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Short communication

Association of the *XPD* and *XRCC3* gene polymorphisms with oral squamous cell carcinoma in a Northeastern Brazilian population: A pilot study

Joabe dos Santos Pereira[a], Fabrícia Lima Fontes[b], Silvia Regina Batistuzzo de Medeiros[b], Roseana de Almeida Freitas[c], Lélia Batista de Souza[c], Márcia Cristina da Costa Miguel[c,*]

[a] Departament of Pathology, Federal University of Rio Grande do Norte, Natal, Rio Grande do Norte, Brazil
[b] Department of Cellular Biology and Genetics, Federal University of Rio Grande do Norte, Natal, Rio Grande do Norte, Brazil
[c] Post-Graduation Program of Oral Pathology, Departament of Dentistry, Federal University of Rio Grande do Norte, Natal, Rio Grande do Norte, Brazil

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ABSTRACT

Objective: to evaluate the association between *XPD* and *XRCC3* polymorphisms and oral squamous cell carcinoma (OSCC).
Design: the sample consisted of 54 cases of OSCC and 40 cases of inflammatory fibrous hyperplasia (IFH). Genotypes were determined by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.
Results: *XPD*-Lys/Gln was more common in IFH ($n = 28$; 70%) than in OSCC ($n = 24$; 44.4%) (OR: 0.3; $p < 0.05$). *XPD*-Gln was more frequent in high-grade lesions (0.48) than in low-grade lesions (0.21) (OR: 3.4; $p < 0.05$). The Gln/Gln genotype was associated with III and IV clinical stages (OR: 0.07; $p < 0.05$). *XRCC3*-Met was more frequent in OSCC (0.49) than in IFH (0.35) (OR: 2.6; $p < 0.05$). The Met/Met genotype was associated with the presence of metastases (OR: 8.1; $p < 0.05$) and with III and IV clinical stages (OR: 0.07; $p < 0.05$).
Conclusions: in this sample, the frequency of *XPD*-Gln in IFH suggests that this variant may protect against OSCC. The presence of the *XRCC3*-Met allele seems to contribute to the development of OSCC, metastases and more advanced stages in these lesions.

1. Introduction

Oral cancer has been the subject of many studies because it is an important cause of morbidity and mortality worldwide. The most common type of oral cancer is oral squamous cell carcinoma (OSCC), which accounts for approximately 90% of all malignant oral neoplasms (Syrjanen, 2005). An important aspect of oral carcinogenesis is individual genetic susceptibility, which is based on differences in the individual's ability to metabolize carcinogens as a result of the presence of different types of polymorphisms that may or may not favor the development of cancer (da Silva et al., 2011). In this respect, DNA repair genes are essential to maintain the integrity of the genome (Huang, Chang, Liu, Lin, & Hsia, 2010).

A common polymorphism in the *XPD* gene is characterized by a nucleotide change in codon 751, which results in the substitution of the amino acid Lys for Gln. This polymorphism has been associated with the occurrence of different neoplasms (Dufloth, Arruda, Heinrich, Schmitt, & Zeferino, 2008; Zhu et al., 2014). On the other hand, one of the polymorphisms found in the *XRCC3* gene is characterized by a non-conservative substitution of the amino acid Thr for Met in codon 241 (exon 7). This polymorphism has been studied regarding its influence on the sensitivity to radiation and induction of DNA damage (Gokkusu et al., 2013). The objective of this study was to evaluate the association between the frequency of the *XPD*-Lys751Gln (rs13181) and *XRCC3*-Thr241Met (rs861539) polymorphisms and the clinical–pathological profile of a series of OSCC cases.

* Corresponding author at: Universidade Federal do Rio Grande do Norte, Departamento de Odontologia, Programa de Pós-Graduação em Patologia Oral, Av. Senador Salgado Filho, 1787, Lagoa Nova, Natal/RN, CEP 59056-000, Brazil.
Fax: +55 84 3215 4138.
E-mail address: mccmiguel@hotmail.com (M.C. da Costa Miguel).

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2. Materials and methods

2.1. Sample

The project was approved by the Ethics Committee of the Federal University of Rio Grande do Norte (Protocol No. 76510) and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from each subject. The sample consisted of 54 cases of OSCC and 40 cases of inflammatory fibrous hyperplasia (IFH) as control. Inflammatory fibrous hyperplasia was chosen as the control since it is a reactional (non-neoplastic) lesion caused by injuries that trigger a chronic process characterized by the formation of excess repair tissue (Zarei, Chamani, & Amanpoor, 2007; Santos, Nonaka, Pinto, & de Souza, 2011). Histological malignancy grading was evaluated according to parameters proposed by Bryne (1998) and adapted by da Silveira et al. (2010). Briefly, four morphologic features were analyzed: degree of keratinization, cellular pleomorphism, pattern of invasion, and inflammatory cell infiltration. Each one received a score ranging from 1 to 4. Cases with a total score ≤ 8 were classified as low-grade, while those >8 were classified as high-grade. Clinical parameters (metastasis and tumor-node-metastasis stage) were also evaluated.

