Original Article

Mitochondria and tumors: A new perspective

Chintha R, Kaipa PR, Sekhar N[1], Hasan Q[2]

Department of Genetics, [1]Center for Bio-informatics Studies, Osmania University, [2]Molecular Medicine, Kamineni Hospitals, L.B. Nagar, Hyderabad, Andhra Pradesh, India

Correspondence to: Dr. Rohini Chintha, E-mail: rohinichintha@gmail.com

Abstract

BACKGROUND: Mitochondrial DNA (Mt DNA) defects have been identified in a variety of Tumors, but the exact role of these defects in the pathogenicity and tumor progression is poorly understood. This study aims at identifying the status of mitochondrial OXPHOS genes in neoplastic transformation and attempts to establish a cause and effect relationship between mitochondrial OXPHOS defects and tumor progression. **MATERIALS AND METHODS**: Mutational, expression and functional analysis of l2 of the 13 mitochondrial OXPHOS genes has been carried out using PCR, Real-Time PCR and protein modeling in 180 sporadic samples of a heterogeneous group of benign and malignant tumors like that of benign, malignant, matched blood and adjacent normal tissue of breast and benign hemangioma. **RESULTS:** Mutations were identified in the ND4L, ND6 and COX-II regions of the mitochondrial OXPHOS genes. All the mutations were limited only to the malignant breast tissues. On relative quantification, a compromised expression of OXPHOS genes was identified in all the malignant tissues irrespective of their mutational states. Protein modeling revealed loss of function mutations of ND6 and COX-II proteins. **CONCLUSION:** This is the first study worldwide wherein a comparative study using different benign and malignant tumors has been carried out to assess the role of Mt DNA defects. Our data reveals mitochondrial dysfunction only in malignant cells and not in their benign counterparts, indicating that the dysfunction may arise after the pro-proliferative pathway has set in. We hypothesize that compromised OXPHOS may be a responsive mechanism of the cell to counter cancers, rather than a mechanism of initiating tumorigenesis.

Key words: Breast Tumor, hemangioma, mitochondria, OXPHOS gene defects, tumors

Introduction

Mitochondria are complex cellular organelles contributing to 90% of the energy needs of the cell. They produce energy through 2 major pathways: i) Oxidative phosphorylation (OXPHOS) and ii) the citric acid cycle. The OXPHOS pathway is the primary source of energy for all aerobic organisms and produces energy 10 times more than the citric acid cycle. The OXPHOS pathway operates through the electron transport chain, a chain of five protein complexes consisting of **Complex I** (NADH dehydrogenase), **Complex II** (succinate dehydrogenase), **Complex III** (cytochrome-c reductase), **Complex IV** (cytochrome-c oxidase), **Complex V** (ATP synthase), embedded in the inner membrane of the mitochondria. Electron transfer proceeds via complex I, III and IV in case of NADH oxidation or complex II, III and IV for FADH oxidation.[1]

Access this article online	
Quick Response Code:	Website: www.indianjcancer.com
	DOI: 10.4103/0019-509X.118732

Of the 87 proteins contributing to cell's OXPHOS system, mitochondrial DNA (mtDNA) encodes 13 of the essential components, spanning 7 subunits of NADH dehydrogenase, 1 subunit of ubiquinone-cytochrome c oxidoreductase and 3 subunits of cytochrome c oxidase and 2 subunits of ATP synthetase.[2] Altered efficiency of the mitochondrial OXPHOS system leads to various human disorders including tumorigenesis.[3] Mitochondrial OXPHOS mutations are a common feature of cancer cells, though the clinical implication of these mutations is still to be understood.[4]

Mutations in the mtDNA-encoded OXPHOS genes are reported in a variety of malignant cancers including breast cancer.[4] The four studies undertaken till date on 'mtDNA mutations in breast cancer' report various

somatic mutations.[2,5-7] The majority of the mt DNA mutations reported in breast cancer are in the D-loop region. Others were detected in the 16*S* rRNA, ND1, ND2, ND4, ND5, ATPase 6 and Cytochrome b genes.[4] All the mt DNA mutations studied till date are found to be associated with malignancy and their status in benign tumors is not yet clear.

Such studies are important to establish cause and effect relationships between mtDNA mutations and malignancy. One study in this direction reports the enhanced expression of mitochondrial cyt-b gene in Hemangioma,[8] a benign self-regressing endothelial tumor, which exhibits different stages of development- a proliferative phase followed by an involuting phase that continues into the involuted phase. Hemangiomas are of two major types: spontaneously regressing infantile capillary hemangioma and non-regressing capillary hemangioma, which continues into the adult life.[9] All the adult hemangiomas are proliferative like other benign tumor. Enhanced expression of mitochondrial cytochrome-b is seen in hemangioma undergoing regression.[8] The status of cyt-b gene in proliferative adult hemangioma has not yet been identified.

