Mutation Screening in the Mitochondrial D-Loop Region of Tumoral and Non-tumoral Breast Cancer in Iranian Patients

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Abstract- The mitochondrial DNA (mtDNA) mutations in mitochondrial coding and non coding regions seem to be important in carcinogenesis. The aim of this investigation was to evaluate coding region (mt-tRNA^{Phe} and tRNA^{Pro}) and non-coding sequence, mitochondrial displacement loop (mtDNA D-loop), in the cancerous and non-cancerous lesions of Iranian patients with breast cancer (BC). Genomic DNA was extracted from 50 breast tumors and surrounding normal tissue pairs as well as from 50 unrelated normal breast tissues from Iranian Kurdish population. Subsequently, PCR amplification was performed using specific primers, and then PCR products were subjected to direct sequencing. 41 genetic variants were identified in mtDNA D-loop among tumoral and non-tumoral tissues but not in tRNA^{Phe} and tRNA^{Pro} sequences. Our findings indicated that C182T, 194insT, 285insA and 16342delT were just found in BC tumors whereas 302insC, C309T and C16069T found in both tumors and surrounding normal tissues. Although our findings showed that the observed genetic variations were not restricted to breast cancer tissues, some genetic changes were found only in BC tumors. Our results, in agreement with the evidence from earlier studies, confirm that the mtDNA genetic alterations might be implicated in tumor initiation, progression and development.

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Keywords: Breast Cancer; D-loop; tRNA^{Phe}; tRNA^{Pro}

Introduction

Breast cancer (BC) is the second most common diagnosed cause of cancer death in the developed countries and accounts for 23% of the total cancers. Different studies reported that BC accounts for 14% of all cancer deaths in females (1,2). It is estimated that, in the developed countries, BC occurs in one out of eight women during her lifetime (2,3). It is well documented that the different factors such as genetics and environment factors are involved in tumorigenesis. Therefore, various risk factors including endogenous or exogenous estrogen exposure, age of menarche, genetic backgrounds, family history, race and ethnicity are thought to be implicated in the development of BC (2,4). The risk is considerably higher for females with a family history of BC or a known mutation in the BC predisposition genes such as BRCA1 and BRCA2 genes (5). The mutated BRCA1 and BRCA2 genes can act alone and the families that carry either of these mutations might have as many as half of their women members with breast cancer. The initial mutation can be inherited from one's parents (familial breast cancer) whereas a significant proportion of BC is due to mutation after conception (sporadic breast cancer). In the western countries approximately 10% of breast cancers are associated with genetic factors, and BRCA 1 or BRCA 2-related cancers constitutes for 2/3 to 3/4 of

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these cases. The lifetime cumulative risk of invasive BC for individuals with BRCA1or BRCA2 mutations ranges from 50% to 87% (2,3).

In addition to the nuclear genome, eukaryotic cells have cytoplasmic genomes compartmentalized in the mitochondria (6). Mitochondria are multifunctional organelles that regulate metabolism, cellular energy production, programmed cell death (apoptosis), unprogrammed cell death (necrosis) and detoxification. Human mitochondrion contains circular DNA (mtDNA) that consists of 16,569 bp and encodes 13 oxidative phosphorylation (OXPHOS) subunits, 22 transfer RNAs (tRNAs), 2 ribosomal RNAs (rRNAs) and the displacement loop (D-Loop) (7). Mitochondria consist of multiple copies of mtDNA genome per mitochondrion (2-10 copies) and are not protected by histones. The mtDNA is directly exposed to the damaging effects of reactive oxygen species (ROS). Studies determined that due to insufficient DNA repair system in this organelle, the mutation rate in mtDNA is higher than nuclear DNA (7-11).

