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Bat 26 Microsatellite Instability in Oral Cavity Cancers in Senegal

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Abstract

Oral cavity cancers are part of head and neck cancers. They have become frequent in the world in general and Senegal in particular. This study evaluates microsatellite instability tumors in oral cavity cancers in Senegal. Forty cancerous tissues, 20 healthy tissues, and 12 blood tissues were included in this study. These tissues were collected from each patient during the biopsy after obtaining consent. DNA extraction, Polymerase Chain Reaction (PCR) and sequencing were carried out to obtain sequences. Mutation surveyor, Bioedit and Dnasp software were used to perform our analyses. High instability was found in 57.5% of patients with cancer. Moreover, 90% of the patients had the same motif on healthy and cancerous tissue. Furthermore, 26.12%, 20.72%, and 11.71% polymorphic sites were found in cancerous, healthy and blood tissue respectively. Thus, a similarity between cancerous and healthy tissues seems to exist. This implies that instability of the Bat 26 microsatellite could occur early in the occurrence of oral cavity cancers.

Keywords

Cancer, Oral Cavity, Microsatellite Instability, Bat 26, Senegal

1. Introduction

Oral cavity cancers are part of the head and neck cancers (HNCs) which remain a major clinical challenge in oncology [1]. Worldwide, HNC was the third most common cancer in 2020. Approximately 1,536,031 patients are diagnosed with HNC, and 1,011,201 die from it each year [2]. Men appear to be significantly

more likely to develop these cancers than women do, with an average age of diagnosis of 50 to 70 years [3]. In recent years, cancers of the oral cavity have become very common in Senegal. Each year, approximately 177 Senegalese develop oral cancer, and 114 die from it [2]. In view of this high mortality, the prognosis of patients with these cancers has not significantly improved in recent years despite the strengthening of diagnostic and therapeutic approaches. This failure is essentially due to a marked clinical heterogeneity of the biological behaviour of these tumours, late diagnosis, lymph node metastasis, and recurrences, driving a high mortality rate of this disease [4]. Moreover, these tumours are observed as a multifactorial disease whose aetiology involves environmental, genetic, and epigenetic factors [5]. Multiple molecular and genetic alterations have been described in these cancers [6]. However, the rate of random mutational events alone cannot explain the number of genetic alterations found in most cancers [7]. For this reason, it has been suggested that microsatellite instability (MSI) may be a prerequisite for the onset of carcinogenesis. *Microsatellites* are DNA segments in which a short motif (usually one to five nucleotides long) is repeated five to 100 times. Their *instability* is defined as the presence of replication errors resulting from base insertions or deletions in nucleotide repeats called microsatellite regions [8]. This phenomenon is generally linked to the dysfunction of mismatch repair genes, in particular MLH1 and MSH2 [6]. MSI is thus considered a biological and molecular factor that has recently been proposed as a prognostic marker for colorectal cancer [9]. The selection of appropriate genetic markers is vital for MSI testing; Bat 26 is one of the most used markers for this purpose. It contains polyadenine repeats, usually 26 adenine repeats, and is considered a quasi-monoform. Therefore, the detection of MSI will be useful in the molecular screening of cancer with genetic or epigenetic mismatch repair defects and thus provide information for the choice of diagnostic and therapeutic modalities [10]. It is in this context that our study evaluates MSI tumours in oral cavity cancers in Senegal.

2. Methodology

2.1. Samples

The study was conducted on Senegalese patients with histologically proven oral cancer treated at the Maxillofacial and Stomatology Department of the Aristide Le Dantec Hospital from February 2021 to July 2022, after obtaining approval from the Cheikh Anta Diop University research ethics committee. In this study, the inclusion criteria were as follows:

- ✓ Senegalese;
- ✓ Diagnosed with cancer of the cavity;
- ✓ Regularly followed in a hospital center;
- ✓ Having signed the consent form.

