Expression of DNA repair genes in oral squamous cell carcinoma using reverse transcription-quantitative polymerase chain reaction

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Objective. The aim of this study was to evaluate the expression of DNA repair genes in cases of oral squamous cell carcinoma (OSCC).

Study Design. Expression of the *MLH1, MSH2, MLH3, ATM, MRE11A, XRCC1*, and *PMS2* genes was evaluated by reverse transcription−quantitative polymerase chain reaction in the OSCC group (32 patients) and the control group (15 patients). The groups were compared by using the Mann-Whitney test, with Bonferroni correction. Associations between gene expression levels and clinical data were explored by using Pearson's and Spearman's correlation coefficients, with P value less than .05 indicating a significant difference.

Results. The *MLH1, MSH2, MLH3, ATM, MRE11A, XRCC1*, and *PMS2* genes were downregulated in the OSCC group compared with the control group, with significant values for *MLH1* ($P < .0001$); *MSH2* ($P = .038$); *MLH3* ($P < .0001$); *ATM* ($P < .0001$); *MRE11A* ($P < .0001$); *XRCC1* ($P = .0004$); and *PMS2* ($P = .008$). Analysis of the correlation between gene expression and clinical data only revealed a significant negative correlation between age and expression of the *PMS2* gene.

Conclusions. Expression of the DNA repair genes *MLH1, MSH2, MLH3, ATM, MRE11 AMRE11A, XRCC1*, and *PMS2* was reduced in OSCC. (Oral Surg Oral Med Oral Pathol Oral Radiol 2020;130:298−305)

Oral squamous cell carcinoma (OSCC) is the most common malignancy in the oral cavity. In Brazil, OSCC is the fifth most prevalent malignant neoplasm in men and the 11th in women,[1] and the tongue is the most commonly affected site.[2] Like other malignant tumors, the development of OSCC involves genetic and epigenetic alterations that can be triggered by hereditary and environmental factors.[3]

Although DNA synthesis is a highly accurate process, errors may occur, and it is the function of the DNA repair systems to identify these anomalies and eliminate them. These systems, therefore, represent an important protection mechanism against the development of the malignant phenotype.[4] Alterations in the DNA repair genes directly influence carcinogenesis because genomic stability depends on the efficiency of DNA repair.[5] Thus, the reduced expression of DNA repair genes might be related to the occurrence of different malignant neoplasms.[5-7]

The most important DNA repair strategies include base excision repair, nucleotide excision repair, homologous recombination, and mismatch repair. Base excision repair is the primary mechanism to remove incorrect and damaged bases, repairing nucleotide damage resulting from oxidation, alkylation, hydrolysis, or deamination. Nucleotide excision repair removes strings of nucleotides. Homologous recombination is a DNA metabolic process that provides template-dependent repair or tolerance to complex DNA damage, including DNA gaps, DNA double-strand breaks, and DNA interstrand cross-links.[8] Mismatch repair corrects errors of DNA replication and gene recombination that result in incorrectly paired nucleotides after DNA replication.[9] Another mechanism of maintaining genome

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Received for publication Jan 17, 2020; returned for revision Apr 14, 2020; accepted for publication Jun 1, 2020.
© 2020 Published by Elsevier Inc.
2212-4403/$-see front matter
https://doi.org/10.1016/j.oooo.2020.06.003

Statement of Clinical Relevance

The reduced expression of the *MLH1, MSH2, ATM, MRE11A, PMS2, XRCC1*, and *MLH3* genes in oral squamous cell carcinoma could imply a decrease in the DNA repair capacity of cells and changes in tumor suppression.

stability in the presence of DNA damage is the precise regulation of the cell cycle. All these pathways contribute to genome stability, and deficiency in one or more relevant genes can lead to deregulated cell growth and, ultimately, to cancer development.[10]

