Published in final edited form as:

J Cancer Res Clin Oncol. 2009 April ; 135(4): 649–651. doi:10.1007/s00432-008-0542-8.

Mitochondrial DNA mutations in the D-loop region may not be frequent in cervical cancer: a discussion on pitfalls in mitochondrial DNA studies

Hezhi Fang,

Zhejiang Provincial Key Laboratory of Medical Genetics, School of Laboratory Medicine, School of Life Science, Wenzhou Medical College, 325035 Wenzhou, China

Jianxin Lu,

Zhejiang Provincial Key Laboratory of Medical Genetics, School of Laboratory Medicine, School of Life Science, Wenzhou Medical College, 325035 Wenzhou, China

Jia Wei,

Zhejiang Provincial Key Laboratory of Medical Genetics, School of Laboratory Medicine, School of Life Science, Wenzhou Medical College, 325035 Wenzhou, China

Li-Jun Shen,

Zhejiang Provincial Key Laboratory of Medical Genetics, School of Laboratory Medicine, School of Life Science, Wenzhou Medical College, 325035 Wenzhou, China

Zhinan Ding,

Zhejiang Provincial Key Laboratory of Medical Genetics, School of Laboratory Medicine, School of Life Science, Wenzhou Medical College, 325035 Wenzhou, China

Hongzhi Li, and

Zhejiang Provincial Key Laboratory of Medical Genetics, School of Laboratory Medicine, School of Life Science, Wenzhou Medical College, 325035 Wenzhou, China

Department of Cellular and Structural Biology, University of Texas Health Science Center at San Antonio, San Antonio, TX 78229, USA

Yidong Bai

Zhejiang Provincial Key Laboratory of Medical Genetics, School of Laboratory Medicine, School of Life Science, Wenzhou Medical College, 325035 Wenzhou, China

Department of Cellular and Structural Biology, University of Texas Health Science Center at San Antonio, San Antonio, TX 78229, USA

Dear Sir,

We read with great interest the recent article by Chen and Zhan (2009). Role of mutations in mitochondrial genomes in cancer has attracted a lot of attentions in recent years. While

jxlu313@163.com, baiy@uthscsa.edu.

Conflict of interest statement None declared.

mtDNA mutations have been detected in most if not all the tumor or cancer cells investigated, there also emerges serious challenges to the procedures and interpretations in those studies (Bandelt et al. 2005; Salas et al. 2005). In this context, we feel it is both important and necessary to discuss this study and draw some alertness in the field as how to avoid experimental pitfalls and false conclusions. We will focus our discussions on four aspects:

1. Materials

In this study (Chen and Zhan 2009), samples were obtained from patients with cervical cancer, and DNA was then extracted from both cancer tissue (CT) and nearby normal tissue (NT). However, apparently the tissue they used for DNA extraction was not the one they used for pathological diagnosis. From our own experiences, the percentages of cancer cells are different in different fractions of CT. We would suggest that DNA used for sequencing analysis should be extracted from the material on slides used for pathological diagnosis with microdissection technology (Eltoum et al. 2002).

2. Polymerase chain reaction (PCR)

Both positive and negative controls should be included in PCR, especially in cases where nested PCR strategy is utilized to improve yields with low amount of materials. Some other standard precautions are listed in Kraytsberg's article on avoiding contamination in PCR (Kraytsberg and Khrapko 2005). Additional efforts should be taken to avoid amplification of mitochondrial pseudogenes in nuclear genome by inclusion of a mtDNA-less rho zero cell as a control (Yao et al. 2008).

3. Sequence electropherograms

It is quite obvious to us that the point mutation G16535A was a reading error after careful examination of the sequence electropherogram. Apparently, a heteroplasmic mutation caused the shift of part of sequence electropherogram. Since error was detected in one of only two figures provided, we are very concerned about the quality of sequencing data which was the foundation of this paper.

