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Implication of exon 4 *TP53* Gene mutations in colorectal cancers in Senegalese patients

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Abstract

Background: Colorectal cancer (CRC) is a major cause of death in men and women, comprising about 10% of cancer deaths after breast and lung cancer. The most significant risk factors currently implicated in the etiology of this cancer are genetic, likely due to several mutations.

Methods: The study explored the involvement of genetic factors in these pathologies; specifically, exon 4 of the *TP53* gene was studied in 15 CRC patients and compared with 10 healthy individuals as controls. The position of the marker mutations was determined with the Mutation Surveyor software version 5.1.2. DnaSP version 5.10, MEGA version 7.014, and the program Arlequin version 3.1 were used to highlight the parameters of variability, differentiation, and the demo-genetic evolution of our study populations. The pathogenicity of the mutations was determined through Polyphen2, TAMISER, and ClinVar.

Results: Our results showed the presence of a recurrent mutation of the *TP53* gene in both tumor and healthy tissue where proline was replaced with arginine at codon 72. This mutation was predicted to be benign. The presence of this mutation in healthy tissue can be considered a relatively late event in colorectal tumorigenesis. In addition, the P47L, D49A, W53S, and D48G mutations appeared to be suspicious because they were predicted to be potentially damaging. This finding suggests the genes' involvement in the pathology of CRCs in our study population. The cancer tissue sequences contained an average of 2.59 nucleotide differences that resulted in amino acid changes. The Nei genetic distance confirms this variability between tumor tissues.

Conclusion: These results suggest that variants in exon 4 of the *TP53* gene may contribute to the development of CRCs. These mutations could constitute molecular markers in CRC and possibly help in the development of early diagnosis

Keywords: Colorectal cancer; *TP53*; Mutation; Variability

1 Introduction

The cell, the structural and functional unit of life, constitutes the simplest form of life. From unicellular organisms to the most complex, the so-called higher organisms, all are composed of varying numbers of cells that are often organized into tissues and organs [1]. These organisms function thanks to a balance governed by both apoptosis and cell renewal. The extremely complex process of cell division mechanism is regulated by many proteins that intervene transiently and

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in a precise order, thus allowing the succession of the different stages of the cell cycle [2, 3]. However, a minor disturbance can quickly lead to either excessive proliferation or cell depletion [4].

In most cases, this disruption leads to a transformation of a normal cell into a tumor cell, causing a successive, sequential accumulation of somatic mutations. As a result, most cancers can be considered genetic diseases. Thus, cancer arises from the pathological clonal proliferation of multiple molecular events (genetic and epigenetic alterations) in a cell of the body [5]. Cancer is a significant public health problem worldwide. With an incidence of 19 million cases and 10 million deaths in 2020, it represents the second leading cause of death worldwide [6].

Among forms of cancer, CRC is, in terms of incidence, about 10.0% of new cases in 2020, one of the most common after skin and lung cancer. It also has a 9.4% mortality rate [7]. CRC incidence and mortality rates continue to rise rapidly in many low- and middle-income countries; however, the highest rates in the world have been reported in highly developed countries [8]. Although the prevalence of CRC is increasing in developing countries, in West Africa, data on the epidemiology and biology of this cancer are scarce, according to Bienfait and collaborators [9]. Nevertheless, CRCs are frequently encountered in hospital practice in Senegal [10].

This cancer has become the most common digestive cancer by incidence, surpassing stomach cancer [11]; it has been associated with many risk factors. The factors involved in the initiation of the pathology are often related to the environment and lifestyle, but they are also related to genetic factors. The study of these genetic factors allows for a greater understanding of the history of tumor cells, the distribution of genetic and phenotypic variability in tissues, and the identification of genes involved in tumorigenesis. Among genetic alterations, the activation of proto-oncogenes and the inactivation of tumor suppressor genes in affected cells are considered the major molecular events that provide selective growth advantage and clonal expansion during the process of carcinogenesis [12]. The tumor suppressor protein *TP53* plays a vital role in maintaining genome integrity, notably by activating and repressing gene expression. However, this gene is mutated in about half of all human cancers. According to Russo and his collaborators, the frequency of *TP53* mutations in CRC is approximately 40-50% [13].