The objective of this study was to analyze expression of the DNA repair genes, *MLH1* (MutL homolog 1), *MSH2* (MutS protein homolog 2), *MLH3* (MutL homolog 3), *ATM* (ataxia telangiectasia mutated), *MRE11A* (meiotic recombination 11 homolog A), *XRCC1* (X-ray repair cross-complementing protein 1), and *PMS2* (postmeiotic segregation increased homolog 2) in OSCC by using reverse transcription−quantitative polymerase chain reaction (RT-qPCR). Previous studies have found altered expression of these genes in smokers, a group at risk for the development of oral carcinogenesis.[11,12]

MATERIALS AND METHODS

Sample selection

The study was approved by the Ethics Committee of the Institute of Science and Technology (ICT), of the São Paulo State University (UNESP; São Paulo, Brazil). All patients signed 2 copies of the informed consent form.

The sample was divided into 2 groups. The OSCC group consisted of OSCC specimens obtained from patients (smokers and nonsmokers) seen at the Head and Neck Surgery Service of The Celso Pierro Hospital and Maternity, PUCCAMP, Brazil, and at the Mario Gatti Municipal Hospital, Campinas, Brazil. The control group consisted of oral biopsy specimens obtained from the epithelial borders of benign lesions. These specimens were obtained from patients matched for age, gender, and smoking habit to the OSCC group, who received outpatient care at the Stomatology Service of the Department of Biosciences and Oral Diagnosis (ICT-UNESP). Patients who had undergone any type of oncologic treatment with surgery, radiotherapy, or chemotherapy of any organ or system were not included.

Tissue fragments of the lesion, measuring approximately 0.5 cm^3, were obtained during biopsy from a nonspecific location. In the case of controls submitted to some type of excision of benign lesions, the border of healthy tissue was used. The samples were stored in Allprotect Tissue Reagent (Qiagen, Valencia, CA) at 4°C overnight and at −80°C after this period.

All patients were submitted to extra- and intraoral clinical examinations and answered a questionnaire, administered via an interview, about the frequency of cigarette smoking and the number of cigarettes smoked. Information about the stage of OSCC was collected from the patient's medical record. Cancer staging was performed according to the tumor−node−metastasis (TNM) classification,[13] per the 8th edition of the *American Joint Committee on Cancer (AJCC) Cancer Staging Manual*. Table I,[13] modified from Huang et al.,[13] describes how the disease stages were determined.

RNA extraction and reverse transcription

Total RNA was extracted by using TRIzol reagent (Ambion, Inc., Carlsbad, CA), according to the manufacturer's recommendations. In brief, 1 mL TRIzol was added to a 2-mL microtube containing the collected cells, and the mixture was incubated for 10 minutes at room temperature. Next, 200 μL of chloroform (Sigma-Aldrich, St. Louis, MO) was added, and the microtubes were centrifuged at × 12,000 *g* for 15 minutes at 4°C. The supernatant was transferred to a new microtube, and 500 μL of isopropanol (Sigma-Aldrich, St. Louis, MO) was added. After centrifugation, the sediment obtained was washed in 70% ethanol (Sigma-Aldrich, St. Louis, MO), centrifuged again, and resuspended in 20 μL of RNA storage buffer (Ambion, Inc., Carlsbad, CA). One microliter of RNA of each sample was used to measure absorbance at 260 nm (A260) and 280 nm (A280) in a NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE). The concentration of RNA was obtained by multiplying the A260 value by 40 (ng/mL). Purity, an indicator of RNA quality, was analyzed by determining the A260/A280 and A260/A230 ratios, with an A260/A280 ratio of 1.8 to 2.0 and an A260/A230 ratio close to 1.7 indicating RNA free from contaminants. RNA integrity was verified by using electrophoresis on 1% agarose gel (Invitrogen, Carlsbad, CA) stained with ethidium bromide (Invitrogen, Carlsbad, CA). Only samples with intact RNA were used. RNA was considered intact when clearly defined bands without smears were

Table 1. TNM Staging Classification for the Lip and Oral Cavity.*

Category	*T*	*N*	*M*
Stage 0	Tis	N0	M0
Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage III	T3	N0	M0
	T1 - 3	N1	M0
Stage IVA	T4a	N0 - 1	M0
	T1 - 4a	N2	M0
Stage IVB	Any T	N3	M0
	T4b	Any N	M0
Stage IVC	Any T	Any N	M1

