA	7 (77.77)	37 (56)	18 (64.28)	62 (60.19)
IVB	1 (11.11)	0 (0)	0 (0)	1 (0.09)
Alcohol History, n (%)				
YES	6 (66.66)	50 (75.75)	21 (75)	77 (74.75)
NO	3 (33.33)	14 (21.21)	6 (21.42)	23 (22.33)
Tobacco Smoking ^a (%)				
1	1 (11.11)	10 (15.15)	8 (28.57)	19 (18.44)
2	5 (55.55)	28 (42.42)	6 (21.42)	39 (37.86)
3	0 (0)	11 (16.66)	4 (14.28)	15 (14.56)
4	2 (22.22)	17 (25.75)	10 (35.71)	29 (28.15)

^aPack years smoked.