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MUTATIONS AND AMINO ACIDS VARIATIONS OF CYTOCHROME B IN 26 OVARIAN TUMOR TISSUES OF SENEGALESE WOMEN

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Abstract

Ovarian cancer is one of the most deadly gynecological cancers. Annual mortality in West Africa is estimated at 76.23% in 2012. To evaluate the implication of the cytochrome B somatic mutations in ovarian cancer among senegaleses women, we analyzed variability of Cytochrome B at twenty six patients with ovarian cancer by PCR-sequencing. Polymorphism, differentiation and genetic evolution of the cytochrome b were highlighted. Results reveal the existence of strong variability of tumours with genetic differentiation within healthy and cancerous tissues, within young and older patients and a moderate demographic evolution of rare mutations of the disease. Our results also show a significant intra-individual increase of Phenylalanine (66.6%), tyrosine (66.6%) and tryptophan (60%) in cancer tissues with different proportions. Chi2 tests performed showed significant p-values. Any increase in the rate of tryptophan, phenylalanine and tyrosin in cancerous tissues could be correlated with an increased risk of developing ovarian cancer.

Keywords: Cancer, Cytochrome b, Variations, Ovary, Senegal.

1. Introduction

The tumor cells generally wear a large number of DNA modifications. The recently acknowledged implication of mitochondria in apoptosis (Green *et al.*, 1998), and probably also tumorigenesis, (Cavalli *et al.*, 1981) have aroused the interest of examining the mutations potential role of the mitochondrial DNA (mtDNA) in the development and maintenance of the cancers. The survival rate for patients with advanced stage disease is around 20% because of the difficulty of early diagnosis. Annual mortality in West Africa is estimated at 76.23% (Globocan, 2012). In Senegal, the number of women who perish because of ovarian cancer has become striking, for that matter in 2008 one hundred and thirsty deaths were noted in the country (Globocan, 2012). Human mtDNA has been entirely sequenced and it is circular and small size (16 569 bp). It contains 37 genes, including the cytochrome b (CYTB) which is generally used to determine the phylogenetic relationship between organisms because of its high sequence variability. Mitochondria are involved in ATP production, and also contribute to thermogenesis, the production of free radicals, to the calcium homeostasis and apoptosis (Duchen, 2004). The mutations rate in mitochondrial DNA is far and away higher than the nuclear DNA one. These modifications are particularly prevalent in pre-neoplastic lesions and in human cancers, including ovarian cancer (Bragoszewski*et al*., 2008). Some studies of mutations in mitochondrial DNA have focused on the genetic penetrance of cytochrome b in Senegalese patients with breast cancer and revealed the existence of intra and inter individual nuclotide variability with genetic differentiation between healthy and cancerous tissues and the correlation between the genetic differentiation and the patient age and the tumor localization (right or left breast). They also showed a significant increase (79.3%) of the number of phenylalanine in cancer tissues with very different proportions between individuals (Mbaye et al., 2012a). This study attempted to evaluate the somatic mutations involvement in ovarian cancer of the women in Senegal by estimating the genetic variability of CYTB, determining the mode of gene evolution and comparing the amino acid profile of tumors ovary in Senegalese women.

2. Materiel et methods

2.1 Patients

This study deals with twenty-six (26) cases of ovarian tumors diagnosed in the cancer ward, Joliot Curie Institute, of the Aristide Le Dantec Hospital in Dakar. The samples were obtained during surgery. They are immediately routed to Joint Laboratory where will be made the different steps of the analysis. All samples are preserved in alcohol 96 °. Healthy ovaries samples (TS) and cancerous ovaries (TC) are obtained for fifteen (15) patients, for other nine (9) patients only ovary cancerous samples were obtained and the other 2 remaining patients had bilateral ovarian tumor . The total number of sample amounts to 43, of which 15TS and 28TC. After receiving the approval of the ethics committee of the Cheikh Anta DiopUniversity of Dakar, an informed consent, written in a standardized form was obtained from patients who have been the subject of this study.