Non-Senegalese patients were excluded from the study**.** Forty (40) individuals with cancer numbered from 1 to 40 and 52 controls numbered from 1 to 52 were

included in this study. All patients were informed about the study in order to obtain their consent before to recruitment. Cancerous tissue, healthy tissue, and peripheral blood were collected from each patient during the biopsy. Blood samples were also taken from control cases.

2.2. DNA Extraction, Polymerase Chain Reaction and Sequencing

DNA from tissues and blood was extracted with the Zymo Research Kit following the manufacturer's instructions for each biological material. The Bat 26 microsatellite was amplified using the primer pair (F 5'-CTGCGGTAATCAAGTTTTTAG3' and R 5'-AACCATTCAACATTTTTAACCC-3'). A reaction volume of 25 µl containing 12.5 µl Master Mix, 1 µl $MgCl_2$, 0.5 µl of each primer, 8.5 µl MilliQ water, and 2 µl cDNA was used. The polymerase chain reaction was performed under the following conditions: initial denaturation at 95˚C for 5 min; 35 cycles of denaturation at 95˚C for 30 s, hybridisation at 47˚C for 1 min, and elongation at 70˚C for 1 min; and final elongation at 70˚C for 10 min. After visualisation under blue light, the polymerase chain reaction products for which the primers hooked were purified and sequenced with an ABI Big Dye Terminator Sequencing Ready Reaction Kit and an ABI PRISM 3730 × l sequencer (Applied Biosystems, Foster City, CA, USA).

2.3. Genetic Analyses

2.3.1. Search for Mutations

The raw sequences were submitted to Mutation Surveyor version 5.0.1 (http://www.sofgenetics.com/), which compares the submitted chromatograms with the reference sequence to determine the presence of any mutation and its position relative to the gene. We used the reference sequence of the hMSH2 gene available in GENBANK with the accession number AH003235.2. The Mutation Surveyor software offers excellent accuracy and sensitivity as well as low false-positive and false-negative rates in DNA analysis the score indicates the level of confidence that a mutation is real. Here we have considered a mutation to be true if the Phred score ≥ 20, for which the accuracy is 99% (https://softgenetics.com/products/mutation-surveyor/).

2.3.2. Microsatellite Instability Analysis

The sequences obtained were carefully checked, aligned, and corrected using the Bioedit software version 7.1.9 [11]. Alignment highlights the similarities between sequences by finding the position of probable deletions or insertions. In view of the difference in the microsatellite repeat motif between the available reference sequences and our study population, a consensus sequence was constructed from our control samples. Thus, MSI was defined as positive when the sequence of any tissue of a patient showed different sizes or mutations from the consensus sequence. An individual analysis was performed between the tissues (cancer, healthy and blood) of the same individual, to determine if the instability was the same

between tissues. We also performed a population analysis to evaluate the frequency of haplotypes in each population.

2.3.3. Genetic Diversity Analysis

An analysis between cancerous tissues, healthy tissues, and blood was performed to identify the number of sites (*N*), the sample size (*n*), the number of variable sites, the number of non-informative sites, the number of informative sites, the total number of mutations (Eta), the number of haplotypes, the average number of nucleotide differences between sequences (k), the nucleotide diversity (π), and the haplotype diversity (Hd). These parameters were determined with DnaSP software version 5.10 [12]. The total number of haplotypes in all populations was output to see which haplotypes were shared by cancerous, healthy, and blood tissues.

3. Results

3.1. Bat 26 Microsatellite Polymorphism

Microsatellite SNP

Sixteen (16) Bat 26 microsatellite variants were found, as shown in Figure 1. The polymorphisms 2397T > A, 2490A > AG, 2491A > AG and 2494G > GT were frequently observed, with respective percentages of 27.32%, 22.5%, 30%, and 20% of cancer patients. The first interruption of the repeat pattern was caused by the 2490A > AG polymorphism and, the second interruption, by the 2491A > AG polymorphism. In patients with a mutation is present in cancérious tissue and health tissue, the score was higher in the cancerous tissue than in the adjacent healthy tissue. None of these mutations has yet been listed in the COSMIC and dbSNP databases.

