The prevalence of mtDNA^{4977} deletion in primary human endometrial carcinomas and matched control samples

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Abstract. mtDNA^{4977}, the most common deletion of the mitochondrial DNA, has been detected in different types of human neoplasia. The aim of the current study was to determine the prevalence of mtDNA^{4977} deletion in primary human endometrial carcinomas (EC) as compared with matched control samples. Thirty-seven matched control tissues and EC samples were enrolled, and the 4977-bp mtDNA deletion was investigated using a PCR-based technique. Deletion of mtDNA^{4977} was detected in 32 out of 37 (84%) matched control samples and in 30 out of 37 (81%) ECs. A statistically significant correlation of mtDNA^{4977}/wild-type mtDNA ratios was noted between normal and cancerous human endometrial tissues (R=0.844, p<0.0001). The intensity ratio of mtDNA bands was significantly higher in normal samples than in malignant human endometrial tissues (p=0.021). The mean mtDNA^{4977}/wild-type mtDNA ratio was significantly higher in the control group (0.655±0.379) than in the cancer group (0.570±0.04, p=0.048) of patients between 50 and 60 years of age. Notably, there was a relationship between clinical stage of the disease (stage II versus III) and the amount of mtDNA^{4977} in ECs (p=0.048). The sequence analysis of two randomly selected EC-positive cases confirmed that amplified fragments originated from mtDNA, and both encompassed deletion. In conclusion, we suggest that mitochondrial 4977-bp deletion is not specific to EC tissues. The accumulation of mtDNA^{4977} may be associated with aging processes, particularly in perimenopausal women affected by EC.

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Abbreviations: EC, endometrial cancer; FIGO, International Federation of Gynecology and Obstetrics; HNPCC, hereditary non-polyposis colorectal cancer syndrome; mtDNA, mitochondrial DNA; MI, microsatellite instability; OXPHOS, oxidative phosphorylation; SD, standard deviation

Key words: deletion, mitochondrial DNA, endometrial cancer

Introduction

Mitochondria are eukaryotic organelles involved in many intracellular metabolic pathways in which bio-fuels, such as fatty acids and carbohydrates, are converted into cellular ATP through the process of oxidative phosphorylation (OXPHOS) (1). The mitochondrion, in addition to the nucleus, possesses its own genome and has genetic machinery capable of producing proteins involved in bioenergetic processes (2). The mtDNA is a compactly organized, double-stranded, closed molecule of a full-length sequence weighing 16.5 kb (2). Human mtDNA encodes 13 polypeptides, all of which are the components of the respiratory chain/OXPHOS system, 2 ribosomal RNAs and 22 transfer RNAs (2,3). Moreover, human mtDNA contains a non-coding region, the D-loop (the displacement loop), which is implicated in the control of replication and transcription (4).

Since the 1930s, mitochondria have been implicated in the process of human tumorigenesis. Otto Warburg suggested that 'respiration damage' may play a pivotal role in cancer (5). Today, mitochondrial dysfunction is one of the most prominent features of cancer, as many reports have revealed a significant relationship between this phenomenon and the development and spread of human neoplasia (4,6,7). Genetic instability, point mutations and abnormal mtDNA expression have been detected in various types of human malignancies, including EC (8-14). For example, Liu and co-workers (8) sequenced the fragment of the mtDNA, encompassing the D-loop, 12S and 16S rRNA genes in 50 endometrial cancers and matched normal controls. Of the tumors, >50% carried one or more somatic changes in mtDNA and MI, in CCCCC TCCCC sequences, was found in the hyper-variable regions I and II of the D-loop and 12S rRNA (8). The frequency of the 16189T>C mitochondrial DNA variant was significantly higher in EC patients (43.1%) versus the control group (21.5%, p=0.009) (9). Somatic D-loop mtDNA mutations were frequently (63%) reported in type II EC-UPSC (uterine papillary serous carcinoma) (10). In another study, a total of 13 D310 mtDNA mutations were found in 56 (23%) primary malignant tumors, including one (1-bp insertion) out of six ECs (11). Notably, the haplogroup D and 5178A mitochondrial polymorphism may be significantly associated with EC risk of women from the Yunnan province in China (12). It is worth pointing out the recently published data of Wang

and co-investigators (14). They suggested that accumulation of mtDNA alterations in primary human endometrial adenocarcinomas occur randomly and independently in individual cells or in a small sub-group of tumor cells. However, to the best of our knowledge (Medline© database), the prevalence of mtDNA[4977] deletion in cancerous human endometrial tissues has yet to be investigated.

This study investigated the incidence of mtDNA[4977] deletion in specimens collected from women affected by ECs and compared the results with the prevalence of this mitochondrial DNA alteration in matched normal control tissues.

