SKIN

Original Research

Association of Single-Nucleotide Polymorphisms of Gene XPC with Susceptibility to Basal Cell Carcinoma in Brazilian Population

Mayara dos Santos Maia[2,5], Otávio Sérgio Lopes[3,4], Poliane da Silva Calixto[2,5], Sylvia Satomi Takeno Herrero[2], Augusto Monteiro de Souza[2,5], Carlos Alberto Longui[4], Ivan Rodrigues de Carvalho Filho[8], Leonardo Ferreira Soares[6], Plínio Delatorre[1,2,5], Renally Barbosa da Silva[9], Danielle Calcagno[7], Rommel Rodriguez Burbano[7], Eleonidas Moura Lima[1,2,5]

[1]Departamento de Biologia Molecular; Universidade Federal da Paraíba; João Pessoa - PB – Brasil
[2]Laboratório de Biologia Molecular Estrutural e Oncogenética - LBMEO; Universidade Federal da Paraíba; João Pessoa - PB – Brasil
[3]Departamento de Dermatologia; Clinica Dermatológica Santa Catarina; João Pessoa - PB – Brasil
[4]Programa de Pós-Graduação em Ciências da Saúde; Faculdade de Ciências Médicas da Santa Casa de São Paulo; São Paulo – SP – Brasil
[5]Programa de Pós-Graduação em Biologia Celular e Molecular, Universidade Federal da Paraíba; João Pessoa - PB – Brasil
[6]Departamento de Farmácia; Universidade Estadual da Paraíba; Campina Grande - PB – Brasil
[7]Instituto de Ciências Biológicas; Universidade Federal do Pará; Belém - PA – Brasil
[8]Depatamento de Patologia, Laboratório de Análises Médicas - UNILAB, João Pessoa - PB – Brasil
[9]Programa de Pós-Graduação em Ciências Naturais e Biotecnologia; Universidade Federal de Campina Grande – UFCG, Campus de Cuité – PB – Brasil

ABSTRACT

Background: Basal cell carcinoma (BCC) is the common neoplasm in humans and its main etiological factor is exposure to solar radiation. Mutations in repair genes can lead to tumor progression and loss of cell integrity leading to the onset of cancer. Nucleotide excision repair (NER) is an important mechanism primarily used to repair injuries caused by UV.
Objective: To evaluate and describe for the first time the single nucleotide polymorphisms *rs745769173*, *rs761106780* and *rs535425175* and risk of developing BCC.
Methods: The present study analyzed 100 samples of paraffin-embedded tissue from patients with histopathological diagnosis of BCC and 100 control samples. The results were obtained by genotyping method, Dideoxy Unique Allele Specific – PCR (DSASP) and molecular modeling.
Results: The SNP *rs535425175* of the *XPC* gene showed a significant association with the BCC in the analyzed samples (P <0.005) and molecular docking showed different binding energy of the complex between the *XPC* region 99-156 and the PH domain of TFIIH p62, being more negative, -710.53 kcal/mol, with the Asn residue at position 108 and less negative, -611.10 kcal/mol, with Lys residue related to the polymorphism.
Conclusion: The results suggest that the SNP *rs535425175* of the *XPC* gene, which causes mutation at codon 108 of the *XPC* protein, which consists of replacing the Ans residue with the Lys, may be considered a risk factor associated with the development of BCC.

INTRODUCTION

Basal cell carcinoma (BCC) is the most common neoplasia in the world and originates from pluripotent basal cells that have lost their differentiation capacity [1,2]. The main etiological factor is exposure to UVB rays that damage DNA [3], which can lead to mutations in important genes regulating cellular functions [4]. Other factors and personal genetic predispositions may also be associated with the development of BCC [5].

The cell has several pathways of repair mechanisms involving more than 130 genes that correct DNA damage, contributing to the integrity and maintenance of genomic stability. However, mutations in repair genes can lead to tumor progression and loss of cell integrity leading to the onset of cancer [6].