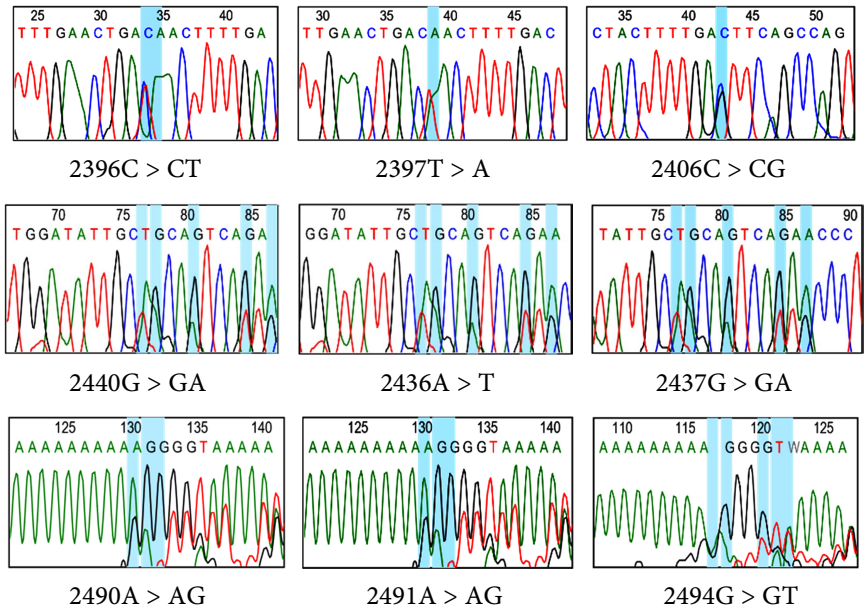

Figure 1. Some Bat single nucleotide polymorphisms in Senegalese patients.

3.2. Repeat Motif Instability

3.2.1. Individual Analysis

Of the 40 cancer patients, 20 had cancerous tissues and healthy tissues and 12 had cancerous tissues, healthy tissue and blood. Ninety percent of the patients had the same motif for cancerous and healthy tissues. On the other hand, 37.5% of the patients showed this resemblance for the cancerous, healthy and blood tissues. Cancerous tissue seems to be genetically closer to the healthy tissue than to blood, given the high frequency of similarity between cancerous tissues and health tissues. These results are shown in Table 1.

3.2.2. Population Analysis

MSI was noted in cancerous tissue, healthy tissue, and blood with a difference in size and pattern (Table 2). The haplotype of the consensus sequence (25A + SubA26 → G) was found in 37.5% of cancerous tissues, 35% of health tissues, and 33.33% of blood. Two cancer patients (5%) showed Bat stability with 26A. The haplotype (AA Del A3 22 + SubA26 → G) was predominantly represented with 30% in cancerous tissues, 20% in healthy tissues, and 41.66% in blood tissues. A shortening of the motif was constantly noted with a deletion of the third and fourth adenines in 37.5% of cancerous tissues 50% of healthy tissues and 58.33% of blood. A remarkably high frequency of mutation of the 26th adenine (A) to guanine (G) was found in our populations with 67.5% of cancerous tissues, 85% of health tissues, and 81.66% of blood tissues.

3.3. Genetic Diversity

Of the 111 sites sequenced, 26.12%; 20.72% and 11.71% polymorphic sites were found on cancerous tissue, healthy tissue, and blood respectively. For cancerous tissues, 55.17% were informative sites in parsimony with a total number of 36 mutations (Eta) and an average nucleotide difference (K) of 4.48. Moreover, 47.82% of the polymorphic sites were informative sites in parsimony in healthy tissue with a total number of 23 mutations (Eta) and an average nucleotide difference (K) of 4.86. For blood, 46.15% were polymorphic sites in parsimony with 14 mutations and an average nucleotide difference (K) of 3.87. This analysis reveals that blood is less polymorphic (11.71% polymorphic sites) compared to cancer and healthy tissues. The strong haplotypic diversity indices and the weak nucleotide indices (cancerous tissue: Hd = 0.963 and π = 0.042, healthy: Hd = 0.958 and π = 0.046, and blood: Hd = 0.909 and π = 0.034) indicate that the microsatellite polymorphism is rapidly evolving in Senegalese patients with oral cavity cancer. These results are shown in Table 3.