*American Joint Committee on Cancer - 8th ed., 2018T: tumor; N: Node; M: Metastasis.Tis: Carcinoma *in situ*Modified from Amin, M.B., Edge, S., Greene, F., Byrd, D.R., Brookland, R.K., Washington, M.K., Gershenwald, J.E., Compton, C.C., Hess, K.R., Sullivan, D.C., Jessup, J.M., Brierley, J.D., Gaspar, L.E., Schilsky, R.L., Balch, C.M., Winchester, D.P., Asare, E.A., Madera, M., Gress, D. M., Meyer, L.R. (Eds). AJCC Cancer Staging Manual. 8 th Ed. New York: Springer, 2017.

observed and the 28 S rRNA band had double the intensity of the 18 S rRNA band.

The extracted total RNA (1 μg) was treated with DNase I (Ambion, Inc., Carlsbad, CA). After DNase treatment, RNA was transcribed into cDNA by using the SuperScript III First-Strand Synthesis SuperMix for qRT (Invitrogen, Carlsbad, CA). The reaction product was incubated in a thermocycler at 25˚C for 10 minutes, at 50˚C for 30 minutes, and at 85˚C for 5 minutes. One microliter of RNase H was added to each tube, and the tubes were incubated for 20 minutes at 37˚C for complete removal of remnant nontranscribed RNA. The cDNA that was obtained was stored at −80˚C until the time of use.

Analysis of gene expression

The amplification efficiency (E) of the target genes and that of the reference genes were approximately the same. Amplification efficiency is determined by using the equation: $E = (10 - 1/\text{slope} - 1) \times 100$, and should ideally range from 90% to 100% ($-3.6 > \text{slope} > -3.1$). An absolute standard curve was constructed for each primer to calculate its amplification efficiency. The mean cycle threshold (Ct) values, measured in triplicate, were used to calculate the expression of the target genes, with normalization to the reference genes. The results are reported as relative gene expression values ($2^{-\Delta\Delta CT}$) compared with the previously selected reference gene, which corresponds to 1.

The sequences of all primers were confirmed at the NCBI/GenBank site, which were specific for *Homo sapiens*. The primers for each reference gene were described by Rentoft et al.[14] Three references genes (*ACTB, GAPDH, TUBA6*) were tested in 2 groups. The results were analyzed by using RefFinder (http://www.leonxie.com/referencegene.phpe) through the geNorm, Normfinder, and BestKeeper programs and the comparative Ct method. Based on the RefFinder results, *TUBA6* was chosen as the reference gene.

The samples from both groups were submitted to evaluation of expression of the *MLH1, MSH2, MLH3, ATM, MRE11A, XRCC1* and *PMS2* genes in relation to the *TUBA6* reference gene. The RT-qPCR method was used to evaluate the amount of cDNA in the exponential phase of the amplification reaction. Platinum SYBR Green qPCR SuperMix-UDG (Invitrogen, Carlsbad, CA) was used for detection

For RT-qPCR, 10 μL of Platinum SYBR Green Super Mix, 1 μL ROX (reference dye), 10 nM forward primer, 10 nM reverse primer, target cDNA solution, and RNAase/DNAase-free DEPEC water were mixed to obtain a final volume of 20 μL, and incubated with the StepOnePlus System (Life Technologies, Carlsbad, CA). The amplification conditions consisted of 2 minutes at 50˚C, followed by 10 minutes at 95˚C and 40 cycles at 95˚C for 15 seconds, followed by 60 seconds at 60˚C. After the last cycle, the samples were submitted to melting curve analysis, and no sign of a bimodal curve or amplification of abnormal signals was observed.