The present study aims at assessing the occurrence of mtDNA mutations and their status and nature in different benign and malignant human tumours, especially those which exhibit altered apoptosis like adult benign hemangioma, spontaneously regressing infant capillary hemangioma and in benign and malignant tumors of the breast. If mutations in the mtDNA encoded OXPHOS genes may alter the OXPHOS cycle such that it promotes proliferation, they may be used as an early diagnostic marker.

Sporadic cases of benign and malignant tissues (invasive ductal carcinomas and benign breast tissue samples), along with matched blood controls were processed for mutational analysis and also expression analysis of the mitochondrial OXPHOS genes. Mutational analysis was done using Polymerase chain reaction (PCR) followed by Single stranded conformational polymorphism (SSCP) to identify mt DNA mutations in all of the mitochondria encoded OXPHOS genes.22 somatic mutations were identified in 13 of the malignant breast samples screened. None of the other malignant or benign cases showed any mutations.

Functional genomic studies using Bio-informatics tools were carried out on the mutated samples to understand the downstream changes brought about by mutations.

Expression analysis of the OXPHOS genes was done using Real-Time PCR to understand the status of mitochondria encoded OXPHOS in benign and malignant tumors. Our data indicates a compromised OXPHOS in all of the malignant cases compared to their benign or matched controls.

Materials and Methods

Institutional Ethical committee approval was obtained for sample collection. The post-surgical tissue samples were collected in saline/trizol immediately after surgery and stored at -80deg for further analysis. 2 ml of matched heparinized whole blood was also obtained along with the demographic and clinical details (Supplementary Tables 1, 2, 3) and family history of the patient. A total of 180 sporadic samples (70 cases of Infiltrating ductal carcinoma along with 55 matched blood samples, 22 cases of benign breast tumors along with 20 adjacent normal breast tissues, 6 cases of proliferative hemangioma, 1 involuted hemangioma and their matched blood samples) were obtained from Various Cancer research Institutes in Hyderabad, INDIA.

Mutational Analysis

Total genomic DNA was isolated from both benign and malignant tissues and matched blood samples using the 'salting out' method.[10] The primer for Cyt b-ND6, ND5, ND1, ND2, COX-1, COX-2, COX-3 are taken from previously published reports.[11-13] The primers for ND3, ND4L, ATPase6 and ATPase 8 were designed in house (Supplementary Table 5). Pseudo amplification was excluded by using a negative control with each set of reaction.

The PCR products less than 350bp were further analyzed for mutation using SSCP. Briefly, 5 ul of PCR product was mixed with 15 ul of denaturing/loading dye. This mixture was then denatured at 95 deg C for 5 minutes and then snap frozen on ice. After 10 minutes, the complete product was loaded onto a 12% poly acrylamide gel and run at 250 volts for 3 hrs 30min. The bands were visualized by silver staining.[14] The DNA fragments from blood and tumor tissues of the same patient were loaded adjacent to each other and analyzed. All the steps were repeated twice to check for the reproducibility of the result. A multiple band pattern indicates heteroplasmic mutation, whereas homoplasmic mutation would be indicated by a single band shift.For PCR products greater than 350bp (ATPase6, COX-1, COX-2), restriction digestion was done. COX-2 was subjected to double digestion using MspI and HaeIII. COX-1 was digested with MspI only.[13] ATPase 6 was digested with HaeIII. The digested products were loaded on to a 12% polyacrylamide gel, run at 250volts for 2hrs and visualized using silver staining. Tissue

samples and their matched blood contols were loaded adjacent to each other. Samples showing altered band pattern as compared to controls were considered to be mutated and were further analyzed by sequencing to identify the exact location and type of mutation.

In order to rule out the possibility of false positives for mutations, and also to rule out the possibility of missing certain mutations due to any draw back in the techniques used, the samples without any mobility differences were also randomly picked up and included for direct sequencing. The PCR products were purified by ammonium acetate precipitation method before processing them for sequencing by ABI 3100 automated DNA sequencer.The sequences obtained were then compared to the wild type sequences using NCBI BLAST. A mutation present in both the control and the case is considered as a germline mutation and those found only in the cases are treated as somatic mutations. Those mutations not reported earlier in the database are considered as novel.

Expression Analysis

Total RNA was isolated from 30 tissue samples (15 malignant breast tissues and 15 matched adjacent normal tissues) using the trizol method regularly followed in our lab. The total RNA was then converted to cDNA using the 'Revert Aid first strand cDNA synthesis kit (# K1621) from MBI Fermentas. The cDNA so obtained was processed for RT-PCR using the SYBR green method. The primers were designed in-house using the primer 3 output software.

