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Review

Analysis of the Mutational Landscape for Head and Neck Squamous Cell Carcinoma in Africans

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ABSTRACT

Reports suggest that there may be differences in the genetic causes of cancer based on ethnic variations in mutations. However, there is a lack of research on the mutational landscape of head and neck squamous cell carcinoma (HNSCC) in African populations to determine if there are any differences among African patients. This study aims to analyze the mutational landscape of HNSCC in Africans by reviewing available data and identifying any unique mutational patterns or distinctions compared to Caucasian data. The search methodology was based on the PRISMA guidelines checklist. MeSH terms and keywords were used and the Cancer Genome Atlas (TCGA) portal was used to analyze data related to the study objective. The TCGA study evaluated 48 African HNSCC samples. The PIK3CA hotspot mutations E545K, E542K and H1047R recorded the highest mutation implicated in Caucasian cohort but were almost non-existence in the African cohort. Equally relevant were the CDKN2A and TP53 mutations recorded amongst the Caucasian cohort were not detected in the African cohort, rather the African cohort recorded no R80* and one R85* - CDKN2A mutations and none of the TP53 mutations seen in Caucasian. In conclusion, studies on HNSCC candidate genes needs to be undertaken in Africa with more of these studies in Sub-Saharan Africa.

Keywords: *Head and neck cancer; Somatic mutations: Sequencing; SNP Arrays*

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INTRODUCTION

In recent years, advances in genomics coupled with innovation in bioinformatics have propelled a better understanding of head and neck squamous cell carcinoma (HNSCC) angiogenesis, tumour progression, and metastasis. There has also been recent advances in the management of HNSCC using targeted therapy. Despite these innovations, the five-year overall survival for stage I and IV HNSCC remains at ~ 80% and ~ 20% respectively thus, screening and early detection are necessary if morbidity and mortality rates are to decrease (Omura, 2014). Head and neck squamous cell carcinomas (HNSCC) belong to the group of malignant tumours in the head and neck region developing from the mucosal lining of the Oral cavity, Paranasal sinuses, Larynx, Nasopharynx, other Pharynx, and Nasal with the heterogeneity of these sites contributing to their individual aetiological and phenotypic characteristics. While the burden of HNSCC worldwide varies from region to region, it remains the sixth most prevalent cancer and the sixth most frequent cause of cancer-related deaths worldwide (Ferlay et al., 2015). Approximately 900,000 new cases and 450,000 deaths are recorded worldwide every year and this is expected to rise by 30% by 2030 (Shield et al., 2017, Bray et al., 2018). Oral squamous cell carcinoma (OSCC) constitutes >50% of HNSCC and develops from the lining of the alveolus, buccal mucosa, floor of mouth, palate, tongue, and other part of the oral cavity. Approximately 450,000 cases with 170,000 deaths are recorded annually (Bray et al., 2018). OSCC has generally been associated with tobacco and alcohol derived carcinogens but there remains no complete understanding of the molecular correlation between nicotine subunits and minor tobacco alkaloids that generates tobacco-specific N-nitrosamines, which is susceptible to oral cancer (Bhat et al., 2018, Rajagopalan et al., 2018). Conversely, oropharyngeal tumours have been linked to high risk human papilloma virus (HR HPV) 16 and to a lesser extent 18 plus other strains (Stein et al., 2015). While the correlation of E6/E7 oncovirus protein expression, to the genotoxic activity of HPV 16/18 in oropharyngeal cancer has been established (Abreu et al., 2012, Leemans et al., 2011), 3-5% of OSCC have transcriptionally active HR HPV subtypes (Upile et al.). Except for HR HPV status used for triaging and prognosis, quite a number of these molecular risk factors have limited clinical use. The Prognostic assessment for HNSCC is by evaluation of the

primary site, staging and histological characteristics of the tumour, although molecular risk factor assessment in HPV status is being implemented (Abreu et al., 2012). Despite surgery, radiotherapy, and chemotherapy, about half of HNSCC cases die of the disease.

HNSCC are a diverse group of cancers with varying aetiology and genetic alterations. Several studies have investigated the genomic landscape of HNSCC using high throughput methods and have reported significant single nucleotide polymorphisms (SNP) and copy number variations (CNV) in candidate genes as well as distinct genetic landscape associated with HPV status (2015, Agrawal et al., 2011, Stransky et al., 2011). These candidate genes comprise; TP53 (40-60%), CDKN2A (12-16%), NOTCH1 (10-12%), PIK3CA (8%), FAT1 (5-8%), HRAS (5-8%), CASP8 (5-8%) and PTEN (3-7%) (2015, Stransky et al., 2011). There has been reports of dissimilarities in genetic aetiology of cancer based on ethnic induced distinctions in mutations (Kuguyo et al., 2018) and to this effect, very little if any paper on the mutational landscape of the African population HNSCC has been reported, to examine if any distinction exists amongst African HNSCC patients.

This study aims to examine the mutational landscape of HNSCC amongst Africans, through review of available data and to interrogate for unique mutational pattern or distinctions compared to the Caucasian data.