Evaluation of the degree of nicotine dependence and smoking profile

Smoking load was quantified by calculating pack-years, which is the number of cigarettes smoked per day divided by 20 and multiplied by the number of years the subject had smoked.[15] The assessment of nicotine dependence was performed by considering a score on the Fagerström Test for Cigarette Dependence,[16] which consists of 6 questions, and the score related to nicotine dependence ranges from very low (0−2 points); low (3−4 points); moderate (5 points); high (6−7 points); and very high (8−10 points).

Assessment of alcohol-related risk

The Alcohol Use Disorder Identification Test was used to evaluate alcohol-related risk. This instrument consists of 10 questions that identify 4 patterns of alcohol consumption: low-risk drinking, at-risk drinking, hazardous drinking, and probable dependence.[17]

Statistical analysis

The data were analyzed by using the GraphPad Prism 5.03 software. The groups were compared by using the Mann-Whitney test, with Bonferroni correction. Associations between gene expression levels and clinical data were explored by using Pearson's and Spearman's correlation coefficients, with P value less than .05 indicating a significant difference.

RESULTS

Thirty-two patients in the OSCC group and 15 patients in the control group were studied. Profile of gender and age of the OSCC group are given in Table I. The tongue was the most commonly affected site, followed by the floor of the mouth. The control group consisted of patients who were matched for gender, age, and smoking habit to the OSCC group. There were 26 (81.25%) smokers in the OSCC group and 12 (80%) in the control group. Tobacco and alcohol consumption in the 2 groups are given in Table II.

Expression of the *MLH1, MSH2, MLH3, ATM, MRE11A, XRCC1*, and *PMS2* genes in the groups studied was analyzed by using RT-qPCR (Figure 1). The *MLH1, MSH2, MLH3, ATM, MRE11A, XRCC1*, and *PMS2* genes were negatively regulated in the OSCC group compared with the control group, with 2.12-, 1.13-, 3.4-, 1.64-, 2.05-, 1.35-, and 1.22-fold decreases in gene expression, respectively. A significant difference was observed for all genes tested: *MLH1* ($P <$

Table II. Profile of gender, age, and tobacco and alcohol consumption in the oral squamous cell carcinoma (OSCC) group

		OSCC group	*Control group*
Age (years)	Average	57.43	50.38
	Range	28−88	32−65
	Standard deviation	13.53	11.86
Gender	Male	23	9
	Female	9	6
Smoking history (pack-years)	Average	44.51	41.85
	Standard deviation	27.59	19.74
Alcohol-related risk score	Low-risk drinking	9	10
	At-risk drinking	15	3
	Hazardous drinking	6	1
	Probable dependence	4	1
Disease stage	I	2	NA
	II	10	NA
	III	5	NA
	IV	15	NA

NA, not applicable.

.0001); *MSH2* (P = .038); *MLH3* (P < .0001); *ATM* (P < .0001); *MRE11A* (P < .0001); *XRCC1* (P = .0004); and *PMS2* (P = .008), with Bonferroni correction 0.0071.

Table III and Table IV show Pearson's and Spearman's correlation coefficients and P values, respectively. Table III shows Pearson's correlation between clinical data (age, cigarettes smoked per day, years of consumption, smoking history [pack-years]) and the expression levels of the genes studied, and Table IV shows Spearman's correlation between clinical data (Fagerstrom score for nicotine dependence, alcohol-related risk score, and disease stage) and the expression levels of the genes studied. A significant correlation was only observed between age and expression of the *PMS2* gene, which were negatively correlated.

DISCUSSION

This study evaluated the expression profile of the *MLH1, MSH2, MLH3, ATM, MRE11A, XRCC1,* and *PMS2* genes in OSCC. Lower expression of these genes was observed in the OSCC samples compared with control samples. These genes are important because their downregulation can influence the DNA repair capacity of cells, triggering carcinogenesis. Thus, a reduction in the expression of these genes might be related to the development of malignant lesions because DNA repair pathways contribute to tumor suppression, reducing mutations and promoting apoptosis in response to some type of DNA damage.[18] Additionally, It is important to highlight that previous studies have also demonstrated the importance of the expression of these genes in smokers, the main risk group for oral carcinogenesis.[11,12]