2.2 DNA extraction, amplification and sequencing

Total genomic DNA was extracted from tissues (healthy and cancerous for each patient) following digestion with proteinase K and then purified on a column (Qiagen). Cytochrome b was amplified using two primers H15915 (TCT-CCA-TTT-CTG-GTT-TAC-AAG-AC) and L14723 (ACC-AAT-GAC-ATG-AAA-AAT-CAT-GGT-T). The amplification reactions were carried out according to these following conditions: preliminary denaturation with 94°C (3 minutes), followed by a repetition of 40 cycles of initial denaturation to 92°C (45 seconds), of hybridization with 50°C (1 minute) and complementary bits of ADN elongation to 72°C during 90seconds and is buckled by a final elongation (10 minutes).

Amplification takes place in a thermocyclor of the Eppendhorf type. Obtained amplified were sent afterwards to Macrogen in South Korea for the purification and the sequençing.

2.3 Molecular analysis

The CYTB sequences of healthy and cancerous tissues are thoroughly checked, adjusted and aligned with BioEdit software, version 7.0.5.3 (Hall, 1995) using the ClustalW multiple aligment editor (Thompson et al., 1994).

The TS and TC nucleotidic frequencies of each individual are calculated with the same software BioEdit (Hall, 1995). For this work, various groups were formed for the study of genetic differentiation. On the one hand, the group of cancerous tissues is compared with the group of healthy tissues to highlight genetic diversities which exist there. On the other hand, a group of cancerous tissues in which patients are older than 50 years (age ≥ 50 years) and another whose patients are younger than 50 years are established. Indeed according to Sando et al. (2010), the age of the predilection ovarian cancer in Africa is between 50 and 59 years. The standard signs of genetic variation: type of mutations, number of variable sites is detailed with MEGA 5 software (Molecular Evolutionary Genetics Analysis 5) Version 5.05 (Tamura *et al*, 2011.). Kimura 2-parameter distance (K2P) and the nucleotide p-distance are calculated for healthy and cancerous tissues by MEGA 5 (Tamura *et al.,* 2011).P-distance and K2P are calculated at the first; 2nd and 3rd position of each codon. We also observed the types and sites of cancer mutations of the CYTB of cancerous tissues of the population targeted with MITOMAP (http://mitomap.org/ MITOMAP) which is a database of human mitochondrial genome. The genetic differentiation analysis will be made with the calculation of genetic distances of Tamura and Nei (Excoffier*et al.,* 2005).

The evolution of cancerous tissues was investigated by calculating haplotype diversity (Hd) and nucleotide diversity (Pi), and analyzing the mismatch distribution (Rogers, 1992) with the editor DnaSP Version 5.10.01. (Rozas *et al.,* 2010). Tajima's D (Tajima, 1989) and Ramos-Rozas index R2 (Ramos-Onsins *and* Rozas, *2002)* were used to test the hypothesis of the deviation to neutrality. They are calculated with the Arlequin software version 3.1 (Excoffieret al., 2005).The average number of synonymous substitution per synonymous site (dS) and the average number of nonsynonymous substitution per nonsynonymous site were calculated with MEGA 5 Modified Nei-Gojobori model Method (proportion). For all tests, the significance level (P-value) is 5%. Significance tests are performed with the chi2 test by Statview Version 5.0.0.0 software.

Among the 26 individuals, fifteen (15) have a normal tissue and a cancerous tissue. And these tissues are used for the comparison of amino acid profiles between healthy and cancerous tissue. A comparison between TS and TC group is also performed. The CYTB gene is coding. This will convert the nucleotide sequences into amino acid sequences using different possible reading frames. The conversion into amino acid sequences is carried out with the following 5 MEGA different reading frames.

3. Results

3.1 Nucleotidic frequency of the sequences

A comparison of the nucleotide frequency at intra-and inter individual shows strong variability of CytB. Intra-individual level, the relative values of nucleotide composition shows a moderate predominance of A and T from healthy tissue to cancerous tissue. It can be noted that in 66.6% of cases, the rate of A + T is higher than C + G.