Despite significant studies of this gene and its effect on the occurrence of cancers, questions arise regarding its importance in colorectal tumors in the Senegalese population. Our study was conducted to understand the genetic mechanisms that govern these pathologies. The general objective was to determine the involvement of genetic alterations of exon 4 of the *TP53* gene in the evolution of CRCs in the Senegalese population.

2 Methodology

2.1 Study populations

The study was conducted on 25 patients with CRC, and 10 control cases were used for comparison. These patients were recruited from the general surgery departments of Aristide Le Dantec Hospital, the main hospital in Dakar, Grand-Yoff, and the cancer department of Le Dantec Hospital. For each patient with CRC, a sample was taken from the fresh surgical specimen in the middle of the tumor, collected in a dry tube, and kept at 20°C, accompanied by the clinical information sheet. The samples were sent to the genomics laboratory of the Department of Animal Biology of the Faculty of Science and Technology of the University of Dakar, where the tissues were preserved in 96% alcohol for the various molecular analyses.

2.2 DNA Extraction, Amplification, and Sequencing of the *TP53* Gene

Total DNA from the tissues was extracted using the standard protocol of the Zymo research kit. After extraction, the exon 4 region of the *TP53* gene was amplified in a reaction volume of 25 µl with two primers: F 5'-TCCCCCTTGCCGTTCCAA-3' and R 5'- CGTGCAAGTCACAGACTT-3'. The PCR was performed in an Eppendorf thermal cycler with the initial denaturation performed at 94°C for five minutes, followed by a repeat of 35 cycles with denaturation at 94°C for 30 seconds, hybridization of the primers at 58°C for 45 seconds, elongation of the complementary DNA strands at 72°C for 40 seconds, and terminated by a final elongation at 72°C for 10 minutes. Each target gene was amplified by performing an electrophoretic migration on a 2% agarose gel. The 1977 F. Sanger method was used to identify the nucleotide sequence of exon 4 of the *TP53* gene.

2.3 Detection of Mutations

The Mutation Surveyor software version 5.1.2 was used to determine heterozygous mutations in the coding region of the *TP53* gene. The chromatograms of the *TP53* exon 4 sequences were compared with a reference sequence of the *TP53* gene (NT_010718_7571220).

2.4 Genetic diversity

Nucleotide compositions of individuals were calculated with BioEdit software. The standard indices of genetic variation were obtained. The number of polymorphic sites, the total number of mutations, average number of nucleotide differences, and haplotypic and nucleotide diversities were determined using DnaSP software version 5.10 [14]. The nature of mutations (i.e., percentage of transitions and transversions), selection test, and amino acid composition were obtained with MEGA software version 7.0.14 [15]. The selection Z-test was performed using the Nei-Gojobori model and the pairwise deletion method. A value of $P < 0.05$ was considered significant.

2.5 Amino acid diversity of exon 4 of the *TP53* gene

Amino acid diversity of exon 4 of the *TP53* gene was determined using MEGA version 7.0.14 [15] to compare sequences from healthy and cancerous tissues with the reference sequence obtained from GenBank and highlight nucleotide substitutions favoring protein changes. The position of the protein mutations was referenced with the *TP53* exon 4 coding region (NG 017013.2). The IARC *TP53* database was used to characterize these amino acid substitutions, and their pathogenicity was determined with PolyPhen-2. TAMISER, which is based on the SIFT program, classified the missense variants as damaging or tolerated, and ClinVav was used for the variants for which their dbSNP was identified.

2.6 Amino acid frequencies

After alignment and correction of the sequences in BioEdit version 7.0.8, the amino acid frequencies of the sequences of healthy and cancerous tissues were determined with MEGA software version 7.0.14. A Chi-squared test was performed with R Studio software to see the amino acids that demonstrated significant differences between healthy and cancerous tissues of the *TP53* gene. A significance level of 5% was chosen.

2.7 Genetic differentiation

The estimation of the genetic differentiation allows accounting for the inter-tissue genetic structure. For this purpose, two parameters have been identified: the dissimilarity index or genetic distance and the genetic differentiation index (Fst).

The determination of the genetic distances of Nei (1087), considering intra- and inter-populations, consists of evaluating the number of allele replacements that have occurred at the locus during evolution. This analysis was completed with MEGA software version 7.0 [15].