Of the 15 malignant cases, 10 had no mutations in any of the Mt OXPHOS genes screened, whereas, the other 5 were those showing mutations in the COX-II region in mutational analysis. This inclusion criterion is followed in order to have a comparison of the status of the OXPHOS genes between the mutated and non-mutated cases. Gene specific primers were employed for the study (Supplementary Table 4).

Four of the mitochondrial OXPHOS genes, ND3, ND4l, ND5 coming under the heavy chain promoter and ND6 gene coming under the light chain promoter were analyzed. The RT-PCR was carried out using ABI 7900 system. GapdH was used as th e endogenous control. Relative quantification was done using the delta-delta Ct method (www. Eurogenetec.com).[15]

Protein modelling

The samples showing mutations in the COX-II and ND6 regions along with their control samples were subjected to functional genomics studies using commercial Bio-Informatics tools.

Sequence alignment of the wild and mutant proteins was done using ClustalX software suite.[16] Percentage identity and similarity between the wild type and mutant forms of the proteins were calculated using GENEDOC software suite (Free Software Foundation Inc). A template with closest homology viz., bovine cytochrome C oxidase at the fully oxidized state from (PDB: 1OCC) was selected for modeling of the mutated COX-II protein and bacterial ubiquinone gene.

The co-ordinates for the structurally conserved regions (SCRs) for COX2 control and mutants and also for ND6 control and mutants were assigned from the template, based on the Needleman-Wunsch algorithm. [17,18]

The initial models of wild and mutant forms of *human* COX2 and ND6 were built by using homology-modeling methods and the MODELLER software 7v7 on windows operating environment.[19]

The structure having the least MODELLER objective function, obtained above, was improved by molecular dynamics and equilibration methods using NAMD 2.5 software, CHARMM27 force field for lipids and proteins along with the TIP3P model for water.[20-22]

The binding pockets of COX2 from *human* were identified using CASTP server [Figure 1] and also based on structure-structure comparison. Structural diagrams were prepared using OPEN EYE (Open Eye Scientific Software, Santa Fe, NM) and SPDBV software.[23]

The substrate including all hydrogen atoms, were built and optimsed with chemsketch software suite. Extremely Fast Rigid Exhaustive Docking (FRED) version 2.1 was used for docking studies (Open Eye Scientific Software, Santa Fe, NM).

Results

A total of 180 sporadic cases of malignant and benign breast cancers (70 malignant breast, 55matched blood controls, 22 benign and 20 adjacent normal breast tissues) and benign proliferating hemangiomas (6 proliferating tissue samples, 6 matched blood controls and 1 matched involuting tissue) along with their matched blood and tissue controls were analyzed for the status of mitochondria encoded OXPHOS genes.

Mitochondrial OXPHOS genes (ND1, ND2, ND3, ND4L, ND5, ND6, Cytochrome b, ATPase 6, ATPase 8, COX-I, COX-II and COX-III) were screened for detection of mutations in benign and malignant breast

cancer cases and in proliferating hemangioma cases using gene specific PCR followed by single stranded conformational polymorphism (SSCP)/restriction digestion (R.D).

The PCR primers included in the study were designed in-house using 'Primer 3 output' software. The primers were validated for the presence of specific bands before proceeding for sample screening.

PCR products of each of the ND1, ND2, ND3, ND4L, ND5, ND6-Cyt b and ATPase 8 genes, whose products were less than 350bp, were analyzed for mutations using SSCP. Those of COX-I, COX-II, COX-III and ATPase6, whose products were more than 350bp were analyzed by R.D. All the cases and controls were loaded adjacent to each other, for convenience and accuracy during analysis.

Analysis of the OXPHOS genes of mitochondria revealed altered band patterns in three infiltrating ductal carcinoma (IDC) case in the ND4L region, one IDC case in the ND6 region [Figure 2] and in nine IDC cases in the COX-II region screened [Figure 3]. None of the other malignant breast, benign breast or hemangioma samples screened showed such alterations. Samples showing altered band patterns were sequenced [Figure 4A-C] to identify the exact type and position of mutation [Tables 1 and 2].

The mutational analysis data indicated the presence of 22 mutations spanning the ND4L, ND6 and COX-II region of mitochondria in 12 malignant breast cancer cases. Of the 22 somatic mutations identified in the study, 19 were novel [Tables 1 and 2].

The mutation identified in the present study in the ND4L region is a silent mutation, whereas, the mutations in the ND6 region [Table 1] were all missense and lead to the alteration of the complete reading frame. The mutations seen in the COX-II region of mitochondria [Table 2] resulted in the formation of a truncated protein.

The mutational analysis of the mitochondria encoded OXPHOS genes revealed the presence of mutations in 12/70 malignant cases included in this study. But all malignant cells are known to have altered energy requirements. Therefore, to assess the status of OXPHOS genes in the cases without mutations, expression analysis was done using Real-Time PCR.