3.2 Variability of the cytochrome B of healthy tissues and cancerous tissues

Table 1 posts the percentage of polymorphic sites which is higher for cancerous samples. The transversions vary between 55,8% and 58,4% and are more numerous than transitions and the transitions / transversions ratio was estimated equal to 0.70 for cancerous tissue and 0.79 for healthy tissue. The nucleotide-p distance for the first codon is higher for normal tissues (89.1%) in contrast to cancerous tissues, where the second codon has a p-distance equal to p-75.1%. Estimates of the p-distance and distance K2P showed a greater degree of substitution at the first nucleotide position followed by the third and then the second for healthy tissues. Contrary to cancerous tissues that showed higher levels of substitution at the second position to the nucleotide and then to the first and third position. The average difference between the nucleotide sequences taken two by two (K) is higher for cancer tissues (125,100).

Table 1: Parameters of genetic variability of healthy tissues and cancerous tissues

	healthy tissues				**cancerous tissues**			
Sequence Number	14				20			
Number of variable sites	249 (55,3%)				278 (61,7)			
Nucleotide frequency (%)	A	T	C	G	A	T	C	G
	26,0	25,7	22,7	25,5	26,0	26,5	22,1	28,2
transitions	110 (44,1%)				116 (41,6%)			
transversions	139 (55,8%)				162 (58,4%)			
Rate Transitions / transversions	0,79				0,70			
Nucleotide p-distance (x100)	1st codon	2th codon	3th codon		1st codon	2th codon	3th codon	
	89,1	67,7	70,5		67,1	75,1	62,9	
	+/-0,125	+/-0,081	+/-0,097		+/-0,083	+/-0,085	+/-0,069	
K2P distance (x100)	1st codon	2th codon	3th codon		1st codon	2th codon	3th codon	
	99,1	72,7	75,9		72,4	82,8	66,3	
	+/- 0,146	+/-0,102	+/-127		+/-0,102	+/-0,121	+/- 0,087	
K	113,374				125,100			

K = average of the nucleotidic differences between the sequences taken two by two.

For healthy tissues and cancerous tissues different mutations observed in different sites were listed in Table 2. For 88.8% of mutations the p-value is significant. There is a strong presence of AT15654d mutation in both healthy and cancerous tissues. The tranversions represent 62.9% against 29.6% transitions and 7.4% deletions. And 33.3% of mutations are types T →A or C → A.

Table 2: Somatic changes of the CYTB in the Senegalese population studied

Sites	Types of mutations	TS %	TC %	P-values	aa
15641	C→T	7,1	10	0,0806	Leu-Phe
15641	C→A	0	5	0,0008	Leu-Ile
15641	C→G	7,1	0	<0,0001	Leu-Val
15642	T→A	7,1	0	<0,0001	Leu-His
15647	C→T	0	5	0,0008	Leu-Leu
15654	AT→d	85,7	80	0,0001	Deletion
15655	A→T	0	5	0,0008	Met-Ile
15656	AA→d	0	5	0,0008	Deletion
15657	T→C	0	5	0,0008	Ile-Thr
15704	C→A	0	5	0,0008	Leu-Met
15777	G→C	7,1	5	0,2751	Ser-Thr
15784	T→C	14,2	5	<0,0001	Pro-Pro
15787	T→G	0	5	0,0008	Phe-Leu
15787	T→A	0	5	0,0008	Phe-Leu
15792	T→A	0	5	0,0008	Ile-Asn
15795	T→A	0	5	0,0008	Ile-Asn
15796	T→A	0	10	<0,0001	Ile-Met
15797	G→A	0	5	0,0008	Gly- Term
15799	A→T	0	5	0,0008	Gly-Gly
15812	G→T	0	5	0,0008	Val-Leu
15813	T→C	0	5	0,0008	Val-Ala
15815	C→A	0	5	0,0008	Leu-Met
15820	C→A	7,1	10	0,0806	Tyr-Term
15824	A→T	0	5	0,0008	Thr-Ser
15824	A→G	14,2	5	<0,0001	Thr-Ala
15827	A→C	0	5	0,0008	Thr-Pro
15828	C→T	7,1	0	<0,0001	Thr-Met

3.3 Genetic differentiation of cytochrome B

To compare the genetic differentiation between healthy tissue and tumor tissue, intragroups genetic distances are highlighted. These parameters reflect the genetic differentiation within samples. The intra-cancerous tissue distance is higher than intra-healthy tissues (Table 3).

