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Somatic Mutations in *TP53* Gene in Colombian Patients With Non-melanoma Skin Cancer

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Abstract. *Background/Aim: Non-melanoma skin cancer is the most common cancer in the world. Somatic mutations in the TP53 gene are associated with the development of this cancer. To describe mutations in exons 5-8 of the TP53 gene in a sample of Colombian patients with non-melanoma skin cancer. Materials and Methods: One hundred and fifteen patients with non-melanoma skin cancer were included. Exons 5-8 were amplified and analyzed by PCR-High Resolution Melting and Sanger sequencing. Results: Fifty-seven patients with basal cell carcinomas and 58 with squamous cell carcinomas were studied. 16% of patients with basal cell carcinoma and 26% of patients with squamous cell carcinoma had mutations in the TP53 gene. The most frequent mutations were substitutions, while three patients had deletions. The most frequent mutation was p.R158G. Conclusion: The analysis showed that Colombian individuals with non-melanoma skin cancer have genetic TP53 variants different from those reported as recurrent for this disease.*

Skin cancer is the most frequent malignancy in the world (1). It constitutes a public health problem and causes high costs for the health system (2, 3). In Colombia, there was an increase in new cases of basal cell carcinoma (BCC) from four new diagnoses per 1,000 individuals in 2003 to 11 per 1,000 individuals in 2005, and squamous cell carcinoma (SCC) increased from one to three new diagnoses per 1,000 individuals in the same period (4). The risk factors related to developing these two types of cancer in our population are having skin phototypes 1 to 3, the presence of actinic keratosis, a family history of skin cancer, living in rural areas and working outdoors (5). However, these factors do not fully explain why some people develop skin cancer and others do not. Therefore, understanding the genetic factors involved in tumor development is of great importance for elucidating skin cancer biology. Non-melanoma skin cancer (NMSC) is divided into SCC, BCC, and other less common cancers (6). BCC is the most frequent skin cancer (80%), (7) and it is caused by intermittent exposure to ultraviolet radiation (UVR), especially during youth (8). SCC is less frequent but is a more aggressive tumor, with a greater probability of metastasis and eventual death (9). SCC is more common among people who work outdoors and are exposed to UVR (8).

UVR induces direct and indirect DNA damage, and these DNA lesions must be repaired to prevent the onset of carcinogenic events (10-12). A key molecule during tumor development is the p53 protein, encoded by the *TP53* gene. In response to cellular stress, p53 modulates the transcription of genes involved in cell cycle arrest, apoptosis, DNA stability and the inhibition of angiogenesis (13-15). Therefore, the alteration of these pathways due to mutation or loss of *TP53* predisposes individuals to tumor development (16). The *TP53* mutations associated with cancer are very diverse and are located throughout the entire sequence of the gene (17-19). However, the most important mutations are located in exons 5 to 8, which encode the DNA-binding domain, and these mutations impair the DNA binding ability (17, 20). In NMSC, *TP53* mutations have been described in up to 50% of cases (21, 22).

High-resolution melting (HRM) scans for mutations by using polymerase chain reaction (PCR) assays. It has been used as an alternative strategy for the screening of variants in DNA due to differences in DNA melting temperatures and curve profiles from samples controls (23). This method is

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fast and much cheaper than other mutation genotyping technologies and does not require post-PCR handling (24). The aim of this study was to describe mutations in exons 5-8 of the *TP53* gene in a sample of Colombian patients with non-melanoma skin cancer using PCR-High resolution melting as prescreening method.

Materials and Methods

Patients and samples. A descriptive cross-sectional study was carried out. We included 116 subjects older than 18 years and diagnosed with non-melanoma skin cancer (58 with BCC and 58 with SCC) between 2012 and 2014. Samples of tumor material from each patient were embedded in paraffin (TTEP) and fixed in formalin. The study was conducted in accordance with ethical standards for human research. The patients were asked to participate voluntarily and signed their consent. The study and the informed consent were approved by the Research Ethics Committee of the Hospital Universitario-Centro Dermatológico Federico Lleras Acosta, E.S.E (Bogota, Colombia).

Cell culture. The cell lines used as controls were HT-29 (Human Colorectal Adenocarcinoma; c.818G>A; exon 8), NCI-H727 (Human lung carcinoma, non-small cell; c.492_493ins9 exon 5), DU145 (c.820G>T; exon 8), HEK-293 (Human kidney; wild type), AU565 (Breast carcinoma; c.524G>A), NCI-H82 (Small cell lung cancer; c.375G>T), NCI-H520 (Lung squamous cell carcinoma; c.438G>A) and PC3 (Prostate adenocarcinoma; c.413del1). Cell lines were kindly donated from Fabio Aristizabal (Universidad Nacional de Colombia, Bogotá, Colombia).

All cell lines were cultured in Corning Glass Culture Flasks (Sigma-Aldrich, St. Louis, MO, USA). All cell lines except HEK-293 were cultured in RPMI 1640 culture medium (Gibco, Life Technologies Corporation, Grand Island, NY, USA), supplemented with 10% fetal serum bovine (v/v). Cultures were maintained at 37˚C, in a humid atmosphere with 5% CO_2. HEK293 cells were cultured using DMEM supplemented with 5% fetal serum bovine (v/v) and streptomycin -penicillin (Gibco, Life Technologies Corporation, Grand Island, NY, USA). Genomic DNA was extracted from confluent cultures (70-80%) and used as control in the PCR-High Resolution Melting.

