Role of XPD, P53 and PTCH Genes Mutations in the Development of Early Onset Basal Cell Carcinoma

Skin cancer is a complex and multistage process that is influenced by multiple genetic and environmental factors such as ultraviolet UVA and UVB light, DNA repair capacity (DRC), and genetic polymorphisms in a number of genes including tumor suppressor, cell growth regulators, and DNA repair capacity. Basal cell carcinoma (BCC) is the most common skin cancer in the Western world. The incidence of sporadic BCC cases increases with age. It is usually manifested in individuals->55 years old. In a survey of our 500 BCC patients database we found seven cases which were diagnosed before 35 years old, an age of onset that is very rare for this condition. In this project, we performed a case-control experimental design to study the single nucleotides polymorphisms (SNPs) in the XPD (DNA repair gene), P53 (tumor suppressor and DNA repair), and PTCH (cell growth regulator) genes in this young population. We hypothesized that the presence of mutant allele(s) in a young person increases the risk for developing BCC at young age. We determined the normal allele frequencies of these genes in the Puerto Rican population in order to evaluate the significance of our results in these seven patients.

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Introduction

The most common type of cancer is the non-melanoma skin cancer, which is classified as squamous cell carcinoma (SCC) and basal cell carcinoma (BCC). In this project, we focused on BCC, which is the most common skin cancer in the Western world. The incidence of sporadic BCC has been shown to increase with age, UV exposure,[1,2] low DNA repair capacity,[3] and DNA mutations in nucleotide excision repair, tumor suppressor or cell growth regulator genes.[4–6] It is rare that individuals < 35 years of age develop this kind of cancer. A survey of our database of nearly 500 Puerto Rican skin cancer patients, from an ongoing skin cancer project, showed that seven patients developed the disease before 35 years of age (early onset). A previous research project studied the influence of mutations in the PTCH and P53 genes in the development of BCC at a young age.[4] Mutations, however, in the XPD gene were not examined at that time. In this project, we performed a case-control study to evaluate the single nucleotide polymorphisms (SNPs) in the XPD (DNA repair gene), P53 (tumor suppressor), and PTCH (cell growth regulator) genes that might influence the early onset of BCC.

Methods

Experimental Design

The experimental design was a case-control study in which seven individuals who developed BCC at <35 years of age were matched with seven participants of the same age range (21–35 years) without BCC.

DNA Extraction

DNA extraction from lymphocytes was performed with the DNA Extractor WB Kit (Wako Pure Chemical Industries, Ltd. Richmond, Va), following manufacturer's recommendations. The concentration of the DNA was calculated spectophotometrically.

Polymerase Chain Reaction (PCR)

The 50 μl PCR reactions for XPD Lys 751, P53 exon 5 and PTCH exons 5, 8, and 9 were carried out with 100 ng of DNA and the HotStar (QIAGEN, Valencia, Calif): 25 μl of HotStarTaq Master Mix, 250 μm final primer concentration, 2 μl of 25 mm $MgCl^{+2}$, and water up to 50 μl. The PCR reactions were performed in an Eppendorf Gradient Mastercycler, with the following conditions: initial denaturation 95°C for 15 min, and 35 cycles of amplification using a step program of 45 sec at 94°C, 1 min. at 72°C, a final extension of 10 min at 72°C and hold at 6°C. Positive controls were included in each reaction. Primers sequences and PCR annealing temperatures are presented in Table 1.

Agarose gel electrophoresis

A 3% agarose gel was run to confirm the amplification of the expected fragment and to exclude contamination of the samples.

PCR product DNA purification

A total of 40 μl of PCR product were purified with the DNA Clean &

Table 1. Primer sequences and PCR annealing temperatures

Studied Genes	Sequence Forward 5′- 3′	Sequence Reverse 5′- 3′	Annealing Temperature °C
XPD Lys751Gln	ATCCTGTCCCTACTGGCCATT	TGTGGACGTGACAGTGAGAAA	56
PTCH Exon 5	GCAAAAATTTCTCAGGAACACC	TGGAACAAACAATGATAAGCAA	55
PTCH Exon 8	GAGGCAGTGGAAACTGCTTC	TTGCATAACCAGCGAGTCTG	55
PTCH Exon 9	GTGCTGTCGAGGCTTGTG	AGAAGCAGGAGCAGTCATGG	55
P53 Exon 5	TACTCCCCTGCCCTCAACAA	CATCGCTATCTGAGCAGCGC	57

Concentrator^{tm-5} (ZYMO RESEARCH Orange, Calif) following manufacturer's protocols. The final elution was 8–16 µl (depending on band intensity in the agarose gel).

DNA Direct Sequencing

The sequencing was performed in the PSM Molecular Biology Core in a LI-COR 4300 DNA Analyzer sequencer using the Infra Red M 13 tail approach.

Sequence Analyses

The revision of the obtained chromatograms was performed with the Chromas LITE software (Version 2.0). The edition, alignment, and search for mutations of the sequences was performed with BioEdit software. The edited sequences were blasted (NCBI GenBank) for confirmation purposes.

Statistical Analyses

The statistical analyses included a Student *t* test performed with SPSS (Version 12.0.1 Chicago, Ill).

Results and Discussions

The mutation in the XPD Lys 751 Gln is a risk factor in the development of early onset BCC (OR: 15; $P=0.03$). The DRC was not statistically significant different in these patients when compared to controls ($p=0.13$). This result may suggest that the XPD gene DNA repair helicase activity was not affected but rather its function as a transcription factor, which interacts with the p53 gene, was affected. In the P53 gene exon 5, 25 mutations were observed in patients in which the deletions were the more prevalent. The analyses for the PTCH gene exons 5, 8, 9 showed 145 mutations with transversions presented as the most frequent. For the p53 and PTCH observed mutations, further analyses are required to evaluate the impact at the protein level. Based on our results, we accepted the hypothesis that mutations in the XPD, P53 and PTCH genes increase the risk for developing basal cell carcinoma at a young age.

Significance

The identification of mutant alleles that predispose for the development of BCC at young age may serve as a screening tool for young persons. If a person is screened and the presence of the predisposing alleles is confirmed, then the individual will be educated in the implementation of environmental preventive measures: decrease or avoid exposure to UV rays, use of sun block and protective clothing, which will compensate for their genetic predisposition.

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