2.2. DNA extraction

DNA was extracted from paraffin blocks using Chelex100® resin (BioRad, Hercules, CA, USA) and the QIAamp DNA Minikit® (Qiagen, Hamburg, Germany), according to manufacturer instructions. The blocks were deparaffinized with xylene, followed by enzymatic digestion with proteinase K (Invitrogen, Grand Island, NY, USA). Next, 200 μl AE buffer (10 mM Tris–HCl, 0.5 mM EDTA, pH 9.0) or distilled water was added.

2.3. PCR amplification

Amplifications were performed in a DNA thermal cycler (Mastercycler® pro, Eppendorf, Hamburg, Germany). Protocols for PCR were adapted from de Souza et al. (2011). PCR amplification of the Lys751Gln polymorphism of the *XPD* gene was carried out in a final volume of 28 μl containing 2 μl genomic DNA (124.5 ng/ml), 0.5 μl (0.1 nM) of each primer, and 25 μl Platinum PCR SuperMix® (Invitrogen Co., Carlsbad, CA, USA). PCR amplification of the Thr241Met polymorphism of the *XRCC3* gene was carried out in a final volume of 34 μl containing 3 μl genomic DNA (124.5 ng/ml), 0.5 μl (0.1 nM) of each primer, and 30 μl Platinum PCR SuperMix®. Primers PCO3+ and PCO4+, flanking a sequence of the human β-globin gene, were used as positive control (Soares, Oliveira, de Souza, Costa Ade, & Pinto, 2008). Amplification of the human β-globin gene fragment was carried out in a final volume of 28 μl containing 2 μl genomic DNA (124.5 ng/ml), 0.5 μl (0.1 nM) of each primer, and 25 μl Platinum PCR SuperMix®. The characteristics of the primers used are specified in Table 1.

The digestion of PCR products was performed according to the manufacturer's instructions. For the Lys751Gln polymorphism of the *XPD* gene, the amplified products were digested in a mixture containing 5 μl of the amplified sample, 2 μl 10X NEBuffer 3 (New England Biolabs, Ipswich, MA, USA), 0.3 μl (20,000 U/ml) PstI, and 12.7 μl Milli-Q water. For the Thr241Met polymorphism of the *XRCC3* gene, the amplified products were digested in a mixture containing 4 μl of the amplified sample, 2 μl 10X NEBuffer 4, 2 μl BSA (100 μg/ml), 0.5 μl NlaIII (10,000 U/ml), and 13.5 μl Milli-Q water.

2.4. Statistical analysis

The data were analyzed using the Statistical Package for the Social Sciences for Windows (SPSS 20.0, Chicago, IL, USA). The results were analyzed statistically using Pearson's chi-square test, adopting a level of significance of $p < 0.05$. The odds ratio was also calculated to determine the degree and direction of correlation between the variables studied, considering a 95% confidence interval and a significant difference when $p < 0.05$.

3. Results

The clinical–pathological characteristics of the selected sample are summarized in Table 2. The frequency distribution of all genotypes was in Hardy–Weinberg equilibrium ($p > 0.05$) for patients with OSCC and controls.

3.1. Analysis of allele and genotype frequencies for XPD

Comparison of OSCCs and IFHs showed a higher frequency of the Lys/Gln genotype in the latter, with this difference being statistically significant ($p = 0.033$). The risk of developing OSCC was lower in patients carrying the Lys/Gln genotype compared to those with the Lys/Lys genotype (OR: 0.373; 95%CI 0.148–0.936) (Table 3). Patients carrying the Gln allele were more than three times more likely to develop high-grade lesions (OR: 3.409; 95%CI 1.101–10.56). Analysis of the frequency of the Gln allele revealed a significant association between this allele and high-grade lesions ($p = 0.031$). Gln/Gln genotype was associated with III and IV clinical stages ($p = 0.01$). Patients carrying Lys/Lys genotype disclosed a

Table 2
Gender, metastasis and histological grade of malignancy of the sample.

Gender *n* (%)		Metastasis *n* (%)		Gradation *n* (%)	
		Presence	Absence	High grade	Low grade
Female	12 (22.2)	3 (13.6)	9 (28.1)	3 (10.7)	9 (34.6)
Male	42 (77.8)	19 (86.4)	23 (71.9)	25 (89.3)	17 (65.4)
Total	54 (100)	22 (100)	32 (100)	28 (100)	26 (100)

Table 1
Genetic polymorphisms, fragment sizes and primers sequences.[a]

Gene	Polimorphism	Size	Primers (5′–3′)	
			Forward	Reverse
XPD	Lys751Gln (rs13181)	161 pb	CTGCTCAGCCTGGAGCAGC	AAGACCTTCTAGCACCACC
XRCC3	Thr241Met (rs861539)	194 pb	AAGAAGGTCCCCGTACTGCT	CTCACCTGGTTGATGCACAG
β-globin	–	110 pb	CTTCTGACACAACTGTGTTCACTAGC	TCACCGCAACTTCATCCACGTTCACC

[a] Primers sequences for XPD gene polymorphism were obtained from Sliwinski et al. (2011) those for XRCC3 gene polymorphism were built using Primer 3® software (0.4.0 version, available at http://primer3.sourceforge.net/), and those for human β-globin were obtained from Soares et al. (2008).