The effect of mtDNA on tumorigenesis or progression to malignant transformation might be achieved via a number of changes in mtDNA such as reducing the mtDNA content (12). In fact, tumor-specific changes in the mtDNA copy numbers have been reported in human cancers. Reduced mtDNA content has been reported in breast (7,13-17), prostate (7,18), ovarian (7,17), renal (7,19) and hepatocellular (7,15,20), head and neck (21), thyroid (22) and gastric (7,23) cancers. The second type of influence of genetic changes in mitochondrial DNA on tumorigenesis can be achieved through a decrease in mitochondrial gene expression (7,24), or alteration in mitochondrial enzymatic activity (25-27).The third type of change in mtDNA is somatic or germ line mtDNA mutations. Mutations in mtDNA have been frequently reported in variety of tumors (7,28,29). It has also been suggested that polymorphisms in a mitochondrial gene encoding for a complex I subunit (ND3) provide an increased risk of invasive breast cancer (7,30).

The displacemental loop (D-loop) is the major control site for mtDNA replication and transcription (31). Genetic variability in the D-loop region has been suggested to affect the function of the respiration chain that is responsible for high ROS levels and could contribute to cancer initiation (32).

In the present study, we performed the mitochondrial mutation screening for somatic mutation in paired cancerous and non-cancerous tissues from 50 patients with breast cancer.

Materials and Methods

Surgical biopsies that included tissue samples from breast cancer tumors and non-tumoral tissues were collected from 50 patients from Kurdish origin referred to Cancer Institute, Tehran University of Medical Sciences (TUMS). Also, 50 normal breast tissues were obtained from unrelated individuals who had reconstruction mammoplasty surgery. To better evaluation of the possible influence of mtDNA genetic changes on BC, all the patients were studied with the same pathological grade (grade II) and specific ethnic group, Iranian Kurdish population. All participants gave their written informed consent, and the study was approved by the TUMS ethics review board as part of the reviewing process of TUMS research projects. A portion of each biopsy was sent for histopathological examination, whereas another portion was immediately snap-frozen in liquid nitrogen and kept at -80^{o}C for DNA extraction.

DNA isolation

Total DNA was isolated using Qiamp®DNA Mini kit according to manufacturer's instructions (Qiagen, Hilden, Germany). In order to amplify the mtDNA D-loop, specific oligonucleotide primers were designed based on Genbank database (NC_012920.1), HV1F 5'-CCAGTCTTGTAAACCGGAGATG-3' and HV2R 5'-CGTGGTGATTTAGAGGGTGAAC-3'. The PCR was performed based on the following conditions: initial denaturation at 94°C for 5 min; followed by 35 cycles including denaturation at 94°C for 35 s, annealing at 58°C for 35 s, and extension at 72°C for 1.30 min; and a final extension at 72°C for 5 min. At the end, 5 µl of the reaction mix was analyzed by 1.8% agarose gel electrophoresis. Then, PCR products were purified using QIAquick PCR Purification Kit according to handbook instruction (Qiagen, Hilden, Germany). The purified PCR products were subjected to direct sequencing. Sequence data searches were conducted in non-redundant nucleic and protein databases using BLAST (http://www.ncbi.nlm.nih.gov/BLAST).

Microsoft Excel spreadsheet and the Statistical Package for Social Sciences (SPSS) version 11.2 were employed for data entry and analysis.

Results

The principal characteristics of samples are indicated in Table 1.

Table 1. Principle characteristics of the breast cancer samples.

	Characteristics	Cancer patients
Age (years)	Mean (95% CI)	41.4 (33.6-50)
	Minimum	36
	Maximum	60
Tumor size (cm^3)	Mean (95% CI)	3.8 (2.6-6)
	Minimum	3.5
	Maximum	9
Grade	II	

C.I.: Confidence interval

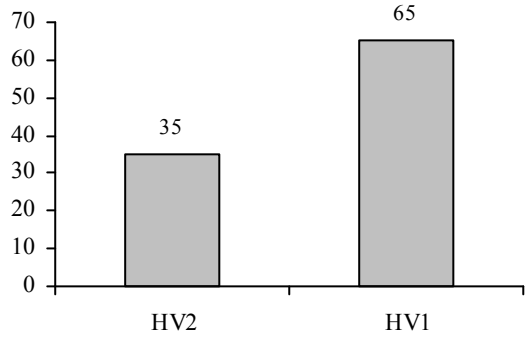

Figure 2. Pie Chart showing the genetic alterations in mitochondrial HV1 and HV2 in the tuomoral and non-tumoral tissues of BC patients.