DNA extraction. From 7 μm-thick histological sections of TTEP stained with haematoxylin and eosin, the tumor tissue was microdissected using light microscopy to ensure 80% morphological tumor characteristics when possible. The DNA was extracted using the commercial QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The DNA integrity was verified by the absorbance ratio at 260/280 nm wavelength measured by NanoDrop 2000c (Thermo Scientific, Waltham, MA, USA), and amplification of the *GAPDH* gene and electrophoresis on 1% agarose gels. Also, DNA from control cell lines were extracted using the same method.

Screening of mutations by PCR-HRM. High resolution melting (HRM) analysis was used to screen mutations in exons 5-8 of the *TP53* gene. PCR and HRM were performed in a CFX96 system thermocycler (Bio-Rad Laboratories, Hercules, CA, USA). For HRM of exons 7 and 8, a single segment was amplified per exon using the primers published by Krypuy *et al*. (23). For exon 5, three segments were amplified using the primers described by Mitchell *et al*. (24). For exon 6, primers were designed in Primer3 (25) (Table I). All analyses were performed in duplicate in 96-well plates with 2 ng of DNA from each sample for a final volume of 20 μl. The Precision Melt Supermix (Bio-Rad) kit was used for HRM. Amplification conditions included an initial denaturation of 5 min at 95˚C, followed by 45 cycles of 95˚ for 10 s, annealing according to Table I and by 10 s at 72˚C. The samples were kept at 60˚C for 30 s, and a denaturation curve was then generated by increasing the temperature by 0.1˚C/s. Electrophoresis was performed on 2% agarose gel stained with SYBR-Safe (Thermo Fisher Scientific) to verify the size and identify the amplified products.

DNA sequencing analysis of TP53 exons 5-8. The sequence of exons 5-8 was determined by direct sequencing from DNA extracted from the cell lines mentioned in Table II. According to the presence mutations or WT sequence in exons 5-8 in each cell line, these were used to optimize the HRM-PCR conditions of each exon as indicated in Table I. The primers used were reported by Krypuy *et al*. (23). The primers sequences and the conditions of PCR are shown in Table I. Samples with differences in the denaturation curves adjusted to temperature and normalized to DNA control of each cell line during the HRM protocol were subjected to amplification by conventional PCR using the primers as shown in Table II. Subsequently, the PCR products were purified using the QIAquick PCR Purification Kit (Qiagen) according to the manufacturer's instructions, and bidirectional direct sequencing was carried out with the Sanger method using BigDye Terminator V1.1 Cycle Sequencing Kit and an ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems, Waltham, MA, USA). Once the sequence was obtained, it was analyzed using the novoSNP program version 3.0 (Department of Molecular Genetics VIB and University of Antwerp) against the *TP53* reference sequence extracted from the ENSEMBL database (ENST00000269305). Additionally, variants were analyzed using the Excel MUT-*TP53* 2.0 open-access data sheet. The variants were verified in the International Agency for Research on Cancer (IARC) mutation database for *TP53* (http://p53.fr) (*IARC TP53 Database*).

TP53 loss of heterozygosity testing. The DNA from anti-coagulated blood was extracted to identify *TP53* loss of heterozygosity (LOH). It was done using QIAamp DNA blood kit (Qiagen) according to the manufacturer's instructions. Peripheral blood DNA samples of patients that showed variants in *TP53* in tumor tissue were amplified and sequenced.

Results

The average age was 61 years for BCC patients and 68 years for SCC patients. 46% (27/58) of patients diagnosed with BCC and 39% (23/58) with SCC were women. 35% of BCC patients (20/57) and 57% of SCC (33/58) patients reported working outdoors. 47% (27/57) and 60% (34/57) of the patients with BCC and SCC, respectively, reported having a family member with a history of cancer. Family history of gastric cancer was the most frequent antecedent, in 25% of patients with BCC (7/57) and 23% (8/34) with SCC. Twenty-three percent (8/34) of patients diagnosed with SCC reported a family history of melanoma.

Table I. *Primer sequences for high resolution melting analysis and conventional PCR for sequencing.*

Gene	Primer sequence	Primer Tm (˚C)	Amplicon size
PCR-HRM			
Exon 5.1	F: 5'CCCTGACTTTCAACTCTGTCTCC3'	61	115
	R: 3'GGTGTGGAATCAACCCACAGC5'		
Exon 5.2	F: 5'GCGCCATGGCCATCTACAAG3'	61	109
	R: 3'CAACCAGCCCTGTCGTCTCT5'		
Exon 5.3	F:5'GCGCAATGGCAATCTACAAGC3'	61	125
	R: 3'CAACCAGCCCTGTCGTCTCTC5'		
Exon 6	F:5'CCAGGCCTCTGATTCCTCAC-3'	68˚C 10 cycles touchdown 1˚C/ cycle and	174
	R: 3'TCCCAGAGACCCCAGTTGC-5'	35 cycles at 58˚C	
Exon 7	F: 5'AGGCGACACTGGCCTCATC3'		
	R: 3'GAGGCTGGGGCACAGCA5'	63˚C 10 cycles touchdown 0.5˚C/	200
Exon 8	F: 5'GACCTGATTTCCTTACTGCCTCTTG3'	cycle and 40 cycles at 58˚C	
	R: 3'AATCTGAGGCATAACTGCACCCTT5'		245
PCR (conventional; used for sequencing)			
Exon 5	F: 5'CCCTGACTTTCAACTCTGTCT3'		
	R: 3'CAACCAGCCCTGTCGTCTCTC5	63	260
Exon 6	F: 5'AGATAGCGATGGTGAGCAGC3'		
	R: 3'ACTGACAACCACCCTTAACC5'	63	255
Exon 7	F:5'CAGGTCTCCCAAGGCGCAC3'	67˚C 10 cycles touchdown 0.5˚C/	219
	R:3'GCAAGCAGAGGCTGGGGCAC5'	cycle and 40 cycles at 60˚C	
Exon 8	F:5'GGAATAGATGGAGCCTGGTT3'	67	287
	R: 3'GTGAATCTGAGGCATAACTG5'		

PCR-HRM: Polymerase chain reaction-High resolution melting; Tm: temperature.