3.4. Haplotypes

Table 4 groups the different haplotypes found between cancerous, healthy, and blood tissues. Thirty-seven (37) haplotypes were identified, of which the majority haplotype, H9, represents 12.5% of the cancerous tissues, 20% of the tissues healthy, and 8.33% of the blood, followed by haplotype H10, which represents

Table 1. Individual analysis of the microsatellite.

Individuals	TC	TS	Sg
37	AAAAAAAAAAAAAAAAAAAAAAAAAA	AAAAAAAAAAAAAAAAAAAAAAAAAA	NS
35	AAAAAAAAAAAAAAAAAAAAAAAAAG	AAAAAAAAAAAAAAAAAAAAAAAAAG	NS
15	AAAAAAAAAAAAAAAAAAAAAAAAGG	AAAAAAAAAAAAAAAAAAAAAAAAGG	AAAAAAAAAAAAAAAAAAAAAAAAAG
10	AAAAAAAAAAAAAAAAAAAAAAAAAG	AAAAAAAAAAAAAAAAAAAAAAAAAG	NS
17	AAAAAAAAAAAAAAAAAAAAAAAAAG	AAAAAAAAAAAAAAAAAAAAAAAAAG	NS
19	AAAAAAAAAAAAAAAAAAAAAAAAAG	AAAAAAAAAAAAAAAAAAAAAAAAAG	AAAAAAAAAAAAAAAAAAAAAAAAAG
20	AAAAAAAAAAAAAAAAAAAAAAAAAG	AAAAAAAAAAAAAAAAAAAAAAAAAG	AAAAAAAAAAAAAAAAAAAAAAAAAG
21	AAAAAAAAAAAAAAAAAAAAAAAAAG	AAAAAAAAAAAAAAAAAAAAAAAAAG	NS
27	AAAAAAAAAAAAAAAAAAAAAAAAAG	AAAAAAAAAAAAAAAAAAAAAAAAAG	AA-AAAAAAAAAAAAAAAAAAAAAAAG
32	AAAAAAAAAAAAAAAAAAAAAAAAAG	AAAAAAAAAAAAAAAAAAAAAAAAAG	NS
34	AAAAAAAAAAAAAAAAAAAAAAAAAG	AA-AAAAAAAAAAAAAAAAAAAAAAAA	AA-AAAAAAAAAAAAAAAAAAAAAAAG
11	AA-AAAAAAAAAAAAAAAAAAAAAAAG	AAAAAAAAAAAAAAAAAAAAAAAAAG	AA-AAAAAAAAAAAAAAAAAAAAAAAG
12	AA-AAAAAAAAAAAAAAAAAAAAAAAG	AA-AAAAAAAAAAAAAAAAAAAAAAAG	AAAAAAAAAAAAAAAAAAAAAAAAAG
18	AA-AAAAAAAAAAAAAAAAAAAAAAAG	AA-AAAAAAAAAAAAAAAAAAAAAAAG	AA-AAAAAAAAAAAAAAAAAAAAAAAG
13	AA-AAAAAAAAAAAAAAAAAAAAAAAG	AA-AAAAAAAAAAAAAAAAAAAAAAAG	NS
29	AA-AAAAAAAAAAAAAAAAAAAAAAAG	AA-AAAAAAAAAAAAAAAAAAAAAAAG	AA-AAAAAAAAAAAAAAAAAAAAAAAG
33	AA-AAAAAAAAAAAAAAAAAAAAAAAG	AA-AAAAAAAAAAAAAAAAAAAAAAAG	NS
8	AA--AAAAAAAAAAAAAAAAAAAAAAG	AA--AAAAAAAAAAAAAAAAAAAAAAG	AA-AAAAAAAAAAAAAAAAAAAAAAGG
27	AA--AAAAAAAAAAAAAAAAAAAAAGG	AA--AAAAAAAAAAAAAAAAAAAAAAG	AA--AAAAAAAAAAAAAAAAAAAAAAG
7	AAAAAAAAAAAAAA-	AAAAAAAAAAAAAA-	AAAAAAAAAAAAAA-