Two mitochondrial coding regions including tRNAs ($tRNA^{Phe}$ and $tRNA^{Pro}$) and mitochondrial displacement loop (mtDNA D-loop) were amplified using PCR amplification (Figure 1). All samples had the same tumor stage (grade II) in similar ethnicity (Iranian Kurdish population). The direct PCR sequencing was conducted as described in the Materials and Methods. The sequencing findings were compared with NCBI database (NC_012920.1).

The direct PCR sequencing revealed 41 genetic alterations were detected in breast cancer samples. Different somatic variants including base substitution, deletion and insertion in tumoral (T) and non-tumoral (NT) were identified. Interestingly, some of the identified somatic mutations were found in both cancerous and non-cancerous tissues (Table 2). About 35% of genetic alterations were located in HV3 whereas 65% located in HV1. The distribution frequency of these findings is shown in Figure 2. A73G, T16519C, A263G, C16067T, 303insC, C310T and T311C somatic mutations were detected in at least 50% of non-tumoral tissues. We identified seven genetic changes including C182T, 194insT, 285insA, 302insC, C309T, C16069T and 16342delT in tumoral and normal adjacent tissues which have not been previously reported.

Table 2 presents as summery of and their location in mitochondrial DNA. Regardless of tumoral and normal classification we found at least one genetic variation among the samples (82%).

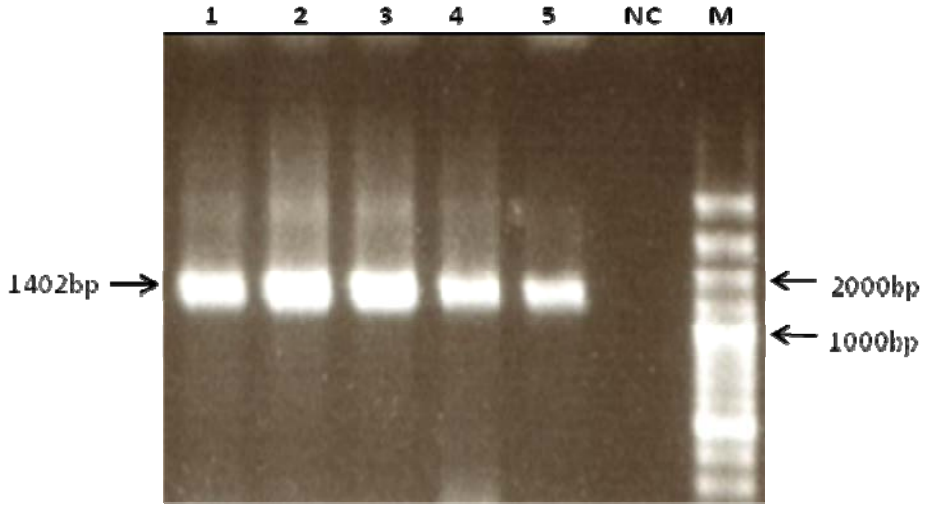

Figure 1. PCR amplification of flanking region of mt D-Loop. Ethiduim bromide stained agarose gel (1.2%) showing amplification (1402 bp) of tumoral (1 and 2) and normal tissues (3, 4 and 5), coding (tRNAPhe and tRNAPro) and non-coding (D-Loop) sequences. M, Molecular weight marker (100bp); NC, negative control (H_2O).

Table 2. A summary of mitochondrial DNA mutations in this study.