Table II. *Cancer cell lines used as mutation controls.*

Cellular line	Origin	N˚ ATCC	Exon	Mutation reported in IARC
HT-29	Colorectal adenocarcinoma	HTB-38	8	c.818G>A
AU565	Adenocarcinoma breast	CRL-2351	5	c.524G>A
NCI-H82	Small cell carcinoma (Lung)	HTB-175	4	c.375G>T
NCI-H727	Lung/Bronchus carcinoid	CRL-5815	5	c.492_493ins9
PC-3	Prostate carcinoma	CLR-1435	5	c.413del1
NCI-H520	Small cell carcinoma (Lung)	HTB-182	5	c.438G>A
HEK-293	Normal kidney tissue	CRL-10852	WT	No mutations
NCI-H520	Small cell carcinoma (Lung)	HTB-182	5	c.438G>A
HEK-293	Normal kidney tissue	CRL-10852	WT	No mutations

IARC: International Agency for Research on Cancer.

None of the patients studied was immunosuppressed. Likewise, none of the patients presented with metastasis. Only one patient with SCC had a history of radiotherapy prior to the tumor diagnosis. Concerning BCCs, 98.2% (56/57) were primary; 64% of these cases were nodular and 31% were mixed. 43% of the patients with SCC histopathology had tumors with moderate differentiation, while 32% of SCC patients had well-differentiated tumors.

To detect samples with probable mutations in exons 5-8 of *TP53,* 115 samples from patients diagnosed with BCC and SCC were analyzed using HRM (Figure 1). The variants present in the cell lines used as controls for the PCR-HRM analysis are found in Table II. The subsequent direct sequencing of samples with differences in the HRM analysis identified 10 pathogenic variants, 4 variants of uncertain significance (VUS) and one benign. Of the variants detected, 80% were substitutions and 20% were deletions (Figure 2 and Table III). 16% of patients diagnosed with BCC and 26% of patients with SCC had variants in the *TP53* gene sequence. The most recurrent mutation in these patients was the missense mutation p.R158P (c.472C>G) resulting in an amino acid substitution from Arginine to Glycine. This mutation was found

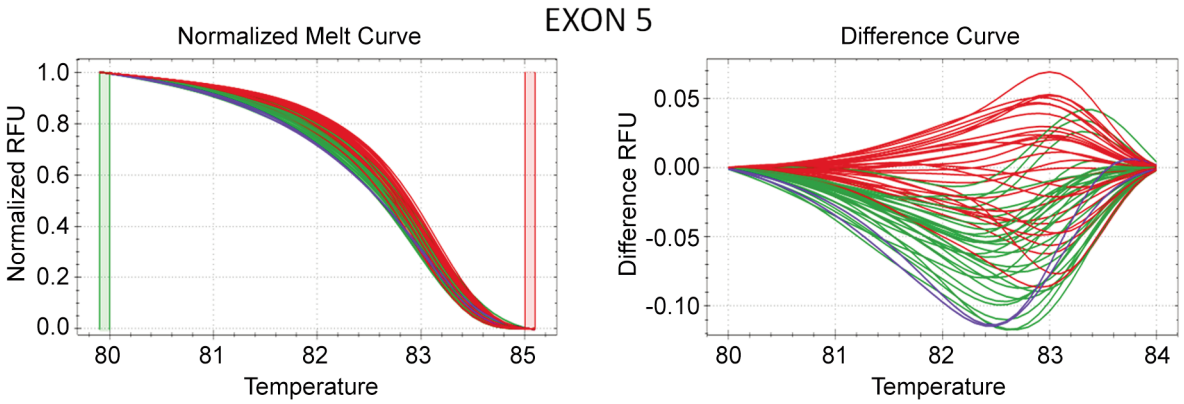

Figure 1. *Differences in the denaturation curve for the different amplicons of exon 5 (fragment 1 of 115 bp). The different colors represent the differences in temperature denaturation.*

in 12% of patients with BCC and 10% with SCC. Of the samples with *TP53* mutations, the most frequent histological subtype was nodular in BCC and poorly differentiated in SCC (Table IV). Any of the variants found in the tumor tissue were found in the matched peripheral blood DNA of each patient.

Discussion

Non-melanoma skin cancer (NMSC; BCC and SCC) has several risk factors, among which are prolonged UVR exposure, skin type, gender, and ability to repair DNA (5, 22, 26). A key molecule during carcinogenesis is the p53 protein, encoded by the *TP53* gene (13, 14).

UVR produces a skin response because it induces the formation of cyclobutane pyrimidine dimers (CPDs) and pyrimidine-pyrimidone photoproducts (6-4 PPs) (9, 12). Despite the clear relationship between *TP53* mutations and the risk of developing skin cancer (18, 19, 27, 28), the mutation rates reported in the literature vary according to the population analyzed (29). In this study, tumor tissue was obtained from biopsies of patients with BCC and SCC diagnoses, and samples were screened for mutations in exons 5 through 8 of *TP53* with HRM analysis and subsequent direct sequencing of the DNA fragments that differed from control cell lines in their denaturation curves.