Nucleotide excision repair (NER) is an important mechanism for repairing UV-induced lesions and removing adducts that prevent replication and DNA transcription. The NER pathway is composed of 28 genes, eight of which are from the XP family. Defects in some of these genes may lead to the development of several syndromes, among them Xeroderma Pigmentosum (XP), which is associated with the deficiency of DNA repair caused by UV radiation lesions [6,7].

The *XPA* gene is located in the chromosomal region 9q22.33 [8]. It is involved in both global and transcriptional genome repair pathways [9]. *XPA* detects the presence of DNA damage, recruits NER factors, stabilizes repair intermediates, positions repair machinery in DNA and interacts with various proteins, such as TFIIH and ERCC1-XPF, RPA, hHRD23B, DDB, PCNA [10,11].

The *XPC* gene is located in the chromosomal region 3p25.1 [12]. *XPC* recognizes and binds to damaged DNA, interacts with hHR23 to protect itself from proteolytic degradation, interacts with CENTN2 to increase its binding affinity for DNA. It recruits the TFIIH, which acts as a helicase and together with the RPA constitutes the pre-incision complex, opening the double helix [12-15].

Several studies have investigated the influence of many SNPs on the *XPA* and *XPC* genes in the risk of developing CBC [16-20]. However, this study is pioneer in the analysis and description of SNPs of *XPA* (*rs745769173*) and *XPC* (*rs535425175* and *rs761106780*) in samples from patients with BCC histopathological diagnosis in Brazil. The first time being in the literature, that these SNPs are described and analyzed.

METHODS

Samples

The present study analyzed 100 samples of paraffin-embedded tissue from patients with histopathological diagnosis of BCC, totaling 200 alleles for each SNP studied and 100 control samples obtained from paraffin-embedded normal tissue from cancer-free patients from the Laboratory of Structural Molecular Biology and Oncogenetic – LABMEO/ UFPB sample bank.

It is a retrospective study and samples has more than five years old and were provided by the bank of data by the Laboratory UNILAB/João Pessoa – PB and LABMEO/ UFPB sample bank. The present study is part of the thematic project approved by the Ethics Committee of the University Hospital Lauro Wanderley - UFPB under the code CAAE: 36522614.2.3001.5883.

DNA Extraction

The samples were submitted to DNA extraction in Laboratory of Structural Molecular Biology and Oncogenetic - LBMEO/ UFPB. The DNA sample was extracted according to the method described by Shang Rong-Shi et al [21] with modifications. The isolated genomic DNA was quantified with the Spectrophotometer NanoDrop™ 2000c from Thermo Fisher Scientific and stored at -20ºC.

Method Dideoxy single allele-specific PCR-DSASP

The DSASP method is based on chain-terminating inhibitors, dideoxynucleotide, established by the method of Sanger et.al. DSASP was previously validated by the Allele Specific PCR-ASP method as described by Lima et al. [22]. The DSASP is a method of genotyping and comprises four stages: I - selection of SNPs of interest, design of the oligonucleotides (primer and complementary sequence) and determination of the dideoxynucleotide to be incorporated; II - asymmetric PCR as dideoxynucleotide of interest; III - hybridization reaction between complementary sequence and asymmetric PCR product; IV - analysis by melting curve by qPCR and was validated and developed by Lima et al. [22].

To genotype the *SNPs rs745769173* (*XPA* gene), *rs761106780* and *rs535425175* (*XPC* gene) by DSASP method, asymmetric PCR was performed for each SNP of interest, complementary sequence and dideoxynucleotide incorporation. The oligonucleotides were obtained by *in silico* validation (GeneRunner Software).

Validation *in silico*

The primers used for DSASP of the *SNPs rs745769173* (*XPA* gene), *rs761106780* and *rs535425175* (*XPC* gene) were designed based on the database Ensembl Genome Browser and using the Gene Runner program to evaluate the annealing temperature, the formation of secondary structure and size of the amplified fragments.