Mutation type and position	Tumoral samples	Non-tumoral samples	Tumoral tissue (%)	Non-tumoral tissue (%)	Region
A73G	16	16	32	32	HV2
T146C	4	4	8	8	HV2
C150T	4	4	8	8	HV2
C152T	5	5	10	10	HV2
C182T	2	2	6.6	6.6	HV2
G185A	4	4	8	8	HV2
194insT	1	0	3.3	0	HV2
T195C	4	4	8	8	HV2
T204C	2	2	4	4	HV2
A257G	2	2	4	4	HV2
A263G	33	33	66	66	HV2
285insA	1	0	3.3	0	HV2
302insC	9	0	33.3	0	HV2
C309T	10	10	36.7	36.7	HV2
310insT	2	2	4	4	HV2
514-523 ins CA	5	5	10	10	HV3
C16069T	2	2	6.6	6.6	HV1
T16172C	12	12	24	24	TAS
A16182C	4	4	8	8	HV1
A16183C	6	6	12	12	HV1
T16189C	4	4	8	8	HV1
T16207C	4	4	8	8	HV1
T16209C	4	4	8	8	HV1
C16223T	8	8	16	16	HV1
T16224C	6	6	12	12	HV1
C16234T	10	10	20	20	HV1
C16278T	4	4	8	8	HV1
C16287T	2	2	4	4	HV1
A16293G	2	2	4	4	HV1
T16298C	2	2	4	4	HV1
T16304C	6	6	12	12	HV1
A16309G	6	6	12	12	HV1
T16311C	10	10	20	20	HV1
G16319A	2	2	4	4	HV1
16342delT	1	0	3.3	0	HV1
A16343G	6	6	12	12	HV1
T16356C	8	8	16	16	HV1
T16362C	12	12	24	24	HV1
T16368C	2	2	4	4	HV1
G16390A	2	2	4	4	*
T16519C	20	20	40	40	*

* Not within HV1/HV2/HV3

* TAS: termination-associated sequence

Table 3. Novel mutations and their frequencies in tumoral and their normal adjacent tissues.

Mutation type	Normal tissue	Tumoral tissue	Normal tissue (%)	Tumoral tissue (%)
C182T	2	2	6.6	6.6
194insT	0	1	0	3.3
285insA	0	1	0	3.3
302insC	0	9	0	33.3
C309T	10	10	36.7	36.7
C16069T	2	2	6.6	6.6
16342delT	0	1	0	3.3

Discussion

The displacement loop (D-loop) is the major control site for mtDNA replication and transcription (31,33). It is a hotbed of mutation and contains two hypervariable regions (31). It is well documented that mutations in D-Loop region result in mitochondrial genome instability and contribute to the process of carcinogenesis (34). Genetic variability in the D-loop region has been suggested to affect the function of the respiration chain that is responsible for high ROS levels and could contribute to cancer initiation (32,33).

We identified 41somatic mutations in mt D-Loop of tumoral and adjacent normal tissues among 50 Iranian patients with BC, but not in coding regions of mitochondrial $tRNA^{Phe}$ and $tRNA^{Pro}$ sequences. 194inT, 285insA and 16342delT variants were found in BC tumors while 302insC, C309T and C16069T detected in both tumors and surrounding normal tissues.

Our results are not the first report suggesting the implication of mitochondrial genetic alterations in breast cancer development. Two previous studies scanning the entire mitochondrial genome in an American population indicated that 14 out of 15 breast cancer samples (93%) and 14 out of 19 samples (74%) displayed at least one somatic mtDNA mutation (35). In the second study, there were as high as 81% of the mutations in the D-loop region (36). It was estimated that 10.8% of breast ductal adenocarcinomas harboring somatic mutation (37). More recent study of the D-loop region in 94 pairs of breast normal and cancer tissues showed 40.42% somatic mtDNA mutations (38). Our data showed genetic mutations in 52% of BC samples. Consistent with our results, it has been demonstrated that the D-loop mutation frequencies to be varied widely, ranging from 10.8% to 65 %.

So far, a variety of diseases including mitochondrial myopathies, diabetes, encephalopathies, deafness and malignancies have been demonstrated to be associated with mitochondrial tRNA (mt-tRNA) mutations. A number of mitochondrial diseases have recently been ascribed to point mutations in mt-$tRNA^{Phe}$ (39-45). A study reported a pathogenic mutation in mt-$tRNA^{Pro}$, in which G36 is changed to A in a seven year-old girl with a pure myopathy (46). However, a few studies described the association of mt-tRNA mutations with human cancers. Sanchez-Cespedes *et al.* identified an A-to-G transition in position 10 448 (inside $tRNA^{Arg}$) in patient with lung cancer (47). Our findings could not detect any genetic variation in the coding sequences of mt-$tRNA^{Phe}$ and mt-$tRNA^{Pro}$.