Table 3: Genetic distances within and between healthy tissue and cancerous tissue

Groups	Genetic distance within groups
Healthy Tissus	0,311
Cancerous Tissus	0,353

According to the age of patients, the genetic distance within old patients is higher than within young patients (Table 4).

Table 4 : Genetic distances in function of age

Groups	Genetic distance within groups
Patients ≥50 ans	0,432
Patients <50 ans	0,339

3.4 Genetic Evolution of cancerous tissues

Table 5 posts the values of haplotypic and nucleotidic diversities of cancerous tissues. It is noticed that the haplotypic diversity is very high (HD=1) and the nucleotidic diversity is equal to 27, 8%. The synonyms substitutions are higher than the non-synonymous substitutions. The statistical value of Tajima's D (2.53662) are positive but not significant. R2 presents a significant p-value that means a demographic expansion.

Table 5: Parameters of genetic evolution in cancer tissues

Geneticparameters	CancerousTissues	p-value
Haplotypic diversity (Hd)	1,000+/-0,00025	
Nucleotide diversity (Pi)	0,27800+/-0,01298	
Synonyms Substitution rate	0,239 +/-0,028	**0,0214**
Non-synonymous substitution rate	0,220 +/-0,024	
Tajima's D	2,53662	0,9980
R2	**0,15924**	**0,00000**

The analysis of the disparity of distribution of cancerous tissues shows a multimode curve (figure 1).

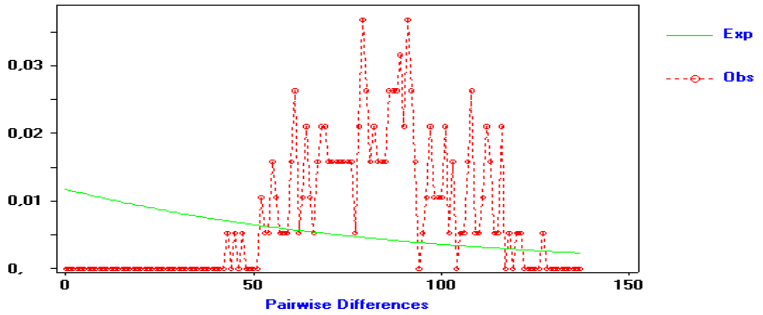

Figure 1 : Mismatch distribution of cancerous tissues

3.5 Variability of the amino acids of the cytochrome B

We note intra-individual variations of the amino acid frequencies from healthy tissue to cancerous tissue of each patient. These modifications are sometimes very significant. The most significant variations remain those of the tyrosin and the phenylalanine which increased for 66, 6% of the patients. This increase is on average 77, 26% for tyrosin and 77, 48% for phenylalanine. Tryptophan has also undergoes an increase of 23.11% on average for 60% of patients. The chi2 test reveals P-values less than 0.0001 and thus significant. The relative values of the frequencies of amino acids are shown in Table 7.