The ND3, ND4L and ND5 genes coming under the influence of the heavy chain promoter were analyzed for expression states. Along with the above genes, the ND6 gene coming under the influence of the light chain promoter has also been analyzed in this study.

Neat specific peaks were obtained during Real-Time PCR as represented by the dissociation curve in Figure 5A-C.

The expression studies data, on relative quantification, reveals a compromised expression of the mitochondrial OXPHOS genes in all the malignant samples, irrespective of the mutational status, compared to their adjacent normal tissues or the benign cases [Figure 1].

Protein modeling data indicated loss of Beta sheets in the mutated proteins as compared to controls [Figures 6 and 7]. The beta sheets are required for interaction of the protein with adjacent molecules and

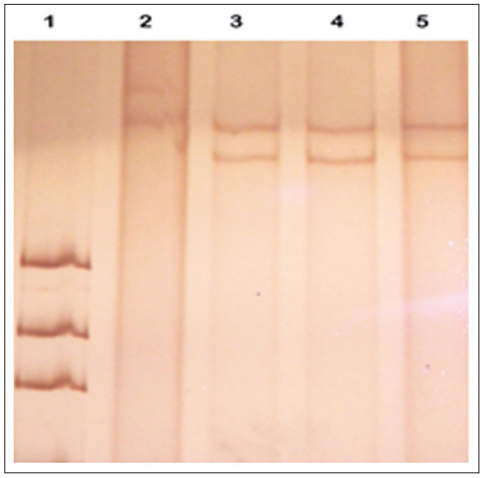

Figure 1: Real-Time graph showing the expression levels of OXPHOS genes in benign and malignant tumors. Y-Axis: Delta Ct values. X-Axis: Sample number

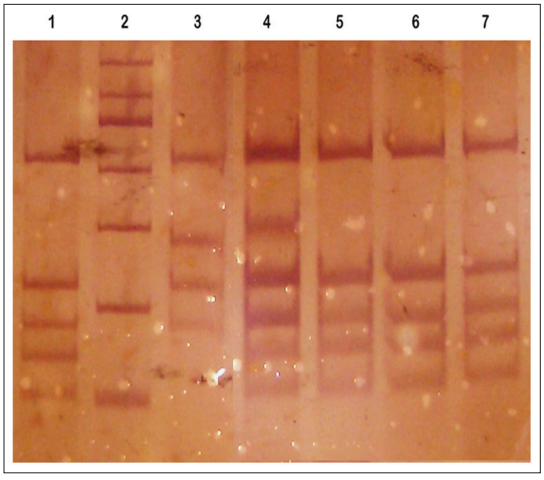

Figure 2: 12% Silver stained polyacrylamide gels showing mutational analysis of ND6 gene of mitochondria by SSCP. Lane 1/Panel 1: shows a 100 bp DNA ladder/Molecular marker. Panel 2/Lane 2: Malignant tissue of case 1 showing altered band pattern. Panel 3: Adjacent normal tissue of case 1

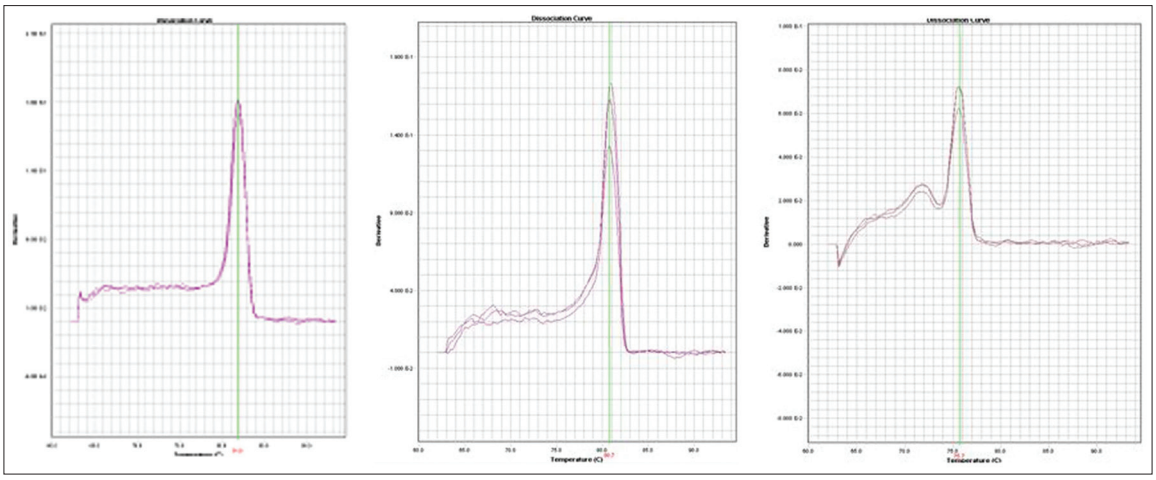

Figure 3: 12% silver stained polyacrylamide gels showing Restriction Digestion analysis of COX-II using MspI and Hae III double digestion. Panel 2/lane 2: shows Molecular marker/100bp DNA ladder. Panel 1: shows adjacent normal tissue of malignant sample loaded in Lane 3. Lane 3 and 4: samples showing loss of restriction site. Lane 5: shows adjacent normal tissue of malignant sample loaded in lane 4

