

Research Article

Microsatellite Instability in Head and Neck Squamous Cell Carcinoma: A Study of a Brazilian Population

Elaine Stur,¹ Eldamaria de Vargas Wolfgramm,¹ Allan Kardec de Castro Neto,² Lucas de Lima Maia,¹ Lidiane Pignaton Agostini,¹ Gabriela Tonini Peterle,¹ Suzanny Oliveira Mendes,¹ Marcelos dos Santos,¹ Flávia de Paula,¹ and Iúri Drumond Louro¹

¹ Núcleo de Genética Humana e Molecular, Departamento de Ciências Biológicas, Centro de Ciências Humanas e Naturais, Universidade Federal do Espírito Santo, Avenida Marechal Campos, 1468, Maruípe, 29043-900 Vitória, ES, Brazil

² Hospital Santa Rita de Cássia, Setor de Patologia, Vitória, ES, Brazil

Correspondence should be addressed to Iúri Drumond Louro; iurilouro@yahoo.com

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Squamous cell carcinoma (SCC) is the sixth most common solid tumor in the world. Apart from known risk factors for head and neck SCC (HNSCC), there is a lack of information about genetic susceptibility regions that may play pivotal roles in the tumorigenesis of these tumors. Therefore, we have aimed to analyze the presence of genetic instability in microsatellite markers distributed in the genome. Microsatellite instability (MSI) was found in 6 HNSCC patients, among which only one was detected by the D17S250 marker, whereas the other 5 occurrences (13.5%) were detected by the D3S1611 marker. No instability was found at markers D5S346, D10S197, D11S922, and D11S988. MSI detected by D3S1611 marker was present in 3 (14.3%) moderately differentiated tumors and in 2 (25.0%) poorly differentiated tumors, but no statistical significance was found. Genotypic frequencies for all markers showed no statistically significant distribution alteration, neither were they related to differentiation grade or patient age. Marker D3S1611 is located in the MLH1 gene, which is part of the mismatch repair system (MMR), helping to maintain genomic stability. We have found a higher rate of D3S1611 MSI in older patients, suggesting that this marker may be affected by aging processes in the DNA repair machinery.

1. Introduction

Head and neck cancer is a significant cause of mortality and morbidity worldwide, presenting approximately 600,000 new cases yearly [1], whereas tumors of the oral cavity contribute with 389,000 new cases per year, with a mortality rate of 50% [2]. Squamous cell carcinoma (SCC) is the most common histological variant, comprising 90% of all cases [3].

SCC is the sixth most common solid tumor in the world [4], found preferentially in 50–70-year-old individuals [5]. Nonetheless, some studies point towards a frequency shift towards younger ages, with several cases in men with less than 40 years of age [6].

The epidemiology of head and neck SCC (HNSCC) shows tobacco, alcohol, and Human Papilloma Virus (HPV) infection as the most important risk factors, as well as genetic susceptibility [7–11]. Therefore, the search for genetic markers has increased significantly over recent years.

Microsatellite regions are genomic 1–5 bp tandem repeats, generally noncoding, and the CA 2 bp repeat is the most common form [12]. These regions may present instability, especially caused by replication errors introduced by DNA polymerase, a phenomenon known as microsatellite instability (MSI) [13].

These replication mistakes may aid tumorigenesis through inactivation of tumor suppressor and repair genes,

TABLE 1: Primers used for STR amplification.

Region	Sequence
D3S1611	5'-GCCGGGTACATTGGCCTGTAATC-3' 5'-AGCTGAGACTACAGGCATTTGCC-3'
D5S346	5'-ACTCACTCTAGTGATAAATCGGG-3' 5'-AGCAGATAAGACAGTATTACTAGTT-3'
D10S197	5'-ACCACTGCACTTCAGGTGAC-3' 5'-CTCAAGTGGCATTGTGAAATCTTCGAAC-3'
D11S922	5'-GGGGCATCTTTGGCTACACTGG-3' 5'-CTCTGACCGCCACCATGTATCC-3'
D11S988	5'-CAGAAAATAGTTCAGACCACCA-3' 5'-GGGACAAGAGAAAAGTTGAACA-3'
D17S250	5'-GGAAGAATCAAATAGACAAT-3' 5'-GCTGGCCATATATATATTTAAACC-3'

altering normal cell functions [12]. The fact that tumor cells harbor several genomic alterations and remarkable instability renders MSI markers a possible tool in the analysis of diverse tumor types. This study aimed to evaluate the MSI rate at six different genomic locations in HNSCC patients.

2. Materials and Methods

2.1. Samples. Samples were obtained from the Pathology Service of Santa Rita de Cássia Hospital (SRCH). Forty-two paraffin blocked HNSCC were analyzed (all from year 2009). Of all patients, 9 (21.4%) were women and 33 (78.6%) were men, with a mean age of 61 years (sd \pm 13.7). This study was approved by the Ethics Committee of the Federal University of Espirito Santo on 30/04/2009 (Protocol no. 010/09).