4. Data analysis

According to the authors, variants were compared with MITOMAP, and then mutations and novel polymorphisms were listed in Tables 1 and 2, respectively. We reanalyzed 13 new polymorphic sites reported in Table 2 of their paper. Contradicting to their claim that 13 new polymorphism changes had not been recorded in MITOMAP, as shown in Table 1 here, we found that 6 (as indicated with # in column 2) of 13 polymorphic sites were already listed in MITOMAP and had been reported before the year of 2008. Moreover, for the rest 7 sites, 5 of them (with the correct sequences indicated in the brackets in column 2) matched with wrong positions at the standard Cambridge reference sequence (CRS). As a result, no polymorphisms were found with sample 7 at 12 and 16,085 positions, and polymorphisms at position 298 and 16,233 were also reported previously (Table 1, column 2). We then did additional database search and found that among the rest 3 “new polymorphisms”, only

G16110T passed the test (Table 1, column 3). As MITOMAP is a only limited database that targeted to medical genetics, for polymorphic sites survey, other mtDNA databases such as mtDB (<http://www.genpat.uu.se/mtDB>), mtDNA (<http://www.ianlogan.co.uk/mtdna.htm>), FBI mtDNA population database (<http://www.fbi.gov/hq/lab/fsc/backissu/april2002/miller1.htm>) or GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>) should also be included. A straightforward Google or Yahoo search can help to define the “novelty” of mtDNA polymorphisms (Bandelt et al. 2006, 2008a, b). The other useful advise is to identify potential pathogenic mutations with phylogenetic methods (Bandelt et al. 2001, 2006; Salas et al. 2005; Yao et al. 2007).

We also briefly checked some “mutations” claimed in their Table 1, and found several possible errors. For examples, position 171 bears a G in CRS, not a mutation as suggested by the authors; 16,303 in samples 1, 3 and 9 should be a base shift for 16,304, as 16,303 has G in CRS; 424 has T in CRS, again not a mutation suggested by the authors; 16,518 in samples 3 and 9 should be a base shift for 16,519. Obviously, the author could have avoided such mistakes by simply using CRS as the reference.

Besides these major problems, some of statements in this paper were also misleading. For examples, “Mutation in D-loop zone is more serious, which will result in disorder of function of whole mitochondrial” is simply not correct; “mtDNA molecule is free in mitochondrial substrate (matrix?)” is also confusing and wrong.

As we discussed, Chen and Zhan's study suffered multiple flaws in experimental design, examination and interpretation of the data. We noticed similar errors were also presented in other publications. On this aspect, we agreed with Salas et al. (2005) that the mtDNA data quality in many cancer studies is quite poor. While we do believe mitochondrial dysfunction and mtDNA mutations could play an important role in tumorigenesis, a call for more careful reassessment of the approach and the interpretation of the raw data seems necessary.

References

- Abu-Amro KK, Gonzalez AM, Larruga JM, Bosley TM, Cabrera VM. Eurasian and African mitochondrial DNA in Xuences in the Saudi Arabian population. *BMC Evol Biol.* 2007; 7:32. [PubMed: 17331239]
- Bandelt HJ, Kong QP, Parson W, Salas A. More evidence for non-maternal inheritance of mitochondrial DNA? *J Med Genet.* 2005; 42:957–960. [PubMed: 15923271]
- Bandelt HJ, Lahermo P, Richards M, Macaulay V. Detecting errors in mtDNA data by phylogenetic analysis. *Int J Legal Med.* 2001; 115:64–69. [PubMed: 11724431]
- Bandelt HJ, Salas A, Bravi CM. What is a ‘novel’ mtDNA mutation—and does ‘novelty’ really matter? *J Hum Genet.* 2006; 51:1073–1082. [PubMed: 17021933]
- Bandelt HJ, Salas A, Taylor RW, Yao YG. Exaggerated status of “novel” and “pathogenic” mtDNA sequence variants due to inadequate database searches. *Hum Mutat.* 2008a in press.
- Bandelt HJ, Yao YG, Salas A. The search of ‘novel’ mtDNA mutations in hypertrophic cardiomyopathy: MITOMAPPING as a risk factor. *Int J Cardiol.* 2008b; 126:439–442. [PubMed: 17482693]
- Brandstatter A, Peterson CT, Irwin JA, Mpoke S, Koech DK, Parson W, Parsons TJ. Mitochondrial DNA control region sequences from Nairobi (Kenya): inferring phylogenetic parameters for the establishment of a forensic database. *Int J Legal Med.* 2004; 118:294–306. [PubMed: 15248073]