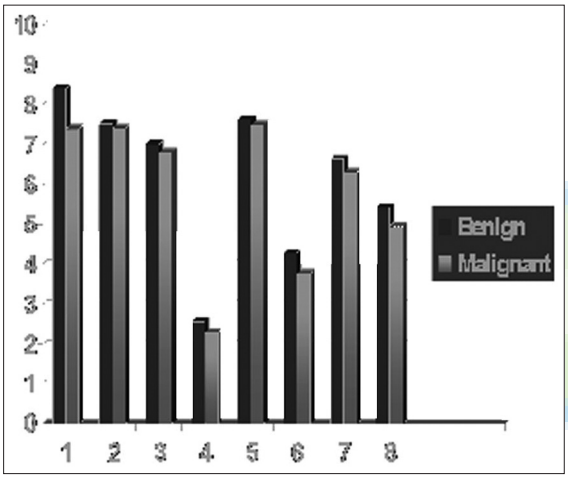

Figure 4: (A) Sequence alteration of ND4L gene indicated by arrow in the malignant sample compared to the matched blood control, (B) Sequence alteration of ND6 gene of two different controls (a,c) compared to their matched malignant breast tissues (b,d). Sequence alteration between control and case is indicated by arrows

Table 1: Mutations observed in nd6 region of mitochondria encoded oxphos in breast cancer*[24]

Position where mutation found	Nucleotide change
14233 *	A to G
14258	G to T
14260	T insertion
14264	G insertion
14270	A deletion
14276	C to G
14307	T insertion
14310	C to G
14312	A to C
14322	G insertion

Table 2: List of mutations observed in COX II in Breast cancer*[24]

Position of Mutation	Nucleotide Change
7957	C insertion
8003	A insertion
8010	T to G
8012	T insertion
8013	T to G
8020*	G insertion
8035	G to A
8047*	T insertion
8055	C insertion
8058	G to A
8070	T insertion

act as the functional domains of the protein. Our study revealed a loss of function of the COX-II and ND6 proteins due to mutations.

Discussion

In this study, we tried to ascertain the Role of Mitochondrial OXPHOS genes in various benign and malignant tumors and their importance in promoting neoplasm. To the best of our knowledge, this is the first report to take up a comparative study on the role of mitochondrial mutations in various benign and malignant tumors. Furthermore, there are few or no previous reports from around the world wherein the status of all the mitochondrial OXPHOS genes has been assessed in tumors. This is the first study worldwide to assess the role of mitochondrial DNA mutations in Hemangioma. Here, we analyzed 12 of the 13 mitochondrial encoded OXPHOS genes, all of which are