Among the 115 samples analyzed, 16% of the BCCs and 26% of the SCCs had mutations in exons 5 to 8. This frequency is similar to that reported for populations in China (20% BCC) (30) and Korea (33%) (31), while it is lower when compared to populations such as England and Sweden (58%) (32) and the United States (between 45 and 58%) (33, 34).

Although Colombia is a tropical country, it has an estimated incidence of skin cancer that is lower than other locations with fewer geographic risk factors. It is possible that skin cancer rates in Colombia are not as high as those reported in Australia or some European countries because of phototype and individual genetic susceptibility. It may be that a combination of certain phototypes with continuous but controlled exposure (not intense enough to produce sunburn) generates skin changes, such as increased melanin production and thickening of the corneum stratum, that protect the skin from the deleterious effects of UVR (5, 32). In addition, the genetic background of Colombian populations is shaped by the mixture of native, European and African migration patterns. This heterogeneity may have an impact on the genetic susceptibility to the development of squamous cell carcinoma or basal cell carcinoma (35).

In this work, we found that 80% of the mutations are substitutions, which is in agreement with the data reported in the IARC database. The most studied genetic alterations are in

Table III. *Mutations found in 116 patients with basal cell carcinoma and squamous cell carcinoma. Genetic variants are confirmed in the IARC database, p53fr and Uniprot -P04637 (P53_HUMAN).*

Variant	Nomenclature protein	Exon	Classification of variant
c.818G>A	p.R273H	8	Pathogenic
c.517G>A	p.V173M	5	Pathogenic
c.475G>A	p.A159T	5	VUS*
c.472C>G	p.R158G	5	Pathogenic
c.464delC	p.T155Xfs	5	VUS*
c.464C>A	p.T155N	5	Pathogenic
c.458delC	p.P153Xfs	5	Pathogenic
c.415delAGGGCAGGTC	p.K139Xfs	5	Pathogenic
c.425C>A	p.P142H	5	VUS*
c.458C>G	p.P153R	5	VUS*
c.639A>G	p.R213R	6	Benign
c.535C>T	p.H179Y	5	Pathogenic
c.458C>T	p.P153L	5	Pathogenic
c.466C>T	p.R156C	5	Pathogenic
c.451C>T	p.P151S	5	Pathogenic

*VUS: Variants of uncertain significance.

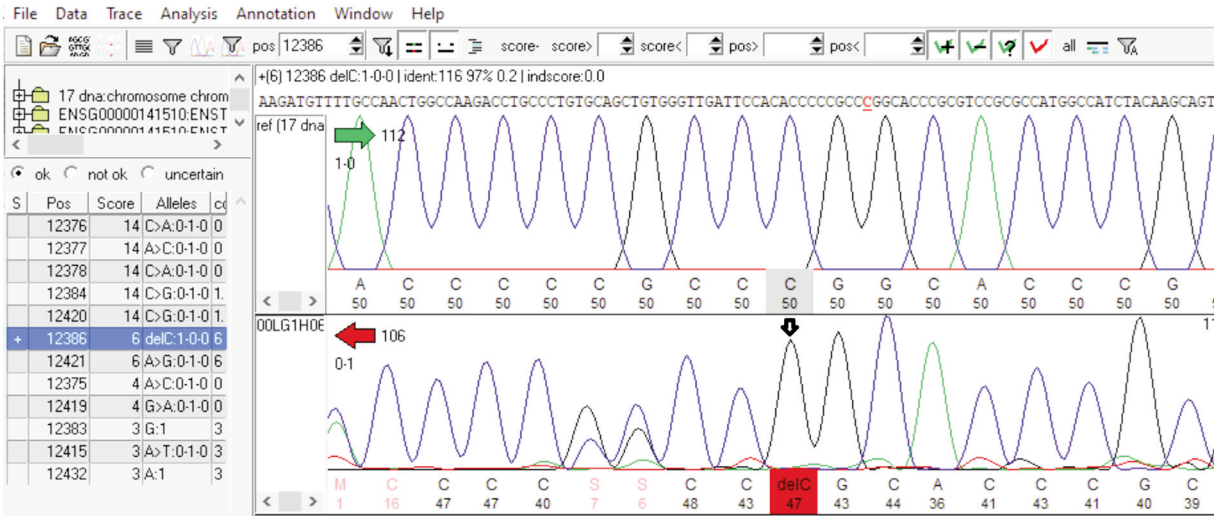

Figure 2. *Sequence of an amplified 184 bp product of exon 5 showing the deletion of a nucleotide (G) that results in the p.P153Xfs mutation in sample-BCC 60. The sequence was analyzed using novoSNP program version 3.0 (Department of Molecular Genetics VIB and University of Antwerp).*

Table IV. *Location and variants in the TP53 gene found in the histological BCC and SCC subtypes.*