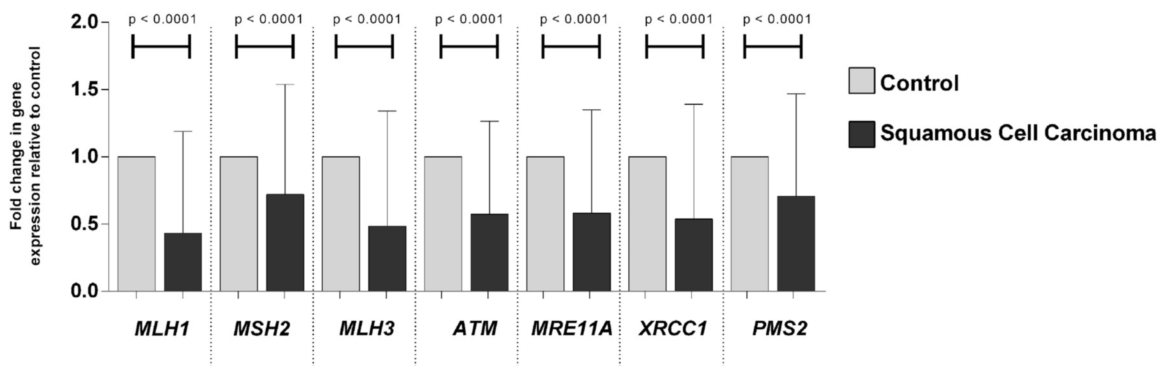

Fig. 1. Relative quantification (Log) of the expression of DNA repair genes (*MLH1, MSH2, MLH3, ATM, MRE11A, XRCC1*, and *PMS2*) in squamous cell carcinoma and control samples by using real-time quantitative polymerase chain reaction (RT-qPCR). Values are expressed as mean and standard deviation. Gene expression was compared by using the Mann-Whitney test. Each gene was normalized and compared with the control samples.

Table III. Pearson's correlation between clinical data (age, cigarettes smoked per day, years of consumption, smoking history [pack-years]) and expression levels of the genes studied

		Age	*Cigarettes smoked per day*	*Years of smoking*	*Smoking history (pack-years)*
MLH1	Pearson's correlation coefficient	−0.04	−0.12	−0.24	−0.16
	P value	.83	.51	.18	.38
MHS2	Pearson's correlation coefficient	0.04	−0.21	−0.20	−0.19
	P value	.84	.25	.26	.29
MLH3	Pearson's correlation coefficient	0.25	−0.33	-0.26	−0.29
	P value	.17	.07	.15	.11
ATM	Pearson's correlation coefficient	0.11	0.22	0.17	0.21
	P value	.54	.23	.34	.24
MRE11A	Pearson's correlation coefficient	0.15	-0.09	0.12	−0.07
	P value	.41	.63	.52	.70
XRCC1	Pearson's correlation coefficient	−0.10	−0.15	−0.19	−0.18
	P value	.60	.41	.30	.33
PMS2	Pearson's correlation coefficient	−0.36	0.26	−0.02	0.15
	P value	.04*	.15	.92	.40

**P* value < 0.05 indicates statistically significant difference.

The control group consisted of patients matched for gender, age, and smoking habit to the OSCC group. Thus, the groups were homogeneous in terms of these variables. The selection of samples with similar characteristics is important to reduce the influence of external variables.

Four (*MLH1, MSH2, MLH3*, and *PMS2*) of the genes studied are involved in the mismatch repair system. This system corrects errors in DNA replication and gene recombination that result in incorrectly paired nucleotides after DNA replication.[9]

Theocharis et al. studied OSCC of the tongue by using immunohistochemistry and, in contrast to our results, observed higher protein expression of MLH1 and MSH2.[19] These authors attributed this higher expression to specific gene mutations. Vasan et al. [20] also evaluated the incidence of MMR protein deficiency by using immunohistochemistry in OSCC. These authors defined deficient MMR protein expression as the loss of nuclear staining within the tumor compared with internal and external controls. MLH1 loss was observed in 12 patients from 21 cases and MSH2 expression was found to be lost in 6 patients. Vasan et al. emphasized that studies examining MMR proteins in OSCC often report conflicting results because of limited sample sizes and heterogeneous cohorts of patients with head and neck squamous cell carcinoma (SCC), including cutaneous and oropharyngeal SCCs, and differing techniques for MMR system analysis, ranging from immunohistochemistry to polymerase chain reaction (PCR) techniques.[20,21]