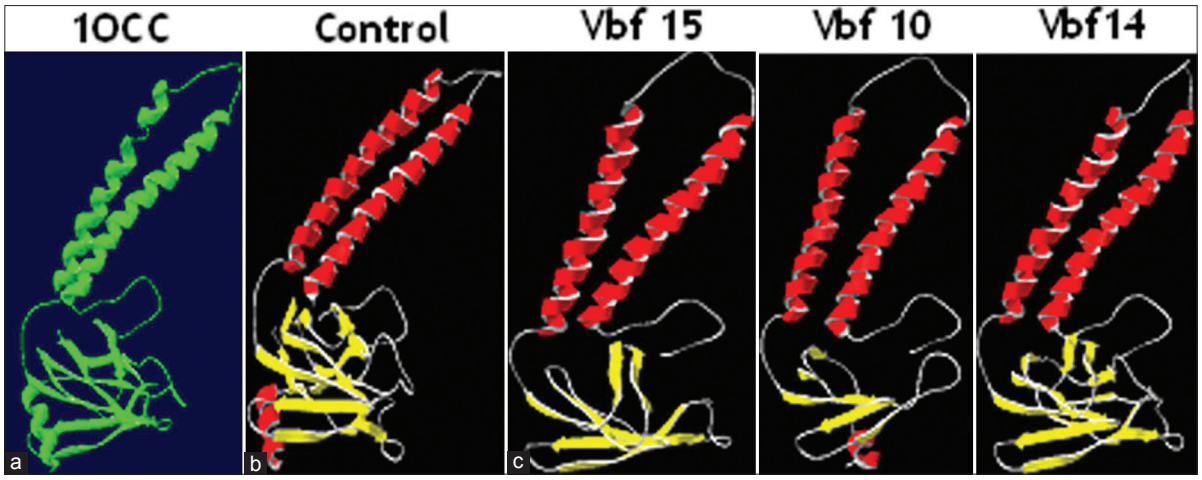

Figure 5: (a) Dissociation curve of ND4L gene, (b) Dissociation Curve of ND5 gene shows the peaks of different samples of different patients coinciding at the same Ct value. The different heights of the peaks indicate the difference in expression level of the said gene in different samples, (c) Dissociation curve of ND6 gene shows the peaks of different samples of different patients coinciding at the same Ct value. The different heights of the peaks indicate the difference in expression level of the said gene in different samples.

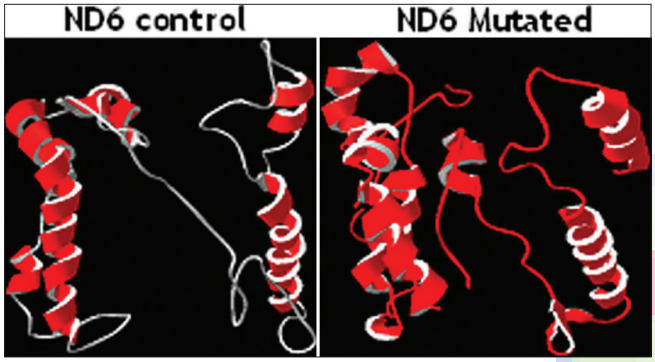

Figure 6: The final 3D structure of control and mutants of COX2. The control contains 3alpha-helices and 11beta-sheets, case15 contains 2alpha-helices and 7beta-sheets,Vdf14 (IDC case 14) contains 2alpha-helices and 11beta-sheets and vdf10 (IDC case 10) contains 3alpha-helices and 5beta- sheets. The alpha helices are represented in red and beta-sheets in yellow ribbons

essential for the energy synthesis.

We studied a total of 180 sporadic cases of malignant and benign breast cancers and benign proliferating hemangiomas. We report the identification of 29 somatic mutations, 10 in the ND6 region of a single case of infiltrating ductal carcinoma and 1 mutation in the region T 10669 C of the ND4L gene of mitochondria in three individual cases of infiltrating ductal carcinomas and 18 mutations in the COX-2 region of nine cases of infiltrating ductal carcinoma.

This is the first study in breast cancer to report mutations in the ND6 and ND4L regions of mitochondria. Mutations in these regions have been reported in various other malignancies, indicating that these regions could be mutational hotspots, though their functional relevance is still to be understood. The mutation identified by us in the ND4L region is a novel silent mutation and hence, may not be contributing to tumor promotion. The mutations in the ND6 region lead to the formation of a truncated protein which may contribute to altered efficiency of the OXPHOS system.

Our data supports the previous studies that all the mutations identified in this study were seen only in the malignant cases. We hypothesize that this could be due to some factors of malignant condition like excess of reactive oxygen species(ROS) generation. No mutations were found in cases of either benign breast tumors or in the involuted or proliferating Hemangiomas, indicating that mutations in the mitochondria may arise after the pro-proliferative pathway has set in.

The four studies taken up till date reported maximum number of mutations in the D-loop region and 27 mutations in the other regions spanning the mitochondria in malignant cases.[4] All the techniques used for identifying mutations in the mitochondrial DNA concentrate on the whole genome analysis using either direct sequencing or TGGE or SSCP and restriction-enzyme analyses.[2,5-7,25] The earlier studies are limited by the sample sizes and the diversity of human neoplasms studied. Also, screening the whole genome for mutation identification could be a laborious and expensive process, which may not be useful for routine diagnostic purposes and the D-loop being highly polymorphic, may result in ambiguity during interpretation. Here we tried to ascertain the importance of the mitochondria encoded NADH and ATPase 8 genes, the key regulators of the OXPHOS pathway, in promoting neoplasm and their relevance in being used as markers for predicting malignancy.