Patient code	Gender	Age (years)	Location	Histological diagnosis	Histological BCC and SCC subtype	Variants
BCC01	F	49	Malar region	BCC	Mixed	p.R158G
BCC04	M	60	Cheeks	BCC	Nodular	p.R158G
BCC 17	F	64	Nose	BCC	Nodular	p.R158G
BCC 24	M	58	Nose	BCC	Mixed	p.P153R p.H179Y
BCC48	M	64	Nose	BCC	Nodular	p.R158G
BCC59	M	30	Nose	BCC	Nodular	p.R158G
BCC60	M	76	Malar region	BCC	Nodular	p.P153Xfs
BCC73	F	46	Nose	BCC	Nodular	p.R158G
BCC75	F	45	Nose	BCC	Nodular	p.R158G
SCC04	F	80	Thigh	SCC	*In situ*	p.P151S p.R273H
SCC 10	M	81	Jaw	SCC	Poorly differentiated	p.R158G
SCC24	M	53	Lip	SCC	Poorly	p.K139Xfs
CEC26	M	66	Parietal region	CEC	Poorly differentiated	p.P151S
CEC30	M	71	Forearm	CEC	Poorly differentiated	p.R158G
CEC35	F	41	Vagina	CEC	*In situ*	p.R158G
CEC36	M	53	Front	CEC	Poorly differentiated	p.R156C
CEC38	M	74	Finger	CEC	Poorly differentiated	p.P151S p.R158G
CEC44	F	65	Nose	CEC	Well differentiated	p.T155Xfs p.A159T
CEC46	M	71	Ear	CEC	Well differentiated	p.R273H
CEC47	M	66	Chest	CEC	Poorly differentiated	p.R158G p.T155N p.V173M
CEC48	F	65	Hand	CEC	Poorly differentiated	p.P153L
CEC49	F	70	Cheek	CEC	Well differentiated	p.P153L
CEC54	F	69	Lip	CEC	Well differentiated	p.P151S
CEC57	F	81	Ear	CEC	*In situ*	p.R158G

the region encoding the DNA-binding domain, which includes exons 5 to 8. The majority of the mutations in this domain (87.9%) are substitutions that generate missense variants (18, 36). These mutations generate proteins that form a p53 tetramer that is less competent to specifically bind DNA, an effect that has been correlated with loss of gene function (18).

On the other hand, frameshift mutations represent 11% of the mutations reported in all types of cancer (37). In this study, deletions were detected in three patients; one of the deletion mutations eliminated 10 nucleotides, resulting in a frameshift mutation and the generation of a premature stop codon.

The most frequent mutations in non-melanoma skin cancer are C:G,T:A, and CC:TT substitution in pyrimidine dimers; this mutation type represents 80% of all *TP53* mutations (31, 38, 39). Twenty-five percent of these substitutions are found in CpG dinucleotides, which have a 10-fold greater mutation frequency than other dinucleotides. The cytosines in CpG dinucleotides are methylated in normal tissue and are frequently deaminated relative to non-methylated cytosines. CpG substitutions generate poor base pairing and represent at least a third of the known *TP53* substitutions (37). In this work, the C>T and G>A substitutions represented 47% of the point mutations, indicating that the *TP53* mutations in these individuals may be related to sun exposure.

The majority of *TP53* mutations reported here are in the DNA-binding domain (DBD). This result is likely because the DBD region contains 22 CpG dinucleotides and three codons (175, 248, and 273) that are considered to be mutation hot spots (40). Likewise, the effect of each genetic variant on protein function depends on its site within the DBD and the type of amino acid substituted. Changes in Loop1 (L1) (residues F113-T123), Loop2 (L2) (residues K164-C176), and Loop3 (L3) (M237-P250) as well as in the amino acids involved in the coordination of zinc binding (R175, C176, H179, C238 and C242) affect the antiproliferative function of p53 and the thermodynamic stability of the DBD (41). In the samples analyzed in this study, a group of mutations located between L1 and L2 (139, 142, 151, 153, 155, 156, 158, and 159) was found, indicating a pattern of variants different from that reported in the literature; however, since the substitutions introduce amino acids with different polarity, these variants impact the protein function.

Three mutations in the DBD region were detected that generate stop codons and frameshifts (p.K139Xfs, p.P153Xfs, and p.P155Xfs); these variants generate premature proteins lacking the DNA-binding segment, and they likely play an important role in carcinogenesis. The p.V173M and p.H179Y mutations detected are located within Loop2 of the DBD; specifically, they are in residue 179, which binds to zinc and coordinates the binding to DNA. These mutations are considered disruptive mutations and impact protein function (41).

The mutation hot spot located at codon 273 was found in two patients diagnosed with SCC. The change from arginine to histidine at position 273 (p.R273H) causes reduced selectivity of DNA-binding compared to the normal protein (40). This variant is associated with carcinogenesis because it participates in invasion, increased migration, increased cell proliferation, and drug resistance, thus preventing the death of the tumor cell (42).

Pfeifer *et al*. (2005) showed that mutation hot spots in skin cancer are found in codons 152, 158, 196, 213, 245, and 282, which contain methylated cytosines and are altered by UVR (44). In contrast, Giglia-Mari and Sarasin report that codon 177 is the most frequently mutated in BCC and codon 278 in SCC (22). In the samples analyzed in this work, we found that codon 158 (p.R158G; c.472C>G) had the highest mutation rate. This codon is between L1 and L2 of the DBD. The prediction analysis on the impact of protein function shows a score of 98.26, indicating loss of function (43, 44).

The discovery of the recurrent p.R158G mutation is important due to its high frequency in this population; 12% (7/57) of the BCC patients and 9% (5/58) of the SCC patients had this mutation. This mutation is reported only 21 times in the p53 database and decreases protein activity by approximately 90% (IARC) (25), which could suggest a preponderant role in the development of NMSC in the population studied. Future studies with a larger sample size are required to verify whether this is a high-frequency (hot spot) variant in our population.

In 80% of patients with identified *TP53* mutations, only one mutation was detected, while the remaining 20% (5/24) had more than one mutation found. The presence of multiple mutations has been reported in human skin cancer, human head and neck cancer, and mouse skin cancer induced by UVR (42, 45-48). This finding is explained by continuous exposure to UVR generating multiple mutational events. On the other hand, the cells that make up the tumor come from multiple clones each with different mutations (49).