Asymmetric PCR Conditions

Asymmetric PCR was performed in a final volume of 25 µL containing 200 µM dNTP (dATP, dCTP, dTTP and ddGTP), 2.0 mM $MgCl_2$, 20 ng/µL DNA, 200 *p*M primer and 0.5 U AmpliTaq Gold (Life Technologies - Carlsbad, CA). PCR conditions for amplification of single stranded DNA were as follows: a pre-denaturation for 3 min at 94°C and 80 cycles of 94°C for 20 s of 50°C for 45 s and 72°C for 30 s with a final extension of 5 min at 72°C.

Hybridization Conditions

The product of the PCR amplification of each sample was subjected to hybridization step under the following conditions: 200 *p*M of the complementary sequence at 4°C for 10 min.

Melting Curve Analysis

The melting curve were analysed to determine the Tm performed by Real Time PCR equipment 7500 Fast Real-Time PCR System (Life Technologies - Carlsbad, CA) following the conditions: preheat starting at 25ºC to 95ºC for 1 min, folding up to 45ºC for 5 min and gradual heating (1°C/min) until a temperature of 95°C for 5 min. For melting curve analysis, SYBR® Green Mix (Life Technologies - Carlsbad, CA) was used.

Molecular Modeling

In the present work to construct the *XPC* region 99-156 the A chain of the complex between the *XPC* acid domain and the PH domain of TFIIH p62 (PDB 2RVB) [23] was used, which was treated with the Swiss-PDB viewer was added the residues Thr 99, Gln 100, Asp 101, Ile 102, Pro 103, Ser 104, Asp 105, Leu 106, Lys 107 and Asn 108 [24]. Optimization and refinement of the structure was performed using WinCoot 0.8.4 [25] and the GalaxyRefine server (http://galaxy.seoklab.org/index.html) [26].

The local and global quality of the amino acids of the three-dimensional structure of the model was analyzed by ProSA [27] and local quality by the QMEAN [28] force fields. The overall quality of the model was determined by the data generated by QMEAN6 [29].

Docking

For the docking tests using Hex 8.0.0 software [30], two anchorages were generated, the first between *XPC* protein with Asn residue at codon 108 and the PH domain of TFIIH p62; And a second anchorage between the mutated *XPC*-modeled protein at position 108, substitution of Asn with Lys and the PH domain of TFIIH p62.

The mutation of *XPC* protein, in which substitution of Asn by Lys at position 108 was performed using the software WinCoot 0.8.4 [25]. Molecular docking was done using the PH domain of TFIIH p62 (B-chain, residues 1-108) which was cut out of the structure resolved by Nuclear Magnetic Resonance (NMR) and deposited in the Protein Data Bank (PDB) code (2RVB) [23]. The binding energies of the complexes were obtained using Hex 8.0.0 and for visualization of the complexes the Pymol was used [32].

Statistical Analysis

The allelic frequencies and genotypic distributions were obtained by the Hardy-Weinberg equilibrium model. The association analysis was performed using Chi-square and Fisher's exact test, using the statistical program BioEstat 5.3, where P <0.05 was considered significant.

RESULTS

The SNPs *rs745769173* of the *XPA* gene and the *rs761106780* of the *XPC* gene were not associated with Basal Cell Carcinoma. Only *rs535425175* of the *XPC* gene was shown to be associated with the development of BCC when compared to control samples (P <0.005) (Table 2.). The genotypic distributions of *rs535425175* was n = 6 C/C (Lys/Lys), n = 14 A/C (Asn/Lys) and n = 80 A/A (Asn/Ans). The observed T m was 70 °C for allele A and 87 °C for allele C (**Figures 1 and 2**).

Our results also suggest that there was a statistical significance between the genotypes of the *XPC* gene *rs535425175* and the gender (p = 0.0384), but for the other variables studied there was no significance: age (p = 0.7281), tumor location (p = 0.3792) and histological type (p = 0.6172) (Table 3.). The modeling and validation of the three-dimensional model for region 99-156 of the *XPC* protein was validated by the ProSA method, which assigned a negative value of approximately -0.13, positioning the model, represented by the black point, within the dark gray area, to Which indicates scores of other structures deposited in the PDB presenting the same size of the model of this research (**Figure 3**).