MATERIALS AND METHODS

Search Strategy: The search methodology was modelled from the checklist of PRISMA guidelines (Page et al., 2021). Cochrane style MeSH terms and keywords comprising; head and neck squamous cell carcinoma, whole exome sequencing (WES), whole genome sequencing (WGS), single nucleotide polymorphism (SNP), SNP arrays, genome analysis, African region, and countries; were used for the initial search by the author through the search tools; Pubmed, Ovid Medline and Web of science. Where appropriate, the cancer genome atlas (TCGA) portal (TCGA) was used to undertake analysis related to this study objective.

Eligibility criteria: English only publications were reviewed, and studies considered were - cross sectional studies, and case control studies. Publication titles and abstracts were reviewed to identify articles suitable for this review and selected manuscripts were proofread with results extracted. All studies must use contemporary or established sequencing methods and bioinformatics for data generation. Methylation and biomarker papers, other omics papers besides genomics, purely clinical or pathological papers, conference papers and abstracts only articles were excluded.

Data Extraction and Analysis: All results were extracted onto a data form for tabulation. Studies that included African samples were further analysed for frequently mutated genes and mutations, and these were compared to Caucasian data. SPSS 27 software package (IBM Company, Armonk, NY, USA) was used for statistical analysis. Sample size, mutation frequency of individuals with specimens, were captured for descriptive statistical information.

RESULTS

The electronic search yielded 182 entries; of which 147 publications were removed for having no correlation to the set search objective and study duplicity, resulting in 35 publications being eligible for full text review. Of these, 8 studies (2015, Al-Hebshi et al., 2016, Su et al., 2017, Consortium, 2013, Agrawal et al., 2011, Stransky et al., 2011) met the eligibility criteria and were included in the study, while 27 publications were removed for being biomarker, methylation, and other omics related papers (Figure 1). The studies cumulatively used 908 HNSCC samples for WES and WGS (Illumina) and SNP arrays (Affymetrix) (Table 1). Four (Su et al., 2017, Pickering et al., 2013, Consortium, 2013, Al-Hebshi et al., 2016) of the eight studies were purely OSCC sequencing studies and only one study (2015) recorded HNSCC samples from Africans. The TCGA HNSCC study (2015) used 527 samples categorized into 451 Whites, 48 Africans and African descent (AAAD), 11 Asians, 2 Indians and 15 unreported.

According to the TCGA study, a total of 20,113 genes with 86,906 mutations were implicated in all 527 cases of which 20,068 genes with 76,340 mutations were implicated in 451 Caucasians and 19,324 genes with 6,930 mutations were implicated in 48 AAAD. The first thirteen HSNCC candidate genes (gene mutations usually implicated in HNSCC) were tabulated across Caucasians and AAAD and their incidences appear to be within their population differential (Table 2).

Table 1.
Genomic sequencing studies - HNSCC characteristics

No.	Studies	Sequencing Method	Sample size	Population
1.	Agrawal et al. 2011	WES (Illumina)	32	Caucasian
2.	Stransky et al 2011	WES & WGS (Illumina), SNP array (Affymetrix)	74	Caucasian
3.	Lui et al. 2013	WES (Illumina)	45	Caucasian
4.	Pickering et al 2013	WES (Illumina), SNP Arrays (Affymetrix)	40	Caucasian
5.	Indian project team ICGC 2013	WES (Illumina)	50	Caucasian
6.	TCGA 2015	WES & WGS (Illumina), SNP array (Affymetrix)	527	Caucasian / Africans
7.	Al-Hebshi et al. 2016	WES (Illumina)	20	Arabian
8.	Su et al. 2017	WES & WGS (Illumina)	120	Taiwanese

Key: ICGC= International Cancer Genome Consortium; TCGA

Table 2:
Frequently mutated genes from TCGA study

No.	Genes	Caucasians (n=464)	AAAD (n=48)
1.	*TP53*	68%	83%
2.	*FAT1*	23%	15%
3.	*CDKN2A*	21%	15%
4.	*MUC16*	20%	30%
5.	*CSMD3*	20%	23%
6.	*PIK3CA*	19%	8%
7.	*NOTCH1*	19%	19%
8.	*LRP1B*	18%	25%
9.	*KMT2D*	14%	19%
10.	*NSD1*	11%	13%
11.	*CASP8*	11%	<1%
12.	*HRAS*	6%	<1%
13.	*PTEN*	<1%	<1%

In addition, the top three mutations with the highest number of case percentage per cohort, were tabulated for Caucasians and AAAD (top two mutations was used for AAAD) (Table 2). The impact for each mutation was also reported with impacts classed as "low" were the amino acid change results in little or no structural protein change, "moderate" were the amino acid change results in limited structural protein change, or "high" where the resulting amino acid change altered the protein's secondary or tertiary structure, or inserted a "stop codon". Further details of 200 and 47 mutated cases for Caucasians and AAAD respectively, and the top 50 mutated genes by simple somatic mutation (SSM) for both, are illustrated in Supplementary data 1 and 2.