Note. TC = cancerous tissue, TS = healthy tissue, Sg = Blood, and NS = none sequenced.

Table 2. Population analysis of the microsatellite.

Motifs	Haplotypes	TC (40) effective percentage	TS (20) effective percentage	Sg (12) effective percentage
AAAAAAAAAAAAAAAAAAAAAAAAG	**25A + SubA26 → G (Consensus)**	**15** **37.5%**	**7** **35%**	**4** **33.33%**
AAAAAAAAAAAAAAAAAAAAAAAAA	26A	2 5%	1 5%	0 0%
AAAAAAAAAAAAAAAAAAAAAAAAGG	24A + SubA25 - 26 → G	4 10%	0 0%	0 0%
AAAAAAAAAAAAAAAAAAAAAAAGGG	23A + SubA23 - 26 → G	1 2.5%	1 5%	0 0%
AA-AAAAAAAAAAAAAAAAAAAAAA	AA Del A3 + 23A	1 2.5%	1 5%	0 0%
AA-AAAAAAAAAAAAAAAAAAAAAAG	AA Del A3 + 22A + SubA26 → G	12 30%	4 20%	5 41.66%
AA--AAAAAAAAAAAAAAAAAAAAG	AA Del A3 - 4 + 21A + SubA26 → G	1 2.5%	2 10%	1 8.33%
AA--AAAAAAAAAAAAAAAAAAAGG	AA Del A3 - 4 + 20A + SubA25-26 → G	1 2.5%	2 10%	0 0%
AA-AAAAAAAAAAAAAAAAAAAAGG	AA Del A3 + 21A + SubA25-26 → G	0 0%	1 5%	1 8.33%
GAGAAAAAAAAAAAAAAAAAAAAAG	SubA1 - 3 → G + 22A + SubA26 → G	1 2.5%	0 0%	0 0%
AAAAAAAAAAAAAA-	14A Del A15 - 26	2 5%	1 5%	1 8.33%

Note. TC = cancerous tissue, TS = healthy tissue, and Sg = Blood.

Table 3. Genetic diversity parameter.

	TC	TS	Sg
Genetic diversity parameter			
Sample size	40	20	12
Numbre of variable sites	29 (26.12%)	23 (20.72%)	13 (11.71%)
Non informatifs sites	13 (44.82%)	7 (30.43%)	7 (53.38%)
Informatifs sites	16 (55.17%)	11 (47.82%)	6 (46.15%)
Total number of mutation (Eta)	36 (32.43%)	23 (20.72%)	14 (12.61%)
Number of haplotypes (h)	25	15	8
Average number of nucleotide differences (K)	4.48	4.86	3.87

Continued

Genetic diversity indice			
Haplotype diversity indice (Hd)	0.963	0.958	0.909
Nucleotide diversity indice (π)	0.042	0.046	0.034

Note. TC = cancerous tissue, TS = healthy tissue, and Sg = Blood.

Table 4. List of haplotypes.