2.2. Genotyping. DNA was extracted according to Goelz et al. [14]. MSI at six different genomic locations was tested by polymerase chain reaction (PCR) in Eppendorf Mastercycler, using D3S1611, D5S346, D10S197, D11S922, D11S988, and D17S250 markers (primers described in Table 1). These regions were selected due to previous descriptions in the literature of genetic instability occurring at these markers in different tumor types [15]. Genomic region and reaction conditions are described in Table 2. After amplification, PCR products were separated by vertical electrophoresis in 15% acrylamide gels and stained with 0.1% silver nitrate. Homozygosity or heterozygosity was characterized by the identification of one or two alleles in the normal tissue, respectively. Genetic instability was determined by the presence of different size bands or extra bands in the tumoral tissue, when compared to the normal tissue.

2.3. Statistical Analysis. The chi square and Fisher exact tests were used for association analysis and confirmation was obtained by the Lilliefors test (significance considered when

$P < 0.05$). Statistical calculations were performed using the Epi Info v3.4.3, 2007 software.

Samples that rendered unsuccessful amplification were excluded from the statistical analysis.

3. Results

MSI was found in 6 HNSCC patients, among which only 1 was detected by the D17S250 marker, whereas the other 5 (13.5%) were detected by the D3S1611 marker. No instability was found at markers D5S346, D10S197, D11S922, and D11S988.

MSI detected by D3S1611 marker was more frequent in SCC of the oropharynx, but without a significant difference when compared to tumors of the mouth ($P = 0.273$) and larynx ($P = 0.279$). In relation to tumor differentiation, MSI detected by D3S1611 marker was present in 3 (14.3%) moderately differentiated tumors and in 2 (25.0%) poorly differentiated tumors, but no statistical significance was found ($P = 0.339$), probably due to the small sample size. Additionally, D3S1611 MSI was significantly associated with older age ($P = 0.034$, Table 3).

Genotypic frequencies for all markers showed no statistically significant distribution alteration, neither were they related to differentiation grade or patient age (Table 4).

4. Discussion

The present study has identified 13.5% MSI for samples analysed with D3S1611 marker. Our results are in agreement with Chakrabarti et al. [16], who identified even higher rates of MSI (40%). In contrast, a lack of MSI in HNSCC was reported by other studies [16, 17]. These discrepancies may be explained by the great variability among repeat regions throughout the genome [18]. Instability frequency is related to the repeat unit length and overall size of the short tandem repeat (STR), affecting the probability of error during DNA replication [19, 20]. Furthermore, individuals from diverse geographic locations may present differences in their STR characteristics [21], possibly affecting accuracy during replication. Therefore, it is possible that populations with a longer average repeat size are more prone to instability than the ones with a smaller repeat size.

Marker D3S1611 is located in the MLH1 gene (*mutL homolog 1*), coding for an enzyme responsible for aiding in the mismatch repair system (MMR), which is pivotal for the maintenance of genomic stability, repairing DNA heteroduplexes generated by replication [22, 23]. MLH1 gene alterations are related to the development of colorectal cancer, suggesting that this enzyme is needed for correct DNA repair at least in some tissues [21].

In addition, we have found a higher rate of D3S1611 MSI in older patients, suggesting that this marker may be affected by aging processes in the DNA repair machinery. According to Hardwick et al. repeat size expansion is an inevitable

TABLE 2: Genomic characteristics and reaction conditions for MSI markers.

Characteristics	MSI marker					
	D3S1611	D5S346	D10S197	D11S922	D11S988	D17S250
Genomic						
Chromosomal location	3p21.3	5q21	10p12	11p15.5	11p15.5	17q11.2
STR sequence	(CA) _n	(CA) _n	(CA) _n	(CA) _n	(CA) _n	(CA) _n
Fragment size	140 pb	115 pb	141 pb	175 pb	120 pb	151 pb
Reaction conditions						
Platinum taq DNA polymerase	0.2 u	0.2 u	0.2 u	0.2 u	0.2 u	0.2 u
25 μM primers F/R	0.3 μL	0.6 μL	0.6 μL	0.6 μL	0.6 μL	0.6 μL
10 mM dNTP	0.2 mM	0.2 mM	0.2 mM	0.2 mM	0.2 mM	0.2 mM
50 mM MgCl ₂	1.5 mM	1.5 mM	1.5 mM	1.5 mM	1.5 mM	1.5 mM
10X PCR buffer	1X	1X	1X	1X	1X	1X
DMSO 100%		0.75 μL				
DNA	1.2 μL	1.2 μL	0.6 μL	1.8 μL	1 μL	0.6 μL
Final volume	15 μL	15 μL	15 μL	15 μL	15 μL	15 μL
Cycling conditions						
Number of cycles	30	30	28	30	32	30
Annealing	70°C for 15seg	55°C; 30seg	70°C; 30seg	68°C; 30seg	69°C; 30seg	50°C; 30seg

TABLE 3: D3S1611 MSI frequency according to clinical and epidemiological features.