Data from the comparison of the six QMEAN parameters for the proposed model for *XPC* region 99-156 with the scores of structures of the same size deposited in the PDB indicated

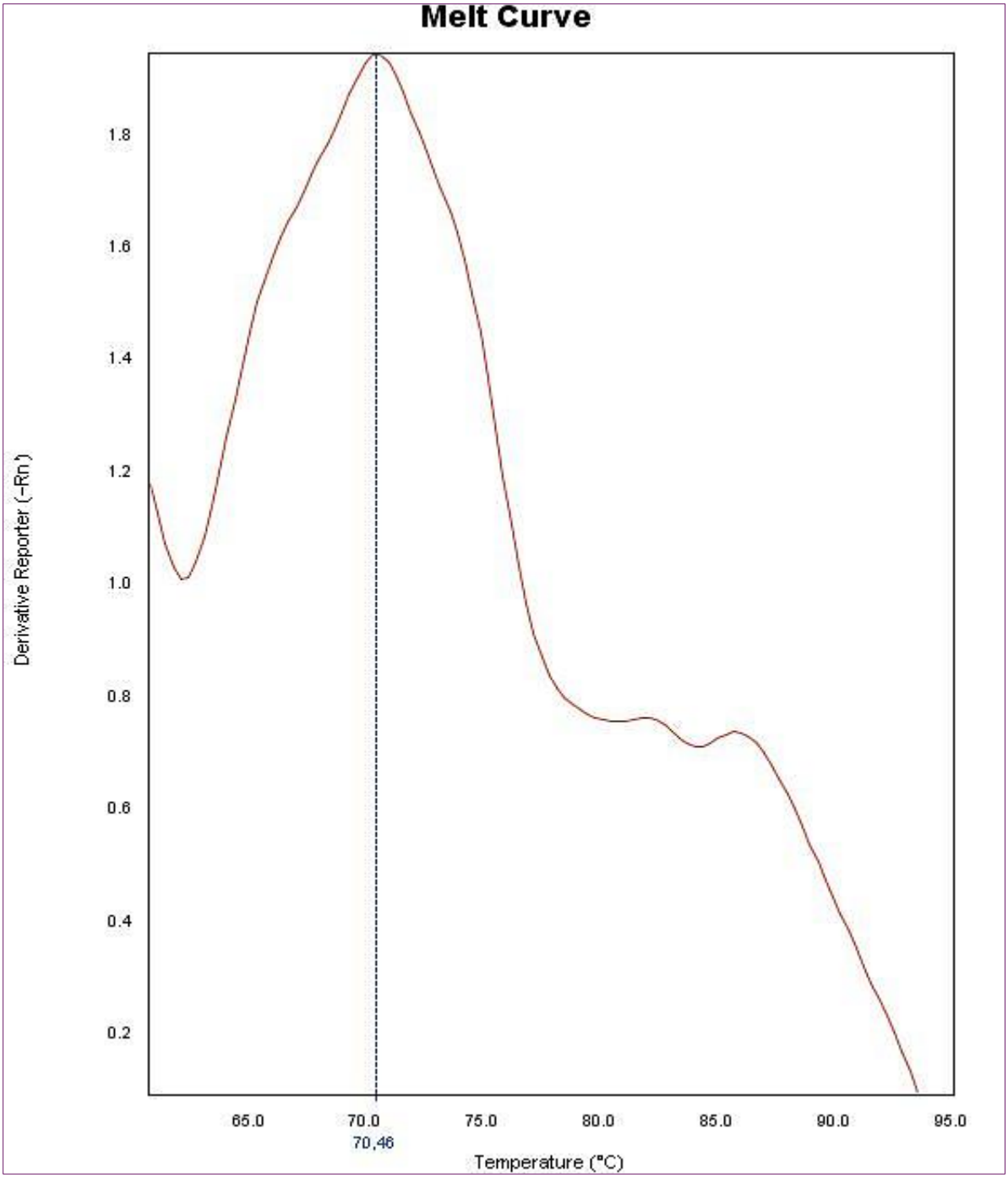

Figure 1. Genotype A/A of the SNP rs535425175 of the XPC gene with Tm 70ºC for the allele A.