Determining Cavalli-Sforza's (1966) genetic differentiation (Fst) consists of measuring the variations of allelic frequency of SNPs between tissues to detect positive selection events. This analysis was completed with the Arlequin program version 3.1 [16]. Its value is between 0 (when there is no structuring) and 1 (if there is genetic structuring).

3 Results

3.1 Nucleotide sequences

After amplifying the 25 CRC tissue samples, we possessed 15 amplicons of exon 4 of the *TP53* gene. Ten sequences of this portion of the *TP53* gene were also obtained from controls.

3.2 Mutations of interest

Table 1 reports the *TP53* mutation profiles of the two groups of colorectal tissues obtained following submission of the chromatograms to Mutation Surveyor software. The results indicated a recurrent non-synonymous transversion mutation (c.11897 C>G = p.72Pro>Arg) where proline was replaced by arginine at position 72. The mutation frequency was 46.7% (7/15) on TC and 70% (7/10) on TS. A synonymous mutation of the transversion type was also found in

both healthy and cancerous tissues with a frequency of 10% (1/10). Table 2 lists the chromatograms showing the nature of the mutations.

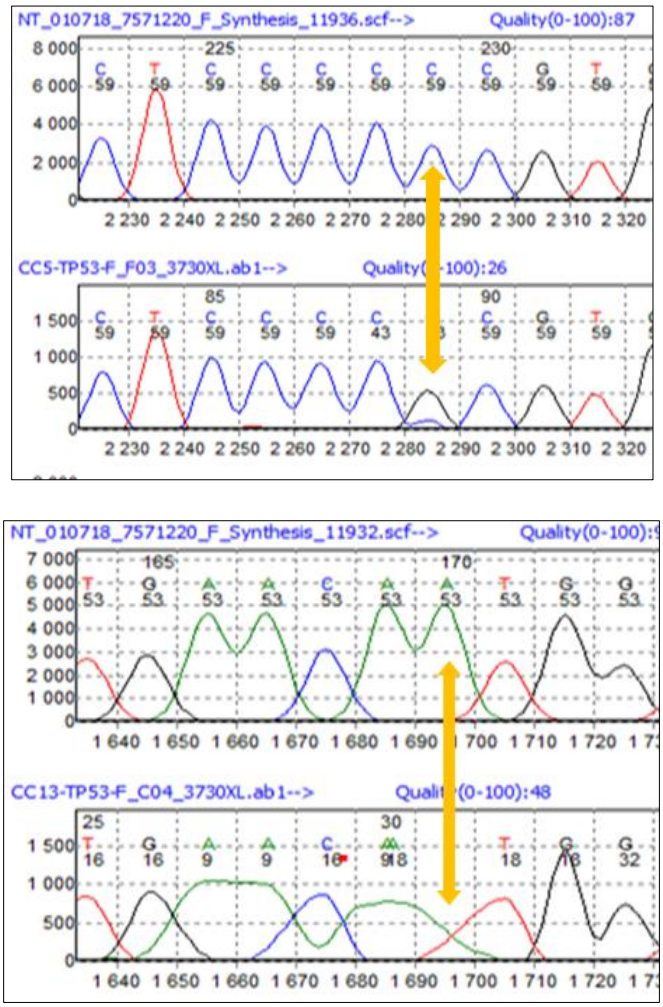
Table 1 Nature and position of the identified heterozygous mutations in exon 4 of the *TP53* gene

Pos. genome (GRCh37 p13.1	Mutations Pos. CDS	Amino acids	Nature of mutations	Affected tissues	Frequency	ID dbSNP
Chr17:7579472	11897C>G	P72R	Transversion (non-syn)	7 TC/7 TS	46,7%TC 70%TS	rs104252 -
Chr17:7579531	11838A>AT	Q52Q	Transversion (syn)	1TC	10%	-
Chr 17:7579453	11916A>AG	A78A	Transition (syn)	1TS	10%	-

TC = Cancerous Tissue, TS = Healthy Tissue, non-syn = Non-Synonym, syn = Synonym

The heterozygous SNPs found are represented in figure 1 below.