In conclusion, mitochondrial DNA mutations have been observed in various types of human malignancies. Mitochondrial functional defects have also been demonstrated due to aberrant expression of mtDNA genes result in defective oxidative/phosphorylation cycle. The clinical phenotypic variations of mitochondrial genome polymorphism create technical challenges for applying biological samples for early detection of tumors. More investigations are required to access the functional role of the different mitochondrial mutations in tumor initiation and progression. Studies on non-coding sequence of mtDNA would help in finding biomarker and developing new therapeutic strategies in cancer treatment.

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References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011;61(2):69-90.
2. Bozovic-Spasojevic I, Azambuja E, McCaskill-Stevens W, Dinh P, Cardoso F. Chemoprevention for breast cancer. Cancer Treat Rev 2012;38(5):329-39.

3. Lillie SE, Brewer NT, O'Neill SC, Morrill EF, Dees EC, Carey LA, Rimer BK. Retention and use of breast cancer recurrence risk information from genomic tests: the role of health literacy. Cancer Epidemiol Biomarkers Prev 2007;16(2):249-55.
4. Chan K, Morris GJ. Chemoprevention of breast cancer for women at high risk. Semin Oncol 2006;33(6):642-6.
5. Wang F, Fang Q, Ge Z, Yu N, Xu S, Fan X. Common BRCA1 and BRCA2 mutations in breast cancer families: a meta-analysis from systematic review. Mol Biol Rep 2012;39(3):2109-18.
6. Yu M, Shi Y, Zhang F, Zhou Y, Yang Y, Wei X, Zhang L, Niu R. Sequence variations of mitochondrial DNA D-loop region are highly frequent events in familial breast cancer. J Biomed Sci 2008;15(4):535-43.
7. Kulawiec M, Owens KM, Singh KK. Cancer cell mitochondria confer apoptosis resistance and promote metastasis. Cancer Biol Ther 2009;8(14):1378-85.
8. Larsson NG, Clayton DA. Molecular genetic aspects of human mitochondrial disorders. Annu Rev Genet 1995;29:151-78.
9. Nagley P, Wei YH. Ageing and mammalian mitochondrial genetics. Trends Genet 1998;14(12):513-7.
10. de Souza-Pinto NC, Mason PA, Hashiguchi K, Weissman L, Tian J, Guay D, Lebel M, Stevnsner TV, Rasmussen LJ, Bohr VA. Novel DNA mismatch-repair activity involving YB-1 in human mitochondria. DNA Repair (Amst) 2009;8(6):704-19.
11. Anderson S, Bankier AT, Barrell BG, de Bruijn MH, Coulson AR, Drouin J, Eperon IC, Nierlich DP, Roe BA, Sanger F, Schreier PH, Smith AJ, Staden R, Young IG. Sequence and organization of the human mitochondrial genome. Nature 1981;290(5806):457-65.
12. Kulawiec M, Safina A, Desouki MM, Still I, Matsui S, Bakin A, Singh KK. Tumorigenic transformation of human breast epithelial cells induced by mitochondrial DNA depletion. Cancer Biol Ther 2008;7(11):1732-43.
13. Yu M, Zhou Y, Shi Y, Ning L, Yang Y, Wei X, Zhang N, Hao X, Niu R. Reduced mitochondrial DNA copy number is correlated with tumor progression and prognosis in Chinese breast cancer patients. IUBMB Life 2007;59(7):450-7.