Haplotypes	TC	TS	Sg
H1	1	-	-
H2	-	1	-
H3	1	-	-
H4	5	2	-
H5	1	-	-
H6	1	-	-
H7	-	1	-
H8	1	-	1
H9	5	4	1
H10	3	2	3
H11	-	1	-
H12	1	-	-
H13	2	-	3
H14	-	-	1
H15	1	-	-
H16	1	-	-
H17	3	-	-
H18	1	-	-
H19	-	1	-
H20	-	1	-
H21	1	-	-
H22	1	-	-
H23	2	-	-
H24	1	-	-
H25	2	-	-
H26	-	1	-
H27	-	-	1
H28	1	-	-

Continued

H29	-	1	-
H30	1	1	-
H31	1	-	1
H32	1	1	-
H33	-	1	1
H34	1	-	-
H35	1	-	-
H36	-	1	-
H37	-	-	1

Note. TC = cancerous tissue, TS = healthy tissue, and Sg = Blood.

7.5% of the cancerous tissues, 10% of the healthy tissues and 25% of the blood. Of the 37 haplotypes, only these two haplotypes group all three tissue types together, demonstrating the great genetic diversity that can exist between tissues in the same individual and between individuals with oral cancer. Nevertheless, there are haplotypes, such as H4, H30 and H32 that group only cancerous and healthy tissues, indicating some similarity between these two tissue types.

4. Discussion

In this study, the Bat 26 status was assessed in individuals with oral cancer in Senegal. A number of 16 SNP types were found on the microsatellite; these SNPs have not yet been listed in the databases or studies we consulted. This lack may be due to studies that are limited to genotyping only and are interested only in the repeated motif and not in the whole microsatellite. However, a mutation in microsatellites that fall within the exon, intron or UTR region of known cancer-associated genes could influence gene expression or protein function by affecting changes in gene transcription or splicing and contribute to generating carcinogenic mutations [13].

Our results showed that in some patients there may be a difference in instability between cancerous and blood tissues; furthermore, 90% of our patients had the same instability between cancerous and healthy tissues. Bat 26 instability was found in 57.5% of our patients with oral cancer. Our results are consistent with those of Demokan *et al.* [14] who found that 19% of their HNC patients were unstable at Bat 26, although there was a difference in incidence. De Schutter *et al.*'s studies from 2007 and 2008 [15] [16] revealed significantly low frequencies of MSI, 2% and 1.25% respectively. Thus both high and low frequencies of MSI in head and neck squamous cell carcinoma have been reported [15]. Differences in these results can be attributed to differences in patient selection, different types of cancers, different ethnic groups, and different sample sizes studied, especially given our study included only patients with cancers of the oral cavity. Thus, we

point out that this study was carried out only on patients with oral cavity cancers and with a sample size of 40 patients. Sometimes the lack of biological material and the non-consent of some patients can be an obstacle for sampling.

Referring to our consensus sequence (25A + SubA26 → G), we can stipulate that contrary to what has been retained in the literature for Bat 26, the Senegalese population may not have all 26 adenines of Bat. We found that the 26th adenine in the Senegalese population is mutated to guanine. This instability found in the population, in general, makes one assume a significantly high instability of the microsatellite in the Senegalese population. The frequency of polymorphism or genetic instability can vary from one population to another. A higher rate of polymorphism was found in the African population for each marker: Biaka Pygmies for NR 27 and Bat 26 (34.7% and 15.3% respectively), South African Bantus for NR 21 (18.8%), and the San for Bat 25 (57.1%). For NR 27, Bat 25 and Bat 26, the 11 populations with polymorphic frequencies above 10% are all from sub-Saharan Africa [17]. Polymorphisms in Bat 26 and BAT 25 have been reported previously for African-Americans and a small population of Nigerians [18] [19]. We can, therefore, suggest that there is a relatively high incidence of single nucleotide repeat polymorphisms in the African population.

Seemingly "normal" individuals in the population may have differences in their ability to repair DNA damage. There may, in fact, be definable sub-populations that have a reduced ability to repair damaged DNA. These individuals are more susceptible to DNA damage from carcinogens and are more likely to develop cancers [20]. We did not observe motif elongation, that is to say insertions, in our study population, but shortenings were observed in most individuals. This result suggests that these deletions have a role in the carcinogenesis process. Research in this window of the genome indicated that there was considerable variation in the extent of genomic alterations between different tumours. Among the genomic alterations, deletions were observed more frequently. Furthermore, the higher frequency of deletion compared to the appearance of new bands suggests that at least some of the deletions reflect true chromosomal loss rather than rearrangement. Continued instability in chromosome number and in the structure are consistent features of primary head and neck tumours and oral cancers, due to genetic instability that allows for an increased mutation rate in developing tumors [21].