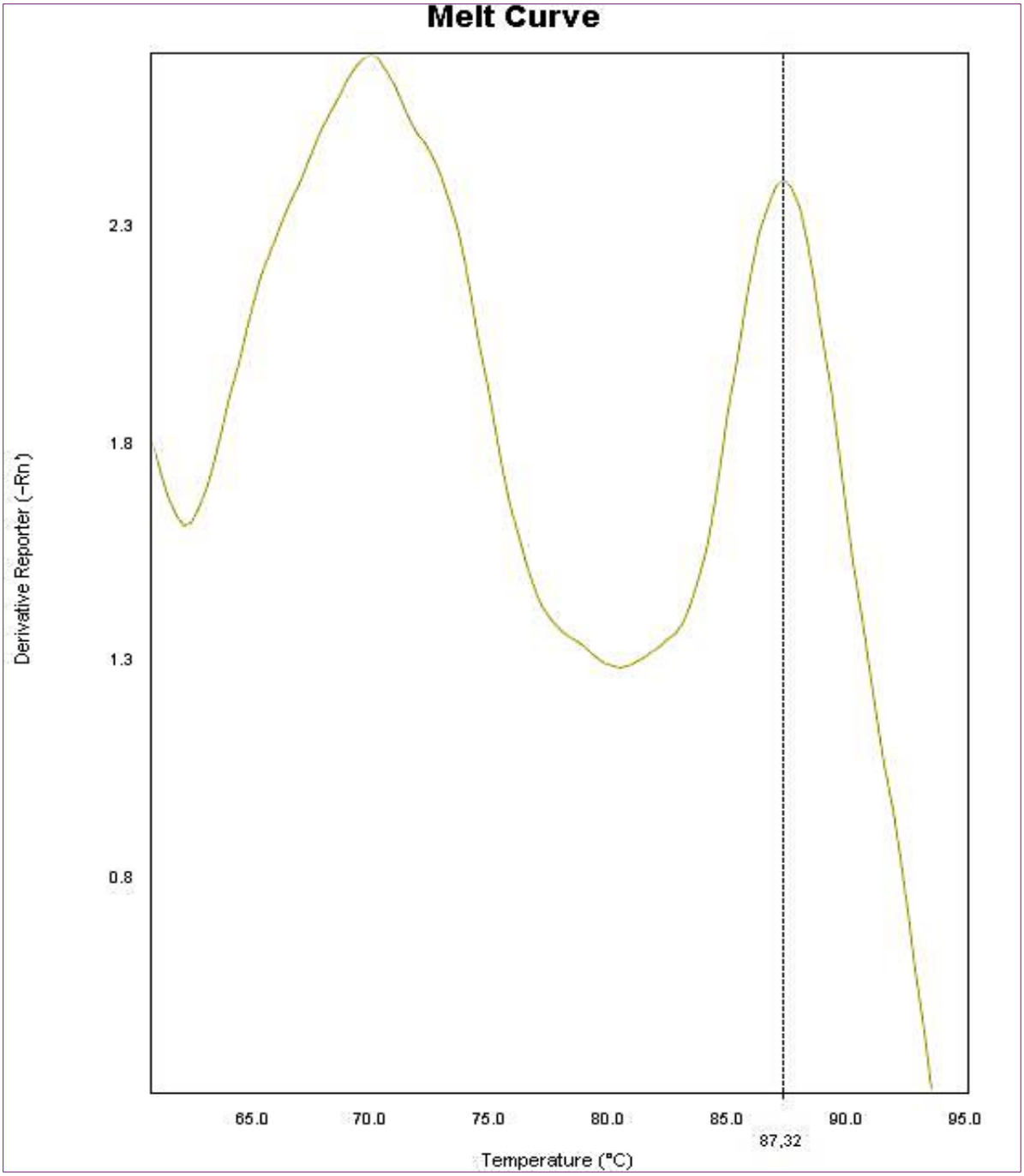

Figure 2. The Genotype A/C of the SNP rs535425175 of the XPC gene with Tm d87ºC for the C allele.