Table 7: Intra-individual frequencies of the amino acids for 15 patients

SEQ	Ala	Cys	Asp	Glu	Phe	Gly	His	Ile	Lys	Leu	Met	Asn	Pro	Gln	Arg	Ser	Thr	Val	Trp	Tyr
TS1	2,8	2,4	2,4	3,2	3,2	7,3	4,9	3,2	4,5	13,9	2	2,4	9,4	4,5	4,5	10,2	7,3	4,5	4	2,4
TC1	2	1,6	2	3,7	2,9	8,2	1,6	2,4	11,2	9,1	2,4	2	10,7	4,9	3,7	12,4	10,3	3,3	1,6	2,9
TS2	6,9	2,4	3,4	5,3	3,4	9,4	4	1,3	5,3	9,4	1,3	4	11	3,4	6,1	6,7	8,3	2,9	1,6	2,9
TC2	4,9	2,1	5,1	4,6	4,3	8,1	4,3	2,7	4,3	11,9	2,7	4,6	7	1,9	4	7,6	7,9	3,8	3,2	4
TS3	1,2	2,5	1,3	5,1	3,8	16,5	1,9	2,5	4,4	20,3	4,4	1,9	1,3	1,9	5,1	3,2	2,5	11,4	7,0	1,9
TC3	3,1	1,8	0,0	2,5	5,0	12,6	1,3	4,4	3,8	17,0	3,8	4,4	6,9	0,6	7,5	5,7	3,1	8,8	5,0	2,5
TS4	6,3	4,3	4,0	4,3	5,6	7,0	4,0	2,6	2,6	9,3	2,3	6,3	8,6	3,0	2,6	8,6	6,6	3,0	2,3	6,6
TC4	4,9	3,0	3,9	3,9	5,9	7,2	3,3	2,6	4,3	10,2	2,3	4,6	11,5	3,3	3,6	9,9	6,3	2,6	2,0	4,6
TS5	3,3	1,2	2,1	2,7	4,5	8,3	2,4	3,9	3,9	15,2	3,6	3,6	10,1	3,3	3,9	9,8	5,4	6,0	3,3	3,9
TC5	4,4	2,4	2,1	3,0	4,1	10,4	2,7	3,3	6,2	12,1	3,3	3,0	11,5	2,7	5,6	6,5	6,2	4,7	3,4	2,7
TS6	3,7	2,2	0,7	3,7	4,0	7,7	2,9	1,5	4,0	13,6	3,3	2,6	12,9	4,0	6,6	9,2	5,5	4,8	4,4	2,6
TC6	4,0	2,2	1,8	6,5	5,0	7,6	2,2	3,2	5,0	13,7	3,2	4,7	9,0	3,6	4,7	8,3	2,9	5,0	4,7	2,9
TS7	9,7	0,0	0,0	1,6	3,2	17,7	1,6	0,0	6,5	19,4	3,2	1,6	1,6	1,6	8,1	3,2	0,0	11,3	8,1	1,6
TC7	6,6	3,3	0,0	1,6	8,2	14,8	1,6	1,6	1,6	24,6	1,6	1,6	4,9	1,6	6,6	1,6	1,6	11,5	3,3	1,6
TS8	3,3	0,0	3,3	3,3	0,0	23,3	3,3	0,0	10,0	16,7	0,0	0,0	10,0	3,3	6,7	0,0	3,3	6,7	3,3	3,3
TC8	3,7	0,0	0,0	3,7	3,7	18,5	0,0	7,4	0,0	25,9	0,0	0,0	3,7	3,7	7,4	3,7	11,1	3,7	3,7	0,0
TS9	4,4	1,5	3,3	5,1	4,0	9,6	1,1	2,2	7,0	11,4	4,0	4,0	5,9	3,3	6,6	7,0	5,9	7,4	4,0	2,2
TC9	4,3	2,2	1,8	2,5	1,4	9,7	3,2	1,1	3,2	16,9	3,6	4,7	8,6	4,0	6,1	7,6	4,7	7,6	4,3	2,5
TS14	6,8	2,3	4,2	4,9	2,9	12,9	1,6	3,6	8,4	6,1	2,3	5,2	8,4	4,9	4,9	8,4	3,9	1,6	2,3	4,5
TC14	4,8	1,9	3,8	4,1	4,5	8,9	1,9	2,5	7,6	8,3	1,9	3,5	14,0	4,8	2,5	7,6	6,7	2,9	2,9	4,8
TS15	0,0	2,8	0,0	2,8	4,2	18,1	0,0	1,4	2,8	26,4	5,6	2,8	0,0	1,4	4,2	4,2	0,0	13,9	8,3	1,4
TC15	0,0	2,8	0,0	2,8	5,6	16,7	0,0	1,4	2,8	25,0	5,6	1,4	0,0	1,4	4,2	2,8	0,0	13,9	9,7	4,2
TS17	0,0	0,0	0,0	2,1	4,2	14,6	0,0	2,1	4,2	31,3	4,2	2,1	0,0	2,1	6,3	4,2	0,0	14,6	8,3	0,0
TC17	0,0	0,0	0,0	2,1	4,2	12,5	0,0	2,1	4,2	31,3	4,2	2,1	0,0	2,1	6,3	4,2	0,0	14,6	8,3	2,1
TS22	4,6	2,1	1,4	3,2	4,6	13,3	2,1	2,5	6,0	11,6	3,5	2,5	9,1	3,2	5,3	7,4	5,3	7,0	3,9	1,8
TC22	4,2	0,7	1,1	1,8	6,7	11,9	1,8	3,5	10,2	11,6	3,2	3,5	10,5	3,5	2,5	6,3	4,9	4,6	4,2	3,5
TS25	3,6	2,5	1,8	1,4	5,7	10,7	2,9	4,3	6,1	14,3	3,9	2,1	8,9	3,2	4,6	7,1	6,1	5,4	3,9	1,4
TC25	2,5	3,2	0,4	3,2	2,5	12,6	2,5	4,3	6,1	14,0	4,3	4,0	9,7	2,2	4,7	4,3	6,1	5,0	5,0	3,2
TS26	2,0	2,4	2,4	3,0	3,0	10,8	1,7	5,1	3,4	14,2	3,0	3,7	9,8	3,7	3,4	7,4	6,8	6,1	5,4	2,7
TC26	4,5	3,4	1,0	2,1	3,8	11,0	1,7	3,4	5,2	14,1	4,1	4,8	5,2	2,4	4,8	8,6	4,5	6,5	4,8	4,1