DISCUSSION

HNSCC are a diverse group of cancers with varying aetiology and genetic alterations. It can develop denovo or from a pre-malignant lesion. Califano et al. (Califano et al., 1996) in 1996 put forward the first multi-step genetic characterisation of the morphological changes in the oral squamous epithelium that gives rise to OSCC. He postulated that loss of heterozygosity (LOH) at chromosomes 3p, 9p and 17p was detected in oral epithelial dysplasia (OED), and this reflected early carcinogenesis. While alterations at chromosome 11q, 4q and 8 indicated late phase carcinogenesis. Another manifestation of genomic instability is DNA repair defect, causing an increased disposition to gene mutation, which is a known pathogenic route for tumorigenesis and malignant transformation (Pino and Chung, 2010, Yu et al., 2007). Patients with Fanconi anaemia (FA), a rare genetic disease caused by FANC gene mutation induced DNA repair impairment, are more inclined to develop OSCC by 500-700 fold (Velleuer and Dietrich, 2014).

Dissimilarities in gene alteration in cancer, based on ethnic induced distinction in mutations have been reported (Kuguyo et al., 2018) and little if any report has evaluated any distinction in the mutational landscape of the HNSCC in African population. Our group did conduct a review on the genetic determinants of HNSCC from an African population and concluded that a research gap exist in the implication of the current HNSCC candidate genes (Okoturo et al., 2020).

This research gap was quite surprising, considering the amount of studies that have been undertaken on HNSCC candidate gene catalogue amongst Caucasians (Tate et al., 2018). The largest genomic study to date on HNSCC is the TCGA where 527 HNSCC samples were sequenced, and their data evaluated (TCGA, 2015) and this present study was premised on this TCGA study.

Table 3.
Mutation frequency case percentage

		Caucasians		
No.	Genes & mutations	Percentage affected cases in caucasians (*n=434*)	Percentage affected cases in AAAD (*n=47*)	Impact
1.	*PIK3CA*			
	• E545K (23)	5.2%	-	Moderate
	• E542K (19)	4.3%	-	Moderate
	• H1047R (11)	2.5%	0.02%	Moderate
2.	*CDKN2A*			
	• R80* (22)	4.9%	-	High
	• R58*(9)	2.0%	0.02%	High
3.	*TP53*			
	• R175H (12)	2.7%	-	Moderate
	• G245S (8)	1.8%	-	Moderate
	• R248W (8)	1.8%	-	Moderate
	• R282W (8)	1.8%	-	Moderate
	• R248Q (8)	1.8%	-	Moderate
		AAAD		
	Genes	Percentage affected cases AAAD (*n=47*)	Percentage affected cases Caucasians (*n=434*)	Impact
1.	*TP53*			
	• Y236C (2)	4.3%	-	Moderate
2.	*NSD1*			
	• R788* (2)	4.3%	-	High

Key: Number of cases with mutation per cohort in parenthesis

While the gene analysis suggested similarities in the frequently mutated (candidate) genes in HNSCC, the mutation analysis tended to suggest a different event i.e., the PIK3CA hotspot mutations E545K, E542K and H1047R recorded the highest mutation implicated in Caucasian cohort but were almost non-existence in the AAAD cohort. Equally relevant were the CDKN2A and TP53 mutations recorded amongst the Caucasian cohort were not reciprocated as the AAAD cohort recording zero R80* and one R85* for CDKN2A mutations and none of the AAAD recorded any of the Caucasian TP53 mutations, rather recording a the Y236C mutation. This mutation is located on 17p, reported to regulate cell cycle and is also present in other cancers like lungs, breast (Liu X, 2015), and interestingly was not recorded in the Caucasian cohort.

Race induced genetic dissimilarity is due mainly to the distinct locus heterogeneity between ethnic groups, and bias premised on available data from Caucasian cases (Kuguyo et al., 2018, 2005). That Caucasians genome contribute only a subset to the human genome and do not fully characterize susceptible variants is well documented. This is particularly relevant considering Africans exhibit a higher genetic diversity and demonstrates a lesser gene linkage disequilibrium (2005). This was further exemplified in this study as 99% (20,068/20,113) of the total genes that recorded 88% (76,340/86,906) mutations were implicated in 451 Caucasians while 96% (19,324/20113) of the total genes which recorded 8% (6,930/86,906) of mutations were implicated in only 48 AAAD cases.

Several genetic disease studies have provided ample evidence of overlap in disease-causal alleles, between ancestry groups and diverse communities (Waters et al., 2010, Saxena et al., 2012). This same overlap of the underlying causal allele at several loci across ancestry, is reported to improve the power to detect disease susceptible loci and improve causal variant mapping (Mahajan et al., 2014). Our group did publish a comprehensive catalogue of susceptible genes and polymorphisms of cancers amongst Africans to create awareness and we recorded limited reports on HNSCC candidate alleles (Okoturo E, 2020). While there are several reports on HNSCC candidate genes (Stransky et al., 2011, Agrawal et al., 2011), there remains no catalogue of implicated genes of HNSCC amongst Africans.

In conclusion, studies on HNSCC candidate genes needs to be undertaken in Africa with more of these studies in Sub-Saharan Africa. Importantly, these studies should be large scale with multiple HNC sites and employing high throughput methods for improved causal mapping.

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