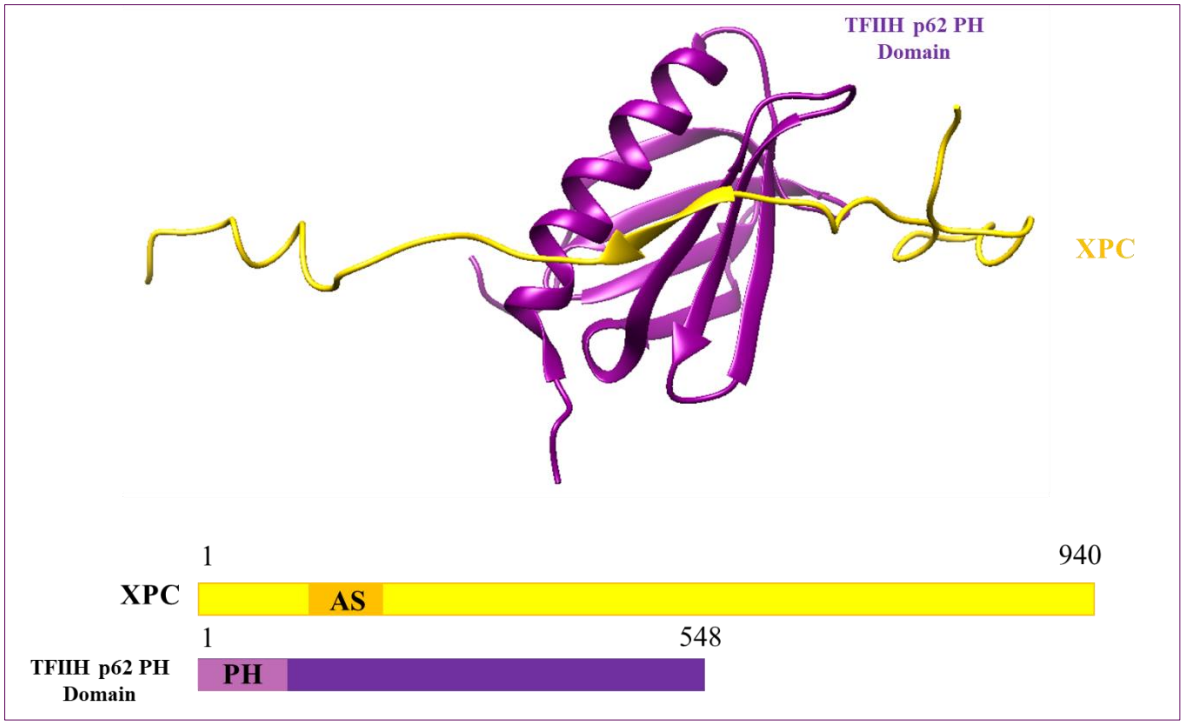

Figure 3. Structure of the Complex between the XPC Acidic String and the TFIIH p62 PH Domain. (A) Domain organization of XPC Acidic String (AS) and and TFIIH p62 (PH). (B) Three-dimensional structure of the acidic string of XPC interacting with the PH domain of TFIIH p62.

that the structure is satisfactory, since it revealed a QMEAN score of 0.75 and Z-score of 0.12 (Figure 4). Thus, the *XPC* model (region 99-156) is considered a good model from the thermodynamic point of view and of structural quality, because it presents values close to proteins already elucidated experimentally.

The molecular docking carried out showed that the binding energy of the complex between the *XPC* region 99-156 and the PH domain of TFIIH p62 is more negative and equivalent to -710.53 kcal/mol compared to the docking between the *XPC* (99-156) with substitution polymorphism, Asn 108 replaced by a Lys, with the PH domain of TFIIH p62, in which the energy of the complex is -611.10 kcal/mol.

DISCUSSION

Several single-nucleotide polymorphisms have been associated with susceptibility to the development of cancer. The SNPs investigated in this work (*rs745769173* - *XPA*, *rs535425175* and *rs761106780* - *XPC*) is the first time they are described and evaluated in neoplastic tissue and were selected because they are close or in regions of interaction with other proteins.