The frequencies of amino acid groups between healthy tissue and cancer tissue in Table 8 show non-significant p-values except for asparagin and lysine. For tryptophan and tyrosin we note an increase which is not significant just as for the phenylalanine which decreased from healthy tissues to cancerous tissues.

Table 8: Frequency of amino acids between healthy tissues in cancer tissues

Aminoacids	HealthyTissues	Cancerous Tissues	p-values
Ala	3,7056	3,1673	0,5931
Cys	2,0812	1,9573	0,1397
Asp	1,8782	1,9217	0,0491
Glu	2,7411	3,3808	0,6979
Phe	4,264	4,1993	0,0729
Gly	10,761	11,032	0,3035
His	1,9289	2,5623	0,6915
Ile	2,7919	2,3132	0,5300
Lys	4,4162	4,4484	0,0363
Leu	14,112	14,875	0,8217
Met	5,2284	5,0178	0,2368
Asn	3,7056	3,6299	0,0854
Pro	8,4772	8,3986	0,0886
Gln	3,1472	4,0925	0,9923
Arg	5,0254	3,9502	0,8942
Ser	6,2944	5,089	0,7880
Thr	4,3147	4,1993	0,1301
Val	8,4264	8,2562	0,1916
Trp	4,9239	5,5872	0,7219
Tyr	1,7766	1,9217	0,1634

4. Discussion

The analysis of the genetic variability of ovarian tumors in Senegalese patients showed a strong variation of tumors and stable genetic evolution of the disease. Our results correlate well with the dominant thesis that ovarian cancer is a heterogeneous disease composed of different tumor types with very different behaviors and clinicopathological characteristics (kurma *et al*., 2010).

In general the percentage of A+T is substantially greater than G+C in cancerous tissues. These differences were noted by Mbaye *et al.* (2012a). The nucleotidic p-distances and the K2P showed a greater rate of substitution at the third nucleotidic position for healthy tissues in contrast to cancerous tissues which showed higher levels of substitution at the first and second position nucleotide. In general a substitution on the 2nd base pair of a codon causes generally, in 100% of cases, a proteinic modification and so a strong disorganization on cancerous tissues. In accordance with other studies, we identified a great number of polymorphisms of ADNmt in the cytochrome B. The majority of polymorphisms are T→A or C→A. This indicates that ADNmt is very sensitive to the mutations, probably by the oxidative stress (Vincent *et al.*, 2001).

The analysis of genetic differentiation revealed that the genetic distance between cancerous tissues is greater than between healthy tissues. The proliferation of abnormal cells appears to be much faster. This report shows that the main characteristic of the cancerous cell resides in the fact that its proliferation is not any more under the control of the body regulating mechanisms, and that it evolves according to its own rhythm. So it would not necessarily divide at the same rate as the normal cell from which it derives, but its proliferation is no longer understood as meeting the unique needs of the organization, it would escape from the different control levels of it (Mbaye et al., 2012a). The genetic distance correlated with the patients 'age, reveals that the distance observed at intra-cancerous tissues of old women is higher than the genetic distance of intra-cancerous tissues of young women. This is explained by greater genetic disorganization in patients aged. Age could therefore affect the genetic variablity of these two groups. But Considering the disparity of the number of sequences, a study with a larger number of samples should be conducted to confirm this.