Conclusion

This study shows that the frequency of *TP53* mutations in tumor tissue from a group of Colombian patients diagnosed with BCC and SCC was similar to that reported for other geographic areas. The sample analyzed has a different pattern of mutations than that reported by other authors for this type of cancer; our discovery of the recurrent p.R158G mutation is most relevant, as it suggests that this mutation could have an important role in the development of SCC and BCC. This study screened for mutations in exons 5 to 8 and did not exclude the presence of mutations in the other exons of the gene.

Data Availability

The informed consent, clinical data, and molecular biology results of this study are restricted by the ethics committee of the Hospital

Universitario-Centro Dermatológico Federico Lleras Acosta E.S.E. and are available from the file with the code 4000-16.6W. These files can be requested in the following e-mail: Comitedeeticaeninvestigacion@dermatologia.gov.co

Conflicts of Interest

The Authors declare no conflicts of interest.

Authors' Contributions

Conceptualization: Luz D Gutiérrez-Castañeda; methodology: Maria Irene Cerezo Cortés and Luz D Gutiérrez-Castañeda; software: Maria Irene Cerezo Cortés and Luz D Gutiérrez-Castañeda; validation: Luz D Gutiérrez-Castañeda; formal analysis: Maria Irene Cerezo Cortés and Luz D Gutiérrez-Castañeda; investigation, Maria Irene Cerezo Cortés and Luz D Gutiérrez-Castañeda; resources: Luz D Gutiérrez-Castañeda; data curation: Maria Irene Cerezo Cortés and John Nova; writing – original draft preparation: Maria Irene Cerezo Cortés and Luz D Gutiérrez-Castañeda; writing – review and editing: Maria Irene Cerezo Cortés, Luz D Gutiérrez-Castañeda and John Nova; supervision: Luz D Gutiérrez-Castañeda; project administration: Luz D Gutiérrez-Castañeda; funding acquisition: Luz D Gutiérrez-Castañeda. All Authors have read and agreed to the published version of the manuscript.

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References

1 Gaddameedhi S, Selby CP, Kaufmann WK, Smart RC and Sancar A: Control of skin cancer by the circadian rhythm. Proc Natl Acad Sci USA *108(46)*: 18790-18795, 2011. PMID: 22025708. DOI: 10.1073/pnas.1115249108

2 Almazán-Fernández FM, Serrano-Ortega S and Moreno-Villalonga JJ: Descriptive study of the costs of diagnosis and treatment of cutaneous melanoma. Actas Dermosifiliogr *100(9)*: 785-791, 2009. PMID: 19889300.

3 Souza RJ, Mattedi AP, Rezende ML, Corrêa Mde P and Duarte EM: An estimate of the cost of treating melanoma disease in the state of Sao Paulo - Brazil. An Bras Dermatol *84(3)*: 237-243, 2009. PMID: 19668936. DOI: 10.1590/s0365-05962009000300004

4 Nova-Villanueva J, Sánchez-Vanegas G and Porras de Quintana L: Skin cancer: a Colombian reference centre's epidemiological profile 2003-2005. Rev Salud Publica (Bogota) *9(4)*: 595-601, 2007. PMID: 18209826. DOI: 10.1590/s0124-00642007000400012

5 Sánchez G, Nova J and de la Hoz F: Risk factors for basal cell carcinoma: a study from the national dermatology center of Colombia. Actas Dermosifiliogr *103(4)*: 294-300, 2012. PMID: 22078143. DOI: 10.1016/j.ad.2011.07.012

6 Jiang DK, Wang WZ, Ren WH, Yao L, Peng B and Yu L: TP53 Arg72Pro polymorphism and skin cancer risk: a meta-analysis. J Invest Dermatol *131(1)*: 220-228, 2011. PMID: 20861852. DOI: 10.1038/jid.2010.270

7 Epstein EH: Basal cell carcinomas: attack of the hedgehog. Nat Rev Cancer *8(10)*: 743-754, 2008. PMID: 18813320. DOI: 10.1038/nrc2503

8 Montes de Oca MK, Pearlman RL, McClees SF, Strickland R and Afaq F: Phytochemicals for the prevention of photocarcinogenesis. Photochem Photobiol *93(4)*: 956-974, 2017. PMID: 28063168. DOI: 10.1111/php.12711

9 White AC, Tran K, Khuu J, Dang C, Cui Y, Binder SW and Lowry WE: Defining the origins of Ras/p53-mediated squamous cell carcinoma. Proc Natl Acad Sci U S A *108(18)*: 7425-7430, 2011. PMID: 21502519. DOI: 10.1073/pnas.1012670108

10 Mitra S: Does evening sun increase the risk of skin cancer? Proc Natl Acad Sci USA *108(47)*: 18857-18858, 2011. PMID: 22084098. DOI: 10.1073/pnas.1116516108

11 Pfeifer GP and Besaratinia A: UV wavelength-dependent DNA damage and human non-melanoma and melanoma skin cancer. Photochem Photobiol Sci *11(1)*: 90-97, 2012. PMID: 21804977. DOI: 10.1039/c1pp05144j

12 Sage E, Girard PM and Francesconi S: Unravelling UVA-induced mutagenesis. Photochem Photobiol Sci *11(1)*: 74-80, 2012. PMID: 21901217. DOI: 10.1039/c1pp05219e