Miller et. al analyzed several SNPs in the *XPA* gene, one in the UTR region (rs1800975) and haplotypes within introns (rs3176633, rs3176689, rs2805835, rs3176719, rs1962592, rs3176751 and rs3176690) in patients with BCC and squamous cell carcinoma (SCC). They found that the G allele of polymorphism rs1800975 is associated with an increased risk of BCC and SCC and the seven haplotypes were in the Hardy-Weinberg equilibrium. This was the first study to relate polymorphisms in the *XPA* gene with the predisposition to the development of non-melanoma skin cancer [15].

Differently from Miller et al [15], Ding et al performed a meta-analysis to analyze the association of rs1800975 polymorphism with cancer risk. They verified that of the 36 control cases analyzed, in general there was no significant association between the polymorphism and the susceptibility to develop cancer. However, in stratified analysis by type of cancer, it was observed that individuals with variant genotypes present a higher risk of lung cancer [32].

Significant associations were also found between the rs2228001 polymorphism (*XPC*) and the risk of lung cancer in the Asian population. Jin et al in the meta-analysis conducted on the basis of 14 studies found that the homozygous genotype Lys939Gln was associated with the risk of developing lung cancer. While the polymorphism rs2228000 was not associated with the risk of this pathogenesis [33].

In squamous cell carcinoma of the head and neck (CCECP), a type of non-melanoma skin cancer, Farnebo et. al sought to investigate PNS as potential risk factors and indicators of survival among Caucasian patients. Four polymorphisms of four genes of the nucleotide excision repair mechanism were studied in 169 patients and 344 controls. The results suggest that all SNPs may affect the risk and survival of CCECP and that rs222800 (*XPC*) is associated with an increased risk of CCECP and laryngeal carcinoma. Statistical analyzes also revealed a twofold increased risk in patients diagnosed with CCECP in the presence of the T allele in the total group, as well as in homozygous men P = 0.02 and a three-fold increased risk in laryngeal carcinoma [34].

No association studies of *XPC* gene SNP with susceptibility to the development of Basal Cell Carcinoma were found. But there are some studies of association of polymorphisms with the risk of developing melanoma. Zhou et al performed meta-analyzes on the association of Lys939Gln polymorphism in the pathogenesis of melanoma in Caucasians, but the results suggest that there was no evidence of an important role of this polymorphism [35].

Our results suggest that there is no association between *rs745769173* and *rs761106780* of the *XPA* and *XPC* genes, respectively, with susceptibility to the development of BCC. However, it was a significant association (P<0.005) between polymorphism *rs535425175* (*XPC*) and susceptibility to the development of BCC.
The *rs535425175* is a SNP near the codon downstream of the site of interaction with TFIIH, a helicase recruited by the *XPC* required repair activity. The analysis revealed that the homozygous genotype C/C confers an increased risk of developing BCC.

Although residue 108 of the *XPC* protein did not participate in the PH domain interaction of TFIIH p62 in any of the complexes. However, energy values suggest that the occurrence of complex binding between *XPC* (99-156) with the Asn residues and the PH domain of TFIIH p62 is more favorable, -710.53 kcal/mol, compared to the domain binding of *XPC* (99-156) with The Lys residues and the PH domain of TFIIH p62, -611.10 kcal/mol. Thus, the results obtained by Docking suggest that the complex protein-protein interaction formed with Lys residue at codon 108 reflects SNP *rs535425175*, which contributes to complex instability, corroborating the association of SNP *rs535425175* to the susceptibility to the development of basal cell cancer by genetic analysis.

CONCLUSION

The results suggest that the mutation at codon 108 of the *XPC* protein, which consists of replacing the Ans residue with the Lys residue related to the SNP *rs535425175* is a risk factor and a potential molecular marker for susceptibility to the development of BCC. As the SNP *rs535425175* was the first time described the need for more studies to corroborate its importance.

Conflict of Interest Disclosures: None

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Corresponding Author:
Eleonidas Moura Lima
Phone: +55 (83) 321674
Email: eleonidas@pq.cnpq.br

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