The analysis of the genetic evolution, revealed a strong value of haplotypic diversity and nucleotidic diversity of cancerous tissues. These results confirm the high variability of the cancerous cells. The average number of synonymous substitutions is higher than the nonsynonymous substitutions one for cancerous tissues. We can make the assumption that synonymous mutations are subject to little or no selection (neutral) because they do not alter the protein encoded by a gene. In this case, the substitution rate is a good estimator of synonymous mutation rate (Kimura, 1983). The rate of non-synonymous substitution depends on the stress level of a protein. As (dn) <(ds), the rate of substitution is less than mutation rate, which indicates the existence of a negative selection eliminating non-synonymous mutations. The weaker (dn) is compared to (ds), the more the number of non-synonymous substitutions is weak (Lopez *et al*, 2002). Neutrality tests performed are not significant. R2 results show an demographic expansion. The curve of mismatch distribution observed is multimode testifying to a population in demographic balance i.e. constant. if combined results we note a moderate evolution of rare mutations that can be scanned over time.

The genetic variability results in a diversity of amino acids between normal tissue and cancerous tissues sequences. The comparison of the amino acid composition of each patient between healthy tissue and tumor tissue showed an increase in phenylalanine, tryptophan and tyrosine for a majority of them. Tryptophan (Trp), which plays an important role in the proliferation of T lymphocytes and phenylalanine (Phe) are among the eight amino acids called essential because they cannot be synthesized by the body and this is our diet which must bring them. Tyrosine (Tyr) is an amino acid generally within the water-soluble proteins or membrane helices in contact with the lipid. This is a non-essential amino acid in human's diet. The Trp, Tyr and Phe are the only amino acid having an aromatic side chain. Tryptophan metabolism in cancer is more and more recognized as an important microenvironment factor that removes antitumor immune responses. It has been suggested that tryptophan, an essential amino acid, is catabolized in the tumor tissue by the enzyme indoleamine-2, 3 - dioxygenase (IDO) expressed in tumor cells or antigen presenting cells. This creates a metabolic pathway immunosuppressive environment in tumors and in lymph nodes draining a tumor by inducing a T cell energy and apoptosis by tryptophan depletion and accumulation of tryptophan catabolites immunosuppressants (Platten *et al.* 2012). Increased levels of Phe in cancer tissues were also observed from 6.6% to 210%. Phe is a component of aspartame which appears to be a poison to the body. Aspartame is transformed in the body into Phenylalanine (50%), aspartic acid (40%), and methanol (10%) which decomposes in the body in formic acid (responsible for brain tumors), and formaldehyde (carcinogen and opposes the DNA replication) (Mbaye et al., 2012b).Tyrosine (Tyr) is an amino acid which readily crosses the blood-brain barrier. Once in the brain, it is a precursor of dopamine, noradrenaline and adrenaline. Kinases are proteins of the organism capable of transferring phosphate groups on proteins or other molecules. Tyrosine kinases are proteins that transfer phosphate groups on tyrosine amino acid in other proteins and in this way regulate the proliferation of cells. Quantifying the rate of Tyr in the body could be considered as means of screening.

5. Conclusion

At the end of this work we observe a high variability of tumors and a moderate evolution of rare mutations. The high rate of cytochrome b mutations found in ovarian cancers suggests a genetic instability of mitochondrial DNA which could play an important role in tumorigenesis. Any modification of Trp, Phe and Tyr leading to a deficiency of these amino acids in normal tissues, as well as increased levels of Trp, Phe and Tyr levels in cancerous tissues, can be correlated with an increased risk of developing ovarian cancer. Moreover, it would be interesting to start searching the rate of Tryptophan, Phenylalanine and tyrosine in the blood as rapid screening test. More advanced studies in targeted regulatory T cells and receptor tyrosine kinases immunology could result to effective treatment or early diagnosis. But these hypotheses need to be confirmed by genetic analysis of a large number of samples.

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