Amaral-Silva et al. concluded that although existing evidences implies that the loss of MMR proteins is important in the development of several oral lesions, further studies are necessary to clarify them as possible therapeutic targets, especially in the OSCC context, where these proteins also remain to be further validated

Table IV. Spearman's correlation between clinical data (Fagerstrom score for nicotine dependence, alcohol-related risk score, and disease stage) and expression levels of the genes studied

		Fagerstrom score for nicotine dependence	*Alcohol-related risk score*	*Disease stage*
MLH1	Spearman's correlation coefficients	−0.41	0.00	−0.24
	P value	.02*	.18	.99
MHS2	Spearman's correlation coefficients	-0.13	−0.18	−0.03
	P value	.46	.32	.89
MLH3	Spearman's correlation coefficients	−0.14	0.06	0.11
	P value	.45	.74	.56
ATM	Spearman's correlation coefficients	0.06	0.18	0.14
	P value	.75	.33	.45
MRE11A	Spearman's correlation coefficients	0.00	0.09	0.07
	P value	.98	.62	.69
XRCC1	Spearman's correlation coefficients	−0.20	0.22	0.17
	P value	.26	.22	.35
PMS2	Spearman's correlation coefficients	0.12	0.18	0.09
	P value	.51	.32	.63

**P* value < .05 indicates statistically significant difference.

as prognostic markers. Interest in the role of MMR proteins in oral carcinogenesis has grown, but their importance in pathogenesis and prognostic potential remains unclear.[21]

Furthermore, Senghore et al. investigated the association between single-nucleotide polymorphisms in MMR pathway genes and survival in patients with OSCC who received adjuvant concurrent chemoradiotherapy. These authors concluded that certain kinds of the GG genotypes (*MSH2*−rs3732183 and *MLH1*−rs1800734) are associated with relatively high survival. Therefore, polymorphisms may contribute to the variability of these findings.[22] In addition, expression variations may be related to differences in sample selection.[20]

Koutsimpelas et al. found a low methylation rate of the *MLH1* promoter in clinical samples and in head and neck SCC cells.[23] However, Pereira et al. observed low expression of *MSH2* in head and neck SCC tissues and associated it with poor survival.[24] These authors suggested that this low expression may contribute to increased genomic instability, resulting in a poor prognosis.

In other studies by our group, we observed reduced gene and protein expressions of *MLH1* and *MSH2* in normal oral mucosa of chronic smokers,[12] a group at high risk for the development of OSCC. However, hypermethylation of these genes was not detected in the same group of patients.[11]

We found no studies associating the expression of the *MLH3* gene with the development or progression of OSCC. However, a relationship between hypermethylation and polymorphisms in this gene and carcinomas at other sites has been reported. This might have happened in the cases in our study, where the reduction of expression was observed. Beggs et al. observed intense methylation in the promoters of *MLH3, MLH1, PMS2*, and *MSH3* and concluded that hypermethylation of these promoters may play a significant role in the carcinogenesis of colorectal cancer.[25] Similar results have been reported by Onrat et al. for *MLH3, MLH1, MSH2, MSH6, MSH3, PMS2*, and *MGMT*.[26] Ioana et al. observed overexpression of DNA mismatch repair genes in polyps, which could be used as a marker of neoplastic proliferation.[27] These authors concluded that malignant transformation is the result of inefficient repair mechanisms resulting from reduced activity of these genes. Ozer et al. found hypermethylation in *MLH3* and *PMS2* in 14.3% of the samples of hepatocellular carcinoma.[28] A polymorphism in the *MLH3* gene has been associated with the development of different carcinomas, such as primary hepatocellular carcinoma,[29] lung carcinoma,[30] and cervical carcinoma.[31]