Features	D3S1611 marker						<i>P</i>
	Total		MSI		MSS		
	No.	(%)	No.	<i>f</i>	No.	<i>f</i>	
Gender							
Female	8	(21.6)	1	0.125	7	0.875	—
Male	29	(78.4)	4	0.138	25	0.862	
Age range, yrs							
≤50	8	(21.6)	0	0.0	8	1.0	0.034
50–70	22	(59.5)	2	0.091	20	0.909	
>70	7	(18.9)	3	0.492	4	0.508	
Tumor site							
Oral cavity	15	(40.5)	1	0.067	14	0.933	—
Oropharynx	8	(21.6)	3	0.375	5	0.625	
Larynx	14	(37.9)	1	0.071	13	0.929	
Differentiation							
Well	8	(21.6)	0	0.0	8	1.0	0.339
Moderately	21	(56.8)	3	0.143	18	0.857	
Poorly	8	(21.6)	2	0.250	6	0.750	
Total	37	(100.0)	5	0.135	32	0.865	

MSI: microsatellite instability; MSS: microsatellite stable; *f*: frequency; *P*: significance value.

and progressive phenomenon along the life span of rodents [24].

In conclusion, the present study reports that D3S1611 marker can identify instability in a fraction of HNSCC patients, being related to older ages.

Ethical Approval

This study was approved by the Ethics Committee of the Federal University of Espírito Santo on 30/04/2009 (Protocol no. 010/09).

TABLE 4: Marker genotypic frequencies, according to clinical and epidemiological features.

Features	Genotypic frequencies														
	D3S1611					D5S346					D10S197				
	HT	<i>f</i>	HM	<i>f</i>	<i>P</i>	HT	<i>f</i>	HM	<i>f</i>	<i>P</i>	HT	<i>f</i>	HM	<i>f</i>	<i>P</i>
Total	26	0.703	11	0.297	—	23	0.821	5	0.179	—	29	0.879	4	0.121	—
Gender															
Female	6	0.750	2	0.250	—	7	0.875	1	0.125	—	8	1.0	0	0.0	—
Male	20	0.690	9	0.310		16	0.800	4	0.200		21	0.840	4	0.160	
Age range, yrs															
≤50	7	0.875	1	0.125	0.448	5	1.0	0	0.0	0.513	6	1.0	0	0.0	0.586
50–70	14	0.636	8	0.364		14	0.778	4	0.222		16	0.842	3	0.158	
>70	5	0.714	2	0.286		4	0.800	1	0.200		7	0.875	1	0.125	
Tumor site															
Oral cavity	9	0.600	6	0.400	—	11	0.786	3	0.214	—	12	0.857	2	0.143	—
Oropharynx	6	0.750	2	0.250		6	1.0	0	0.0		7	0.875	1	0.125	
Larynx	11	0.786	3	0.214		6	0.750	2	0.250		10	0.909	1	0.091	
Differentiation															
Well	8	1.0	0	0.0	0.115	7	1.0	0	0.0	0.334	7	1.0	0	0.0	0.518
Moderately	13	0.619	8	0.381		11	0.786	3	0.214		15	0.833	3	0.167	
Poorly	5	0.625	3	0.375		5	0.714	2	0.286		7	0.875	1	0.125	
Features	Genotypic frequencies														
	D11S922					D11S988					D17S250				
	HT	<i>f</i>	HM	<i>f</i>	<i>P</i>	HT	<i>f</i>	HM	<i>f</i>	<i>P</i>	HT	<i>f</i>	HM	<i>f</i>	<i>P</i>
Total	19	0.760	6	0.240	—	26	0.867	4	0.133	—	28	0.875	4	0.125	—
Gender															
Female	7	0.875	1	0.125	—	8	1.0	0	0.0	—	7	0.875	1	0.125	—
Male	12	0.706	5	0.294		18	0.818	4	0.182		21	0.875	3	0.125	
Age range, yrs															
≤50	4	0.800	1	0.200	0.413	5	1.0	0	0.0	0.171	5	0.833	1	0.167	0.912
50–70	11	0.688	5	0.313		13	0.765	4	0.235		17	0.895	2	0.105	
>70	4	1.0	0	0.0		8	1.0	0	0.0		6	0.857	1	0.143	
Tumor site															
Oral cavity	9	0.692	4	0.308	—	11	0.917	1	0.083	—	12	0.923	1	0.077	—
Oropharynx	6	1.0	0	0.0		7	0.875	1	0.125		8	1.0	0	0.0	
Larynx	4	0.667	2	0.333		8	0.800	2	0.200		8	0.727	3	0.273	
Differentiation															
Well	6	0.857	1	0.143	0.586	7	1.0	0	0.0	0.436	6	0.857	1	0.143	0.986
Moderately	10	0.769	3	0.231		12	0.800	3	0.200		15	0.882	2	0.118	
Poorly	3	0.600	2	0.400		7	0.875	1	0.125		7	0.875	1	0.125	

HT: heterozygote; HM: homozygote; *f*: frequency; *P*: significance value.

Conflict of Interests

The authors declare that they have no competing interests.

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