13 Benjamin CL, Ullrich SE, Kripke ML and Ananthaswamy HN: p53 tumor suppressor gene: a critical molecular target for UV induction and prevention of skin cancer. Photochem Photobiol *84(1)*: 55-62, 2008. PMID: 18173701. DOI: 10.1111/j.1751-1097.2007.00213.x

14 Benjamin CL and Ananthaswamy HN: p53 and the pathogenesis of skin cancer. Toxicol Appl Pharmacol *224(3)*: 241-248, 2007. PMID: 17270229. DOI: 10.1016/j.taap.2006.12.006

15 de Gruijl FR, van Kranen HJ and Mullenders LH: UV-induced DNA damage, repair, mutations and oncogenic pathways in skin cancer. J Photochem Photobiol B *63(1-3)*: 19-27, 2001. PMID: 11684448. DOI: 10.1016/s1011-1344(01)00199-3

16 Levine AJ: p53: 800 million years of evolution and 40 years of discovery. Nat Rev Cancer *20(8)*: 471-480, 2020. PMID: 32404993. DOI: 10.1038/s41568-020-0262-1

17 Kim E, Giese A and Deppert W: Wild-type p53 in cancer cells: when a guardian turns into a blackguard. Biochem Pharmacol *77(1)*: 11-20, 2009. PMID: 18812169. DOI: 10.1016/j.bcp.2008.08.030

18 Menendez D, Inga A and Resnick MA: The expanding universe of p53 targets. Nat Rev Cancer *9(10)*: 724-737, 2009. PMID: 19776742. DOI: 10.1038/nrc2730

19 Petitjean A, Achatz MI, Borresen-Dale AL, Hainaut P and Olivier M: TP53 mutations in human cancers: functional selection and impact on cancer prognosis and outcomes. Oncogene *26(15)*: 2157-2165, 2007. PMID: 17401424. DOI: 10.1038/sj.onc.1210302

20 Joerger AC and Fersht AR: Structure-function-rescue: the diverse nature of common p53 cancer mutants. Oncogene *26(15)*: 2226-2242, 2007. PMID: 17401432. DOI: 10.1038/sj.onc.1210291

21 Giglia-Mari G and Sarasin A: TP53 mutations in human skin cancers. Hum Mutat *21(3)*: 217-228, 2003. PMID: 12619107. DOI: 10.1002/humu.10179

22 Rivlin N, Brosh R, Oren M and Rotter V: Mutations in the p53 tumor suppressor gene: Important milestones at the various steps of tumorigenesis. Genes Cancer *2(4)*: 466-474, 2011. PMID: 21779514. DOI: 10.1177/1947601911408889

23 Krypuy M, Ahmed AA, Etemadmoghadam D, Hyland SJ, Australian Ovarian Cancer Study Group, DeFazio A, Fox SB, Brenton JD, Bowtell DD and Dobrovic A: High resolution melting for mutation scanning of TP53 exons 5-8. BMC Cancer *7*: 168, 2007. PMID: 17764544. DOI: 10.1186/1471-2407-7-168

24 Mitchell G, Ballinger ML, Wong S, Hewitt C, James P, Young MA, Cipponi A, Pang T, Goode DL, Dobrovic A, Thomas DM and International Sarcoma Kindred Study: High frequency of germline TP53 mutations in a prospective adult-onset sarcoma cohort. PLoS One *8(7)*: e69026, 2013. PMID: 23894400. DOI: 10.1371/journal.pone.0069026

25 Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M and Rozen SG: Primer3 – new capabilities and interfaces. Nucleic Acids Res *40(15)*: e115, 2012. PMID: 22730293. DOI: 10.1093/nar/gks596

26 Welcome - IARC TP53 Database. Available at: https://p53.iarc.fr/ [Last accessed March 13, 2020]

27 Khalesi M, Whiteman DC, Doi SA, Clark J, Kimlin MG and Neale RE: Cutaneous markers of photo-damage and risk of Basal cell carcinoma of the skin: a meta-analysis. Cancer Epidemiol Biomarkers Prev *22(9)*: 1483-1489, 2013. PMID: 23833126. DOI: 10.1158/1055-9965.EPI-13-0424

28 Ateenyi-Agaba C, Dai M, Le Calvez F, Katongole-Mbidde E, Smet A, Tommasino M, Franceschi S, Hainaut P and Weiderpass E: TP53 mutations in squamous-cell carcinomas of the conjunctiva: evidence for UV-induced mutagenesis. Mutagenesis *19(5)*: 399-401, 2004. PMID: 15388813. DOI: 10.1093/mutage/geh048

29 Olivier M, Hussain SP, Caron de Fromentel C, Hainaut P and Harris CC: TP53 mutation spectra and load: a tool for generating hypotheses on the etiology of cancer. IARC Sci Publ *(157)*: 247-270, 2004. PMID: 15055300.

30 Pellegrini C, Maturo MG, Di Nardo L, Ciciarelli V, Gutiérrez García-Rodrigo C and Fargnoli MC: Understanding the Molecular Genetics of Basal Cell Carcinoma. Int J Mol Sci *18(11)*: 2485, 2017. PMID: 29165358. DOI: 10.3390/ijms18112485

31 Wang YM, Huang YS, Ma ZH, Bu DF, Wang Y, Tu P and Li H: Frequency and features of TP53 mutation in 30 Chinese patients with sporadic basal cell carcinoma. Clin Exp Dermatol *39(7)*: 829-834, 2014. PMID: 25196205. DOI: 10.1111/ced.12411

32 Kim MY, Park HJ, Baek SC, Byun DG and Houh D: Mutations of the p53 and PTCH gene in basal cell carcinomas: UV mutation signature and strand bias. J Dermatol Sci *29(1)*: 1-9, 2002. PMID: 12007715. DOI: 10.1016/s0923-1811(01)00170-0

33 Brash DE, Rudolph JA, Simon JA, Lin A, McKenna GJ, Baden HP, Halperin AJ and Pontén J: A role for sunlight in skin cancer: UV-induced p53 mutations in squamous cell carcinoma. Proc Natl Acad Sci USA *88(22)*: 10124-10128, 1991. PMID: 1946433. DOI: 10.1073/pnas.88.22.10124

34 Rady P, Scinicariello F, Wagner RF Jr and Tyring SK: p53 mutations in basal cell carcinomas. Cancer Res *52(13)*: 3804-3806, 1992. PMID: 1617650.