- Chen D, Zhan H. Study on the mutations in the D-loop region of mitochondrial DNA in cervical carcinoma. *J Cancer Res Clin Oncol*. 2009; 135:291–295. [PubMed: 18584207]
- Eltoum IA, Siegal GP, Frost AR. Microdissection of histologic sections: past, present, and future. *Adv Anat Pathol*. 2002; 9:316–322. [PubMed: 12195221]
- Kong QP, Yao YG, Sun C, Bandelt HJ, Zhu CL, Zhang YP. Phylogeny of east Asian mitochondrial DNA lineages inferred from complete sequences. *Am J Hum Genet*. 2003; 73:671–676. [PubMed: 12870132]
- Kraytsberg Y, Khrapko K. Single-molecule PCR: an artifact-free PCR approach for the analysis of somatic mutations. *Expert Rev Mol Diagn*. 2005; 5:809–815. [PubMed: 16149882]
- Liu VW, Shi HH, Cheung AN, Chiu PM, Leung TW, Nagley P, Wong LC, Ngan HY. High incidence of somatic mitochondrial DNA mutations in human ovarian carcinomas. *Cancer Res*. 2001; 61:5998–6001. [PubMed: 11507041]
- Metspalu M, Kivisild T, Metspalu E, Parik J, Hudjashov G, Kaldma K, Serk P, Karmin M, Behar DM, Gilbert MT, et al. Most of the extant mtDNA boundaries in south and southwest Asia were likely shaped during the initial settlement of Eurasia by anatomically modern humans. *BMC Genet*. 2004; 5:26. [PubMed: 15339343]
- Salas A, Yao YG, Macaulay V, Vega A, Carracedo A, Bandelt HJ. A critical reassessment of the role of mitochondria in tumorigenesis. *PLoS Med*. 2005; 2:e296. [PubMed: 16187796]
- Yao YG, Bandelt HJ, Young NS. External contamination in single cell mtDNA analysis. *PLoS ONE*. 2007; 2:e681. [PubMed: 17668059]
- Yao YG, Kong QP, Salas A, Bandelt HJ. Pseudomitochondrial genome haunts disease studies. *J Med Genet*. 2008; 45:769–772. [PubMed: 18611982]

Table 1

Reanalysis of “novel polymorphism”

Sample	“New polymorphism” ^a	Literature ^b	Haplogroup ^c
2	T146A	Kong et al. (2003) *	M7
3	A16553G	Metspalu et al. (2004)	R2
7	C(T)12T		H2
7	T(C)16085C		H2
12	A183G [#]	Kong et al. (2003) *	R9b, N9a
15	A244G [#]	Brandstatter et al. (2004) *	L4b2a1, L4b2a2, L3d, L3g
17	G53A [#]	Liu et al. (2001)	?
18	T16157C [#]	Kong et al. (2003) *	P2b, G
18	G16470A [#]	Kong et al. (2003) *	M8
19	T(A)16233C	Abu-Amero et al. (2007)	?
21	C431T [#]	Kong et al. (2003) *	D
22	G(C)298T	Liu et al. (2001) *	?
23	C(G)16110T	—	—

?indicates that haplogroup status could not be determined based on the given information

^aNew polymorphisms as claimed in Table 2[#]indicates the polymorphic site was already reported in MITOMAP; in brackets we indicate the correct sequence according to MITOMAP^bReferences where polymorphic sites were reported previously

* indicates more than one reference was found and only one was presented

^cIn which haplogroup that polymorphic sites have been reported