14. Mambo E, Chatterjee A, Xing M, Tallini G, Haugen BR, Yeung SC, Sukumar S, Sidransky D. Tumor-specific changes in mtDNA content in human cancer. Int J Cancer 2005;116(6):920-4.
15. Lee HC, Yin PH, Lin JC, Wu CC, Chen CY, Wu CW, Chi CW, Tam TN, Wei YH. Mitochondrial genome instability and mtDNA depletion in human cancers. Ann N Y Acad Sci 2005;1042:109-22.
16. Tseng LM, Yin PH, Chi CW, Hsu CY, Wu CW, Lee LM, Wei YH, Lee HC. Mitochondrial DNA mutations and mitochondrial DNA depletion in breast cancer. Genes Chromosomes Cancer 2006;45(7):629-38.
17. Desouki MM, Kulawiec M, Bansal S, Das GM, Singh KK. Cross talk between mitochondria and superoxide generating NADPH oxidase in breast and ovarian tumors. Cancer Biol Ther 2005;4(12):1367-73.
18. Mizumachi T, Muskhelishvili L, Naito A, Furusawa J, Fan CY, Siegel ER, Kadlubar FF, Kumar U, Higuchi M. Increased distributional variance of mitochondrial DNA content associated with prostate cancer cells as compared with normal prostate cells. Prostate 2008;68(4):408-17.
19. Selvanayagam P, Rajaraman S. Detection of mitochondrial genome depletion by a novel cDNA in renal cell carcinoma. Lab Invest 1996;74(3):592-9.
20. Yin PH, Lee HC, Chau GY, Wu YT, Li SH, Lui WY, Wei YH, Liu TY, Chi CW. Alteration of the copy number and deletion of mitochondrial DNA in human hepatocellular carcinoma. Br J Cancer 2004;90(12):2390-6.
21. Lièvre A, Blons H, Houllier AM, Laccourreye O, Brasnu D, Beaune P, Laurent-Puig P. Clinicopathological significance of mitochondrial D-Loop mutations in head and neck carcinoma. Br J Cancer 2006;94(5):692-7.
22. Tong BC, Ha PK, Dhir K, Xing M, Westra WH, Sidransky D, Califano JA. Mitochondrial DNA alterations in thyroid cancer. J Surg Oncol 2003;82(3):170-3.
23. Wu CW, Yin PH, Hung WY, Li AF, Li SH, Chi CW, Wei YH, Lee HC. Mitochondrial DNA mutations and mitochondrial DNA depletion in gastric cancer. Genes Chromosomes Cancer 2005;44(1):19-28.
24. Weber K, Ridderskamp D, Alfert M, Hoyer S, Wiesner RJ. Cultivation in glucose-deprived medium stimulates mitochondrial biogenesis and oxidative metabolism in HepG2 hepatoma cells. Biol Chem 2002;383(2):283-90.
25. Espineda CE, Chang JH, Twiss J, Rajasekaran SA, Rajasekaran AK. Repression of Na,K-ATPase beta1-subunit by the transcription factor snail in carcinoma. Mol Biol Cell 2004;15(3):1364-73.
26. Isidoro A, Martínez M, Fernández PL, Ortega AD, Santamaría G, Chamorro M, Reed JC, Cuezva JM. Alteration of the bioenergetic phenotype of mitochondria is a hallmark of breast, gastric, lung and oesophageal cancer. Biochem J 2004;378(Pt 1):17-20.
27. Eng C, Kiuru M, Fernandez MJ, Aaltonen LA. A role for mitochondrial enzymes in inherited neoplasia and beyond. Nat Rev Cancer 2003;3(3):193-202.
28. Modica-Napolitano JS, Kulawiec M, Singh KK. Mitochondria and human cancer. Curr Mol Med 2007;7(1):121-31.