There are no studies in the literature that associate expression of the *PMS2* gene with the development or progression of OSCC. However, loss of the PMS2 protein has been described in individual cases of different carcinomas, such as colorectal carcinoma,[32] endometrial carcinoma,[33] lymphoepithelioma-like gastric carcinoma,[34] neuroendocrine carcinoma of the endometrium,[35] and T-cell leukemia.[6]

As observed for the other cited genes, reduced expression of the *ATM* gene has been reported in different carcinomas, such as breast carcinoma,[36] gastric carcinoma,[37] adrenocortical carcinoma,[38] and pancreatic carcinoma.[39] Decreased expression has been attributed to deletions or rearrangement within or close to the *ATM* gene.[36] In OSCC, the mean expression of *ATM* was found to be 2.33 times lower in carcinomatous tissue compared with normal oral tissue; a reduction in *ATM* expression can lead to lack of DNA damage detection and contribute to tumor cell proliferation.[40] In the present study, we also observed lower expression in carcinoma (1.64-fold). Mansour et al. demonstrated that this reduced expression is mediated by the microRNA miR-421.[41] However, Rigi-Ladiz et al. found no significant difference in the methylation profile between OSCC cases and controls.[42]

Few studies have investigated the expression of *MRE11* in OSCC. Ziółkowska-Suchanek et al.,[43] studying peripheral blood samples, concluded that genetic variations in the *MRE11* gene do not contribute to an increased risk of malignant head and neck tumors. These authors investigated a variety of malignant tumors at different sites, with SCC being the most prevalent.[43] However, we examined fresh OSCC tissues and observed reduced expression of the *MRE11* gene. With respect to the expression of this gene in carcinomas at other sites, expression of the MRE11 protein was reduced in epithelial ovarian carcinomas[44] and endometrial carcinomas.[45]

We found no studies in the literature that associated expression of the *XRCC1* gene with the development or progression of OSCC. However, Bisarro dos Reis et al. demonstrated that allelic variants are not adequate markers for susceptibility or progression in OSCC.[46] Majumder et al. concluded that *XRCC1* variant haplotypes are associated with an increased risk of malignant transformation of oral leukoplakias.[47] Yadav et al. observed that tobacco use by individuals carrying polymorphic genotypes increases the risk of malignant transformation of oral leukoplakia.[48] According to Farnebo et al., polymorphisms in *XRCC1* are associated with the risk of and survival in head and neck SCC, acting synergistically with other polymorphisms and contributing to the development of the disease.[49]

With respect to carcinomas at other sites, Meng et al. associated mutations in the *XRCC1* gene with the development of cervical cancer.[50] Liu et al. concluded that loss of expression of this gene was

significantly correlated with the progression of clear cell renal cell carcinoma, as well as with poor survival.[51] Bajpai et al. demonstrated a significant association between reduced expression of the *XRCC1* gene and increased risk of cervical cancer, irrespective of the presence of human papillomavirus infection, with decreased expression being the initial event in cancer progression.[52]

CONCLUSIONS

Expression of the DNA repair genes *MLH1, MSH2, MLH3, ATM, MRE11A, XRCC1,* and *PMS2* is reduced in OSCC. However, we found no correlation between expression of DNA repair genes and use of carcinogenic factors. It is important to highlight that reduced expression of the DNA repair genes could be a cause or consequence of disease. The cause−effect relationship could not be addressed in our study because of its design. Future studies to assess the epigenetic events involved in gene expression, such as methylation and protein expression, are required for better understanding of this process.

ACKNOWLEDGMENTS

We acknowledge the National Council of Technological and Scientific Development,

Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and São Paulo Research Foundation, Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Brazil, for their support of this research; and Professor Ivan Balducci, for his statistical assistance.

FUNDING

This study was funded by the National Council of Technological and Scientific Development, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (Process 402717/2016-3) and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (Grants #2016/08633-0, #2017/21227-4, and #2017/23179-7).

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