Nature of heterozygous mutations



The position of the mutation is indicated by the yellow arrows

Figure 1 Heterozygous SNPs in exon 4 of the *TP53* gene

3.3 Variability of the *TP53* gene in healthy and cancerous tissues

In both groups of tissues, transversions were much higher than transitions: 100% *vs*. 0.0% in healthy tissues with zero mutation rate and 61.9% *vs*. 38.1% in cancer tissues with a mutation rate of 0. 55%. Similarly, non-synonymous substitutions were higher in cancer tissues than in healthy tissues, with values of 0.013 (0.005) and 0.008 (0.005), respectively. No positive selection test was identified for healthy and cancerous tissues with p values of 1.693 and 1.06, respectively. Polymorphism analysis revealed a high haplotypic diversity value (HD = 0.911 for TS; 0.724 for TC) and a low nucleotide diversity value (π = 0.007 for TS; π = 0.010 for TC). Table 2 reports the relative values of genetic variability of exon 4 of the *TP53* gene from healthy and cancerous tissues.

Table 2 Genetic variability parameters of healthy and cancerous tissue sequences

Nature of tissue / Parameters		TS				TC			
Number of sites		238				238			
Monomorphic sites		234				223			
Polymorphic sites		04				15			
Total number of Eta mutations		04				17			
Average number of nucleotide differences K		1.644				2.590			
Rate of transitions (%)		0.0				38.1			
Rate of transversions (%)		100				61.9			
Mutation rate R		0.0				0.55			
Nucleotide frequency (%)		A 19.29	T 20.39	C 36.60	G 23.82	A 19.27	T 20.20	C 36.86	G 23.67
Type of substitution	Ks	0.0				0.007 (0.005)			
	Kns	0.008 (0.005)				0.013 (0.005)			
Z-test (dN > dS)		0.048 (1.693)				0.146 (1.06)			
Hd haplotypic diversity		0.911				0.724			
Nucleotide diversity Pi		0.007				0.010			

A= Adenine; T= Thymine; C= Cytosine; G= Guanine; TC = Cancerous Tissue, TS = Healthy Tissue

3.4 Amino acid diversity

Figure 2 shows the amino acid sequence of a reference sequence (NG 017013.2) and each tissue (TS and TC). All eight nucleotide-level variable sites show an amino acid change (highlighted in yellow), with 25 changes total. The positions of these amino acids were referenced to that of the reference protein (P04637) of the *TP53* gene. Among these substitutions, the one inducing a change from proline to arginine at codon 72 was found in both healthy and cancerous tissues with a frequency of 20% (5/25). The substitution at codon 47 occurred only in healthy tissues at a rate of 16% (4/25). Ten other amino acid changes were found in cancerous tissue, and one was found in healthy tissue, with a frequency of 4% (1/25) for each variant. The variant inducing a stop codon in place of glutamic acid was also found at codon 68 and was specific to healthy tissue. All of these variants were also listed in the IARC database of *TP53,* and their characteristics are presented in Table 3.

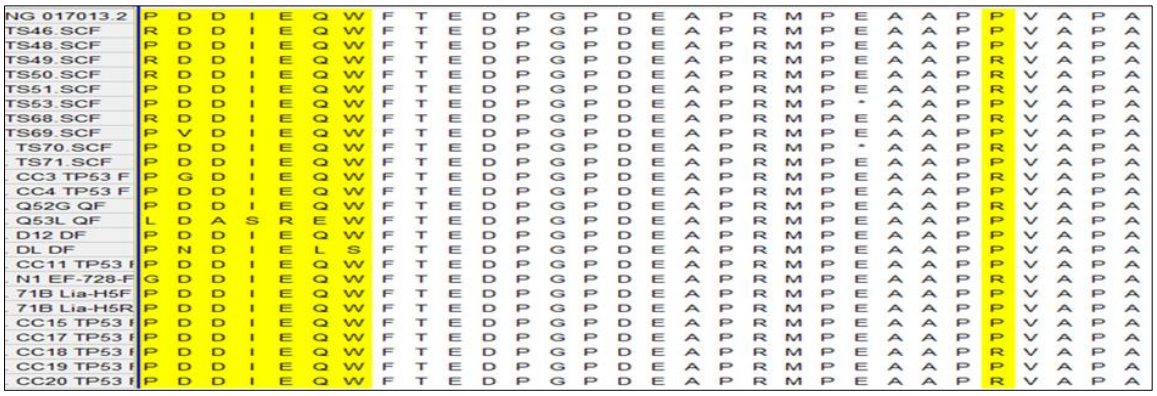


Figure 2 Substitutions on amino acid sequences aligned to MEGA version 7.0

The characteristics of the amino acid substitutions obtained are presented in Table 3