35 Ossa H, Aquino J, Pereira R, Ibarra A, Ossa RH, Pérez LA, Granda JD, Lattig MC, Groot H, Fagundes de Carvalho E and Gusmão L: Outlining the ancestry landscape of Colombian admixed populations. PLoS One *11(10)*: e0164414, 2016. PMID: 27736937. DOI: 10.1371/journal.pone.0164414

36 Olivier M, Hollstein M and Hainaut P: TP53 mutations in human cancers: origins, consequences, and clinical use. Cold Spring Harb Perspect Biol *2(1)*: a001008, 2010. PMID: 20182602. DOI: 10.1101/cshperspect.a001008

37 Pfeifer GP and Besaratinia A: Mutational spectra of human cancer. Hum Genet *125(5-6)*: 493-506, 2009. PMID: 19308457. DOI: 10.1007/s00439-009-0657-2

38 Leroy B, Anderson M and Soussi T: TP53 mutations in human cancer: database reassessment and prospects for the next decade. Hum Mutat *35(6)*: 672-688, 2014. PMID: 24665023. DOI: 10.1002/humu.22552

39 Setlow RB and Carrier WL: Pyrimidine dimers in ultraviolet-irradiated DNA's. J Mol Biol *17(1)*: 237-254, 1966. PMID: 4289765. DOI: 10.1016/s0022-2836(66)80105-5

40 Ziegler A, Jonason AS, Leffell DJ, Simon JA, Sharma HW, Kimmelman J, Remington L, Jacks T and Brash DE: Sunburn and p53 in the onset of skin cancer. Nature *372(6508)*: 773-776, 1994. PMID: 7997263. DOI: 10.1038/372773a0

41 Saha T, Kar RK and Sa G: Structural and sequential context of p53: A review of experimental and theoretical evidence. Prog Biophys Mol Biol *117(2-3)*: 250-263, 2015. PMID: 25550083. DOI: 10.1016/j.pbiomolbio.2014.12.002

42 Kotler E, Shani O, Goldfeld G, Lotan-Pompan M, Tarcic O, Gershoni A, Hopf TA, Marks DS, Oren M and Segal E: A systematic p53 mutation library links differential functional impact to cancer mutation pattern and evolutionary conservation. Mol Cell *71(1)*: 178-190.e8, 2018. PMID: 29979965. DOI: 10.1016/j.molcel.2018.06.012

43 Muller PA and Vousden KH: Mutant p53 in cancer: new functions and therapeutic opportunities. Cancer Cell *25(3)*: 304-317, 2014. PMID: 24651012. DOI: 10.1016/j.ccr.2014.01.021

44 Pfeifer GP, You YH and Besaratinia A: Mutations induced by ultraviolet light. Mutat Res *571(1-2)*: 19-31, 2005. PMID: 15748635. DOI: 10.1016/j.mrfmmm.2004.06.057

45 Neskey DM, Osman AA, Ow TJ, Katsonis P, McDonald T, Hicks SC, Hsu TK, Pickering CR, Ward A, Patel A, Yordy JS, Skinner HD, Giri U, Sano D, Story MD, Beadle BM, El-Naggar AK, Kies MS, William WN, Caulin C, Frederick M, Kimmel M, Myers JN and Lichtarge O: Evolutionary action score of TP53 identifies high-risk mutations associated with decreased survival and increased distant metastases in head and neck cancer. Cancer Res *75(7)*: 1527-1536, 2015. PMID: 25634208. DOI: 10.1158/0008-5472.CAN-14-2735

46 Chung KY, Mukhopadhyay T, Kim J, Casson A, Ro JY, Goepfert H, Hong WK and Roth JA: Discordant p53 gene mutations in primary head and neck cancers and corresponding second primary cancers of the upper aerodigestive tract. Cancer Res *53(7)*: 1676-1683, 1993. PMID: 8453641.

47 Kanjilal S, Pierceall WE, Cummings KK, Kripke ML and Ananthaswamy HN: High frequency of p53 mutations in ultraviolet radiation-induced murine skin tumors: evidence for strand bias and tumor heterogeneity. Cancer Res *53(13)*: 2961-2964, 1993. PMID: 8319202.

48 Kanjilal S, Strom SS, Clayman GL, Weber RS, el-Naggar AK, Kapur V, Cummings KK, Hill LA, Spitz MR and Kripke ML: p53 mutations in nonmelanoma skin cancer of the head and neck: molecular evidence for field cancerization. Cancer Res *55(16)*: 3604-3609, 1995. PMID: 7627969.

49 Marusyk A, Almendro V and Polyak K: Intra-tumour heterogeneity: a looking glass for cancer? Nat Rev Cancer *12(5)*: 323-334, 2012. PMID: 22513401. DOI: 10.1038/nrc3261

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