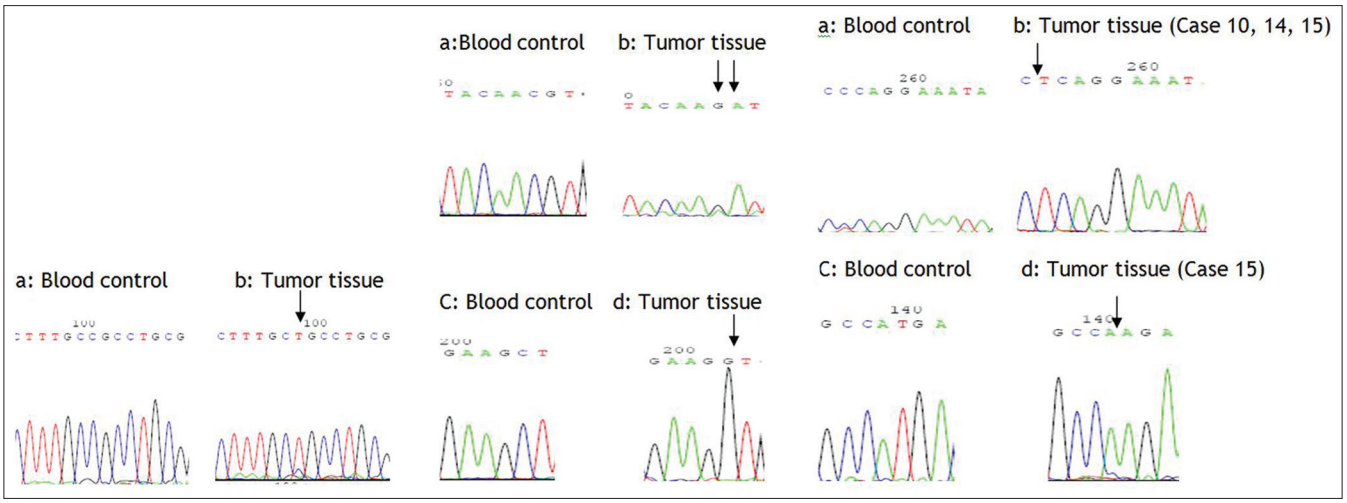

Figure 7: ND6 structure. The control contains 8alpha-helices and mutant contains 9alpha-helices and 2beta-sheets(C) Sequence analysis of COX-2 gene. The altered sequences between cases (b,d) and their matched controls(a,c) is indicated by arrows. Three malignant breast tissues (Case 10, 14 and 15) lodged the mutation at the same region in COX-2. In addition case 15 showed a mutation at a different site too

Our findings do not indicate any significant correlation of mitochondrial OXPHOS gene mutations in malignancies. The use of this study as a predictor for malignancy is limited because of the low frequency of the mutation occurring in the INDIAN population. It is still to be established if i) the mutations identified in the mtDNA directly contribute to a pro-proliferative pathway and to malignancy or ii) the malignancy could be a result of malfunctioning of the nuclear genes and the mutations in the mitochondria enhance the apoptotic process, but are not very effective, as the critical number of mutated mtDNA's required for the malfunctioning of the OXPHOS may not be present.

The expression analysis data revealed a down regulation of the Mt OXPHOS genes in malignant cases when COMPARED to their adjacent tissues, though in certain cases the decrease in expression might have been relatively low. The same observation confirmed by protein modeling studies. We hypothesize that reduced expression of the OXPHOS genes leads to an increased oxygen tension in tissues, contributing to higher ROS generation which in turn leads to damage of the vital components of the cell, especially DNA, thus countering malignancy.

Conclusion

The overall result of the study is that mitochondrial impairment is a feature of malignant cells and results in compromised OXPHOS. Based on the observations of the study, we propose that if mutations are required for tumor initiation, they would be more or less consistent at a particular position in at least few of the various tumors screened. But the literature shows a wide variation in the mutations identified.

Therefore, in conclusion, we hypothesize that the mutations and reduced expression of the Mt OXPHOS genes seen in malignant cases sets in after the pro-proliferative pathway has been established in the cell and this compromised pathway could be a responsive mechanism of the cells to counter the effects of cancers rather than a mechanism of tumorigenesis.

Acknowledgements

KPR sincerely acknowledges Prof Judd Aiken, Department of Animal health nd Bio-medical Sciences, University of Wisconsin, Madison, for the initial insights into mitochondrial research. RC was a recipient of the LadyTATAmemorialTrust Researchfellowship. RC is a Guest Faculty, University of Hyderabad, Andhra Pradesh, India. The partial financial assistance is received from University grants commission, New Delhi, India. Sanction no: F.3-40/2004(SR). RC is greatful to Dr. L.S. Shashidhara, CCMB for all the support he extended. RC also thanks her family for all the help and support.