Table 3 Characteristics of the amino acid sequence substitutions in exon 4 of the *TP53* gene

Pos. genome (GRCh38 p13.1)	Mutation Pos. CDS	Amino acid	Nature	Tissue affected	ID dbSNP
g.7676167	c.202G>T	p.E68*	Non-sens	2TS	rs869312782
g.7676154	c.215C>G	p.P72R	Non-syn	6TS/5TC	rs1042522
g.7676229	c.140C>G	p.P47R	Non-syn	4TS	-
g.7676229	c.140C>T	p.P47L	Non-syn	TC	-
g.7676228	c.139C>G	p.P47G	Non-syn	TC	-
g.7676226	c.143A>T	p.D48V	Non-syn	TS	-
g.7676227	c.142G>A	p.D48N	Non-syn	TC	-
g.7676223	c.146A>C	p.D49A	Non-syn	TC	-
g.7676220	c.149T>G	p.I50S	Non-syn	TC	-
g.7676216	c.151A>G	p.E51R	Non-syn	TC	-
g.7676215	c.154C>G	p.Q52E	Non-syn	TC	-
g.7676214	c.155A>T	p.Q52L	Non-syn	TC	-
g.7676211	c.158G>C	p.W53S	Non-syn	TC	-
g.7676226	c.143A>G	p.D48G	Non-syn	TC	-
g.7676069	c.300G>A	p.Q100Q	syn	TC	-

Non-syn = Non-synonymous, syn = synonymous, TC = Cancerous Tissue, TS = Healthy Tissue

Most amino acid changes were located in the N-terminal region of the *TP53* gene. Among these mutations, those at codons D48G, D48N, D49A, Q51L, and Q52E in cancerous tissues had functional transactivation activity except for P47G and E51R. All were predicted to be benign on PolyPhen-2 and tomere by TAMISER from the IARC *TP53* database and D48V from a healthy tissue sequence. P47L, I50S, and W53S from cancerous tissues showed partially functional transactivation activity and were predicted to be damaging with both programs and P47R from the four healthy tissues with supertans activity. The replacement of proline to arginine at codon 72 in the five cancerous and five healthy tissues was located in the proline-rich region of *TP53* and contained several copies of the PxxxP motif. PolyPhen-2 and ClinVar predicted the substitution to be benign, and TAMISER predicted it to be tolerated. The impact of the E68* mutation on the protein's function was not available in the IARC database of *TP53*. The PolyPhen-2 tool and TAMISER ClinVar did not predict an amino acid change at the stop codon. Variants without dbSNP identifiers (P47R, P47L, P47G, D48V, D48N,

D48A, I50S, E51R, Q52E, Q52L, W53S, and D48G) were absent from the ClinVar database. Table 4 reports all of these results.

Table 4 Effect of non-synonymous mutations on protein function and pathogenicity

Characteristics of the Protein			**Pathogenicity**		
Mutations	**Location**	**Transactivation Class**	**PolyPhen-2 (Score)**	**TAMISER**	**ClinVar**
E68*	-	-	-	-	-
P72R 5TS/5TC	SH3/riche en Pro	-	Benign (0,379)	Tolerated	Bénigne
P47R TS	N-terme/ Transactivation	Supertrans	Potentially harmful (0,947)	Damaged	-
P47L	N-terme/ Transactivation	Partially functional	Potentially harmful (0,848)	Damaged	-
P47G	N-terme/ Transactivation	-	Benign (0,379)	-	-
D48G	N-terme/ Transactivation	Functional	Potentially harmful (0,633)	Tolerated	-
D48V TS	N-terme/ Transactivation	Functional	Benign (0,113)	Tolerated	-
D48N	N-terme/ Transactivation	Functional	Benign (0,153)	Tolerated	-
D49A	N-terme/ Transactivation	Functional	Potentially harmful (0,945)	Tolerated	-
I50S	N-terme/ Transactivation	Partially functional	Benign (0,163)	Damaged	-
E51R	N-terme	-	Benign (0,220)	-	-
Q52E	N-terme	Functional	Benign (0,00)	Tolerated	-
Q52L	N-terme	Functional	Benign (0,006)	Tolerated	-
W53S	N-terme	Partially functional	Potentially harmful (0,995)	Damaged	-

We found that the frequency of each amino acid of *TP53* differed slightly between healthy and cancerous tissues without any statistical significance (Table 5). However, we observed a significant value of 0.02 for valine and 0.0455 for glutamine and arginine.