29. Brandon M, Baldi P, Wallace DC. Mitochondrial mutations in cancer. Oncogene 2006;25(34):4647-62.
30. Canter JA, Kallianpur AR, Parl FF, Millikan RC. Mitochondrial DNA G10398A polymorphism and invasive breast cancer in African-American women. Cancer Res 2005;65(17):8028-33.
31. Suzuki M, Toyooka S, Miyajima K, Iizasa T, Fujisawa T, Bekele NB, Gazdar AF. Alterations in the mitochondrial displacement loop in lung cancers. Clin Cancer Res 2003;9(15):5636-41.
32. Lièvre A, Chapusot C, Bouvier AM, Zinzindohoué F, Piard F, Roignot P, Arnould L, Beaune P, Faivre J, Laurent-Puig P. Clinical value of mitochondrial mutations in colorectal cancer. J Clin Oncol 2005;23(15):3517-25.
33. Ye C, Shu XO, Pierce L, Wen W, Courtney R, Gao YT, Zheng W, Cai Q. Mutations in the mitochondrial DNA D-loop region and breast cancer risk. Breast Cancer Res Treat 2010;119(2):431-6.
34. Kuo SJ, Chen M, Ma GC, Chen ST, Chang SP, Lin WY, Chen YC, Lee TH, Lin TT, Liu CS. Number of somatic mutations in the mitochondrial D-loop region indicates poor prognosis in breast cancer, independent of TP53 mutation. Cancer Genet Cytogenet 2010;201(2):94-101.
35. Zhu W, Qin W, Bradley P, Wessel A, Puckett CL, Sauter ER. Mitochondrial DNA mutations in breast cancer tissue and in matched nipple aspirate fluid. Carcinogenesis 2005;26(1):145-52.
36. Tan DJ, Bai RK, Wong LJ. Comprehensive scanning of somatic mitochondrial DNA mutations in breast cancer. Cancer Res 2002;62(4):972-6.
37. Alazzouzi H, Farriol M, Espín E, Armengol M, Pena M, Zeh K, Schwartz S, Schwartz S Jr. Molecular patterns of nuclear and mitochondrial microsatellite alterations in breast tumors. Oncol Rep 2003;10(5):1561-7.
38. Pavicic WH, Laguens M, Richard SM. Analysis association between mitochondrial genome instability and xenobiotic metabolizing genes in human breast cancer. Mol Med 2009;15(5-6):160-5.
39. Deschauer M, Swalwell H, Strauss M, Zierz S, Taylor RW. Novel mitochondrial transfer RNA(Phe) gene mutation associated with late-onset neuromuscular disease. Arch Neurol 2006;63(6):902-5.
40. Hanna MG, Nelson IP, Morgan-Hughes JA, Wood NW. MELAS: a new disease associated mitochondrial DNA mutation and evidence for further genetic heterogeneity. J Neurol Neurosurg Psychiatry 1998;65(4):512-7.
41. Mancuso M, Filosto M, Mootha VK, Rocchi A, Pistolesi S, Murri L, DiMauro S, Siciliano G. A novel mitochondrial tRNAPhe mutation causes MERRF syndrome. Neurology 2004;62(11):2119-21.
42. Moslemi AR, Lindberg C, Toft J, Holme E, Kollberg G, Oldfors A. A novel mutation in the mitochondrial tRNA(Phe) gene associated with mitochondrial myopathy. Neuromuscul Disord 2004;14(1):46-50.
43. Darin N, Kollberg G, Moslemi AR, Tulinius M, Holme E, Grönlund MA, Andersson S, Oldfors A. Mitochondrial myopathy with exercise intolerance and retinal dystrophy in a sporadic patient with a G583A mutation in the mt tRNA(phe) gene. Neuromuscul Disord 2006;16(8):504-6.
44. Chinnery PF, Johnson MA, Taylor RW, Lightowlers RN, Turnbull DM. A novel mitochondrial tRNA phenylalanine mutation presenting with acute rhabdomyolysis. Ann Neurol 1997;41(3):408-10.
45. Kleinle S, Schneider V, Moosmann P, Brandner S, Krähenbühl S, Liechti-Gallati S. A novel mitochondrial tRNA(Phe) mutation inhibiting anticodon stem formation associated with a muscle disease. Biochem Biophys Res Commun 1998;247(1):112-5.
46. Moraes CT, Ciacci F, Bonilla E, Ionasescu V, Schon EA, DiMauro S. A mitochondrial tRNA anticodon swap associated with a muscle disease. Nat Genet 1993;4(3):284-8.
47. Sanchez-Cespedes M, Parrella P, Nomoto S, Cohen D, Xiao Y, Esteller M, Jeronimo C, Jordan RC, Nicol T, Koch WM, Schoenberg M, Mazzarelli P, Fazio VM, Sidransky D. Identification of a mononucleotide repeat as a major target for mitochondrial DNA alterations in human tumors. Cancer Res 2001;61(19):7015-9.