References

1. Suomalainen A, Majander A, Haltia M, Somer H, Lönnqvist J, Savontaus ML, *et al*. Multiple deletions of mitochondrial DNA in several tissues of a patient with severe retarded depression and familial progressive external opthalmoplegia. J Clin Invest 1992;90:61-6.
2. Delsite R, Kachhap S, Anbazhagan R, Gabrielson E, Singh KK. Nuclear genes involved in mitochondria-to-nucleus communication in breast cancer cells. Mol Cancer 2002;1:6.
3. Rudin CM, Thompson CB. Apoptosis and disease: Regulation and clinical relevance of programmed cell death. Annu Rev Med 1997;48:267-81.
4. Carew JS, Huang P. Mitochondrial defects in cancer. Mol Cancer 2002;1:9.
5. 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;26:145-52.
6. Tan DJ, Bai RK, Wong LJ. Comprehensive scanning of somatic mitochondrial DNA mutations in breast cancer. Cancer Res

2002;62:972-6.
7. Parrella P, Xiao Y, Fliss M, Sanchez-Cespedes M, Mazzarelli P, Rinaldi M,, *et al*. Detection of mitochondrial DNA mutations in primary breast cancer and fine-needle aspirates. Cancer Res 2001;61:7623-6.
8. Hasan Q, Tan ST, Gush J, Davis PF. Altered mitochondrial cytochrome b gene expression during the regression of hemangioma. Plast Reconst Surg 2001;108:1471-6.
9. Hasan Q, Tan ST, Xu B, Davis PF. Effects of five commonly used glucocorticoids on hemangioma in vitro. Clin Exp Pharmacol Physiol 2003;30:140-4.
10. Lahiri DK, Nurnberger JI Jr. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. Nucleic Acids Res 1991;19:5444.
11. Kirches E, Krause G, Warich-Kirches M, Weis S, Schneider T, Meyer-Puttlitz B, *et al*. High frequency of mitochondrial DNA mutations in glioblastoma multiforme identified by direct sequence comparision to blood samples. Int J cancer 2001;93:534-8.
12. Ling XL, Fang DC, Wang RQ, Yang SM, Fang L. Mitochondrial microsatellite instability in gastric cancer and its precancerous lesions. World J Gastroenterol 2004;10:800-3.
13. Kirkali G, Gezer S, Sercan ZY. Mitochondrial Cytochrome Oxidase activity in Renal Cell Carcinoma. Turk J Canc 2000;30:24-30.
14. Orita M, Iwahana H, Kanazawa H, Hayashi K, Sekiya T. Detection of polymorphisms of human DNA by gel electrophoresis as single stranded conformation polymorphisms. Proc Natl Acad Sci U S A 1989;86:2766-70.
15. Livak KJ, Schmittgen TD.. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T))Method. Methods 2001;25:402-8.
16. Hofmann K, Stoffel W. TMBASE-a database of membrane spanning protein segments. Biol Chem Hoppe Seyler 1993;374:166 ff.
17. Needleman SB, Wunsch CD. A general method applicable to the search for similarities in the amino acid sequence of two proteins. J Mol Biol 1970;48:443-53.
18. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalities and weight matrix choice. Nucleic Acids Res 1994;22:4673-80.
19. Sali A, Blundell TL. Comparitive protein modeling by satisfaction of spatial restraints. J Mol Biol 1993;234:779-815.
20. MacKerell AD Jr, Bashford D, Bellott M, Dunbrack RL Jr, Evanseck J. Self-consistent parameterization of biomolecules for molecular modeling and condensed phase simulations. FASEB J 1992;6:A143.
21. Kale L, Skeel R, Bhandarkar M, Brunner R, Gursoy A, Krawetz N, *et al*. K: NAMD2: Greater scalability for parallel molecular dynamics. J Comput Phys 1999;151:283.
22. Schlenkrich M, Brickmann J, MacKerell AD Jr, Karplus M. Emperical potential energy function for phospholipids: Criteria for parameter optimization and applications. In: Merz KM, Roux B, editors (Birkhauser), Biological membranes. Boston, MA: 1996. p. 31-81.
23. Guex N, Peitsch MC. SWISS-MODEL and Swiss-pdb Viewer: An environment for comparative protein modeling. Electrophoresis 1997;18:2714-23.
24. Ozawa T. Genetic and functional changes in mitochondria associated with aging. Physiol Rev 1997;77:425-64.
25. Tseng LM, Yin PH, Chi CW, Hsu CY, Wu CW, Lee LM, *et al*. Mitochondrial DNA mutations and mitochondrial DNA depletion in breast cancer. Genes Chromosomes and Cancer 2006;45:629-38.

How to site this article: Chintha R, Kaipa PR, Sekhar N, Hasan Q. Mitochondria and tumors: A new perspective. Indian J Cancer 2013;50:206-13. **Source of Support:** Nil. **Conflict of Interest:** Authors declare no conflict of interest.

News

8th SAARC Federation of Oncology (SFO) Conference
13th to 15th December 2013
Kathmandu, Nepal

Abstract submission deadline is October 30th 2013.

For further details please:

visit: www.sfon.org.np

Contact: saghimire@hotmail.com

Dr. Sarita Ghimire

General Secretary, Conference organising committee