Table 5 Amino acid frequencies of exon 4 of *TP53*

Amino acid	TS	TC	P-value	Amino acid	TS	TC	P-value
Ala	15.22	15.27	0.3763	Met	1.26	1.26	0.07684
Cys	1.26	1.26	0.07684	Asn	00	0.08	0.4142
Asp	4.94	4.81	0.1474	Pro	19.16	19.66	0.4036
Glu	4.82	5.06	0.07684	Gln	3.80	3.62	0.0455 *
Phe	3.80	3.79	0.07684	Arg	3.68	3.03	0.0455 *
Gly	6.34	6.49	0.7942	Ser	10.15	10.29	0.7942
His	1.26	1.26	0.07684	Thr	7.61	7.59	0.07684
Ile	1.26	1.18	0.06182	Val	3.93	3.79	0.02699 *
Lys	2.53	2.53	0.07684	Trp	2.53	2.44	0.06182
Leu	3.80	3.96	0.7942	Tyr	2.53	2.53	0.07684

*: indicates a significant value (P< 0.05)

3.5 Genetic Differentiation

The analysis of genetic distances between healthy and cancerous tissues of exon 4 of the *TP53* gene revealed low intra-tissue genetic distances of 0.007 and 0.011 in healthy and cancerous tissues, respectively. This analysis demonstrated a low inter-tissue genetic distance of (0.009) and a significant Fst of 0.52889 with a p-value of 0.00598. Table 6 reports each of these results.

Table 6 Intra and intergroup genetic distances

Amplified genes	Groups	Genetic distances Intra-group	Genetic distances Inter-group	Fst
TP53	TS	0,007 (0,004)	0,009 (0,003)	0.52889 (0.00598)
	TC	0,011 (0,003)		

4 Discussion

This study aimed to determine the involvement of genetic alterations in exon 4 of the *TP53* gene in the evolution of CRCs in the Senegalese population. The formation of cancers is governed by the mutation-selection process specific to our evolution, which allows us to understand the history of tumor cells and to set a certain number of specific objectives, among which are the identification of mutations, the study of genetic variability, genetic differentiation, genetic evolution at the level of colorectal tissue sequences. In this study, we analyzed exon 4 of the *TP53* gene of 15 Senegalese patients with colorectal cancer and compared tissues to a group of 10 controls.

Our results showed recurrent heterozygous mutations at codon 72 of the *TP53* gene resulting in the change of proline to arginine. The mutation occurred in both affected and healthy tissues, resulting in a change in the amino acid sequence. This finding suggests that these variations are not involved in the development of colorectal cancer. This result is consistent with findings from Olschwang and colleagues [17]. They compared the characteristics of a frequent polymorphism in the coding sequence of the *TP53* gene in patients with colon cancer and a control population. They could not find any evidence that this polymorphism was associated with a marked predisposition to colorectal cancer. Nanda and his teammates [18] and Dastjerdi [19] indicated that overexpression of p53 is synonymous with mutation, which could explain the significant frequency of this mutation in both tissue groups.

In contrast, studies by Wan et al. [20] demonstrated that the single nucleotide polymorphism (SNP) at codon 72 of the *TP53* gene was associated with an elevated risk of developing various neoplasms. Therefore, these mutations could be

considered a relatively late event in colorectal tumorigenesis. Thus, the development of a field of carcinogenesis is a possible cause for the presence of a mutation in healthy tissues, which could therefore be considered pre-neoplastic. Although many research groups have studied *TP53* gene mutations in CRC, controversy still exists regarding the prognostic significance of these alterations. The likely explanation for this finding pertains to the genetic variability at the intra- and inter-individual levels. In this sense, our study showed a low nucleotide variability of the *TP53* gene. This finding could be explained by its regulatory features in the primary apoptotic pathways: the extrinsic "death" receptor signaling pathway and the intrinsic "mitochondria" pathway. Despite its regulatory activity, *TP53* polymorphism is very marked in Senegalese individuals as CRCs tend to have a high haplotypic diversity of cancer cells and a low nucleotide diversity. This trend leads us to hypothesize that mutations of this molecular marker are rapidly increasing in colorectal carcinomas in a small ancestral population. Braakhuis and his collaborators [21] found a common clonal origin in cancer cells recognized based on a mutation within *TP53*. These findings have been reported in head, neck, lung, skin, and breast cancer [21]. This could also explain the high frequency of nucleotide variations resulting in changes from proline to arginine in healthy tissues, which may lead to pathogenicity.