We also found 26.12% polymorphic sites in cancerous tissue, 20.72% in healthy tissue, and 11.71% in blood. For blood, the mutations were found only on the part of the repeated motif, so blood seems to be less polymorphic than the other two tissues. The fact that metastasis occurs via the bloodstream and that the tumours we recruited were primary cancers could explain that blood is less polymorphic. On the other hand, we found mutations in both cancerous and healthy tissues, with a slightly higher number of mutations in the former. This result is in agreement with the results on haplotype groups where we have cancerous and healthy tissues that sometimes share the same haplotypes. During a biopsy, the healthy tissue is obtained from the side adjacent to the affected part of the can-

cer. In other words, if the disease affects the tongue or the lower lip, it seems appropriate to obtain the healthy tissue from the same organ (tongue or lip). As the oral cavity is a very small entity and some patients are late to be diagnosed, the supposedly healthy tissue may not be healthy at all and could be in the process of carcinogenesis. It would therefore be important to perform a pathological analysis on both tissues to confirm that the healthy tissue is not affected by the cancer.

El Naggar *et al.* [22] found that MSI was an important factor in the development of invasive squamous cell carcinomas. They studied the level of instability in DNA extracted from peripheral blood and fresh tissue of the tumour and adjacent dysplastic tissue in 20 patients with HNCs. This group found that MSI was absent from peripheral blood DNA. Instability was reported in 15% of the patients with pre-invasive lesions and 30% of patients with invasive cancer. They concluded that instability might develop as pre-neoplastic tumours progress to invasive carcinoma. From their data, MSI appears to be a tumor phenomenon and not an intrinsic host phenomenon. The rate of MSI was non-existent in DNA extracted from peripheral blood lymphocytes. We can also state that Bat 26 mutations can be an early stage in the cancer process. Cancers evolve over a variable period (ranging from 1 to 50 years), and the clonal structure, genotype and phenotype may change over time in each patients. In effect, each cancer is a multiple of different cancers that occupy distinct or overlapping tissue habitats. The number of mutations in a cancer can vary from a handful (10 to 20) to hundreds or thousands. The vast majority are transient, and a modest but indefinite number are functionally relevant drivers. As clones and sub-clones develop, migrating cells invade new habitats within and between tissues, where they experience new selective pressures that may result in a greater diversity of cancer cells. This malignant feature and its associated morbidity characterise the terminal stage of cancer [23]. Gryfe *et al.* [7] suggested that the high-frequency MSI strongly associated with lower cancer stage, even after controlling for the depth of tumour invasion, is intriguing. The high haplotypic diversity and low nucleotide diversity indicates a rapid evolution of the polymorphism on the Bat 26 microsatellite, making it more unstable in Senegalese patients with oral cavity cancers. Defects in the mismatch repair system can lead to an accelerated accumulation of mutations in critical genes and result in progression to malignancy. A variety of sporadic tumours exhibit frequent MSI associated with defects in DNA mismatch repair pathways. This process is particularly affected by abnormal functions of the hMLH1 and hMSH2 [24].

5. Conclusion

Senegalese patients with oral cavity cancers are highly unstable at Bat 26 microsatellite. Although a similarity was found between cancerous and healthy tissues, an increase in the number of blood samples to the same as healthy tissues would reinforce this result. Future studies should focus on the role of MSI in the ex-

pression of cancer-related genes and whether changes in the length of intronic regions affect the expression of these genes. The future direction of genomic instability research should occur in both the clinical and laboratory settings. Clinicians should focus on utilising genomic instability as a biomarker for high-risk patients. Laboratory scientists should work to develop new tests to improve the detection of patients with genomic instability.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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