Exon 4 is the only one in the *TP53* gene that partially encodes three functionally significant domains of the protein [22]. The low total number of mutations (Eta = 4 in TS vs. 17 in TC) in this study reveals that exon 4 is highly variable, but all nucleotide substitutions were of the transversion type. These caused more extensive changes in DNA structure and were more likely to result in an amino acid substitution [23]. This could explain our finding that almost all of the transversions lead to missense mutations. This corroborates the results of other studies because most *TP53* mutations in cancers are known to be caused by missense mutations [24]. The superiority of non-synonymous mutations demonstrates that exon 4 of the *TP53* gene is likely under positive selection. Thus, a proliferative advantage is conferred to cancer cells with mutations in this gene, further implicating colorectal cancer progression.

Inter-individual variability in the amino acid sequences of *TP53* cancer tissues distinguished changes from proline to arginine at codon 72 in both tissue groups and codon 47 only in healthy tissues. The mutation at codon 72, located in the proline-rich region involved in apoptosis, was the most frequent of all mutations found in this study. This finding is consistent with Batta and Pandey's study, which focused [25] on oral squamous cell carcinomas, and the work of Sina and his teammates [26], where this variant of the *TP53* gene had already been identified in oral cavity squamous cell cancers. This variant (P72R) had previously been classified as an SNP of the gene. The newly listed variant (P47R) is predicted to have the ability to increase the transactivation activity of P53 (supertrans protein) in the IARC *TP53* database, is classified as potentially damaging on PlyPhen-2 with a score of 0.947, and, according to TAMISER, may have an impact on the occurrence of CRC. This phenotype could be due to variations in promoter specificity and binding affinity that may be associated with p53 mutations or post-translational modifications [27]. During transcription, the role of *TP53* is of crucial importance in carcinogenesis and neoplastic progression [22]. According to Monti and colleagues [28], this sequence-specific transcription factor can transactivate multiple sets of genes, and the promotion of this factor includes appropriate response elements and germline mutations in *TP53* that can result in cancer predisposition syndromes. Thus, these supertrans alleles may be useful in cancer gene therapy protocols aimed at restoring *TP53* function to tumor cells. Another transversion (G>T) present only in 2 TS, where a stop codon replaces glutamic acid at position 68 (E68*), led to a truncated protein. This finding is at odds with the results of Diatta and colleagues' study of [29] oral cavity cancer, where mutations were found in both TS and TC. Nucleotide variations causing amino acid changes occurred at codons P47L, I50S, and W53S, and their transactivation activity was partially functional and predicted to be damaging, underscoring an essential role of *TP53* in carcinogenesis. Indeed, in some cancer cells where the p53 protein is inactive, the G1/S checkpoint of the cell cycle is defective, resulting in anarchic cell growth [30]. This may be correlated with the pathogenicity of these variants.

The analysis of the genetic distance and the genetic differentiation index (Fst) shows the beginning of genetic differentiation between the two groups of tissues, characterized by a low inter-tissue genetic distance of (0.009) and a highly significant Fst with a p-value of (0.00598). This finding reflects the malignant state of the tumor cells. The low intra-tumoral genetic distance (0.011) can be explained by the monoclonal origin of colorectal tumors [21], whose development is based on a succession of waves of clonal expansions.

5 Conclusion

The results of our genetic analyses revealed a low variability of exon 4 of the *TP53* gene, with the presence of a recurrent mutation found in both cancerous tissues. The penetrance of this recurrent mutation in CRCs, where proline was

replaced by arginine at codon 72, was incomplete and possibly pre-neoplastic. The presence of variants (P47L, D49A, W53S, and D48G) in cancerous tissues predicted to be damaging could impact the evolution of CRCs. These mutations could constitute molecular markers in CRC, and possibly contribute to early diagnosi.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest.

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