Materials and methods

Patients. Forty specimens of primary human ECs and their matched normal control tissues (ovary, omentum or uterine cervix) were collected. The patients had undergone surgery at the Second Department of Gynecology, Lublin Medical University, Lublin, Poland because of EC between 2000-2006. Three patients were excluded due to insufficient data. Altogether, 37 women were enrolled. All patients gave informed consent before the specimens were collected according to institutional guidelines. The tumors were considered sporadic because none of the patients met the Amsterdam criteria for HNPCC syndrome. Neither initial chemotherapy nor hormonal or radiation therapy was performed prior to surgery. The standard surgical procedure in our Department was total abdominal hysterectomy with bilateral salpingo-oophorectomy. Lymph nodes were removed when the tissue collected at the dilatation and curettage procedure was found to be non-endometrioid, G3 (poorly-differentiated) endometrioid-type EC, or cancer infiltrating for more than a half of the myometrial wall at surgery. Clinical and pathological features of EC patients are summarized in Table I. The clinical stage of the disease was classified based on the FIGO staging system (15), whereas pathological assessment was performed at the Department of Pathology, Lublin Medical University, based on the World Health Organization classification (16).

Table I. Clinical and pathological features of EC patients.

Features	No. of cases (%)
Patient age (years)	
<50	7 (19)
50-60	17 (46)
>60	13 (35)
FIGO stage	
I	25 (67)
II	8 (22)
III	4 (11)
Histological grade	
Endometrioid	35 (94)
Non-endometrioid	2 (6)
Myometrial invasion	
None	3 (8)
≤	13 (35)
>	21 (57)
Lymph nodes metastases	
Present	2 (6)
Absent	24 (64)
Not evaluated	11 (30)
Coexistence of hyperplastic and neoplastic endometrium	
Yes	10 (27)
No	27 (73)
Metastases to the ovary/ovaries	
Positive	4 (11)
Negative	33 (89)
Infiltration of the neoplasm in the uterine cervix	
Present	11 (30)
Absent	26 (70)
Total	37 (100)

PCR analysis. At surgery, the material collected was immediately snap-frozen in liquid nitrogen and stored at -80˚C. DNA was isolated based on a standard technique using SDS-proteinase K, RNase digestion, phenol-chloroform extraction and ethanol precipitation (17). The two sets of PCR primers applied in the experiments, WT1 (5'-AATCAATTGGCGACCAATGG-3'; 7878-7897 bp) and WT2 (5'-CGCCTGGTTCTAGGAATAATGG-3'; 7979-7958 bp), amplified the D-loop segment of the mitochondrial genome (18). The second set of primers, L4 (5'-GCCCGT ATTTACCCTATAGC-3'; 8251-8270 bp) and H3 (5'-GTCT AGGGCTGTTAGAAGTC-3'; 13845-13826 bp), was used to evaluate the existence of the 'common' 4977-bp deletion of mtDNA (19). The 5594-bp mtDNA fragment was flanked by the L4 and H3 set of primers, whereas despite deletion, the product had a size of 617-bp. PCR amplification was carried out by applying the Advantage 2 PCR Kit (BD Biosciences, Palo Alto, CA) in a 50 μl reaction volume with 100 ng DNA template, 1 μl of Advantage 2 Polymerase Mix, 1 μl dNTP and 1 μl (50 pmol/μl) of each primer. After denaturation at 95˚C for 5 min, the reaction mixture was cycled 30 times at the conditions described below: 95˚C for 45 sec, 60˚C for 45 sec and 72˚C for 1 min, finally extended at 72˚C for 5 min. The PCR-products were purified and separated on a 1.2% agarose gel at 50 V in 1X TBE buffer, and the products were visualized by ethidium bromide staining and photographed. DNA bands were analyzed densitometrically using Kodak D1 3.6 software, and the intensity of the mtDNA bands was measured. For each positive sample, the percentage of deleted mtDNA[4977], with respect to wild-type mtDNA, was determined by the ratio between the deleted and wild-type mtDNA band densities.

To confirm the presence of mtDNA[4977] in the cancerous human endometria, we randomly selected two positive cases (numbers 164 and 238). PCR-products were cut from agarose

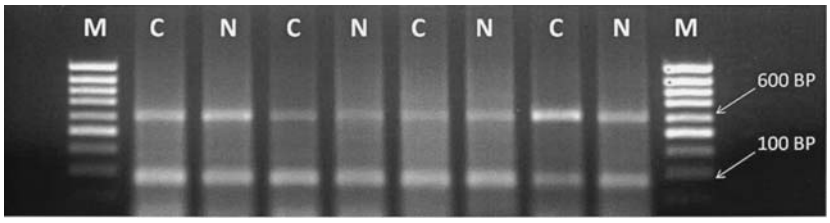

Figure 1. Detection results of 4977-bp deletion in the mtDNA of cancerous human endometrial specimens (C) and matched control samples (N). Four matched normal tissues and cancerous endometrial specimens showed mtDNA^{4977} deletion (numbers from left to right: 161/162, 164/165, 172/173 and 182/183). M, Mass Ruler™ (M) and ladder of low range (MBI Fermentas, Lithuania).

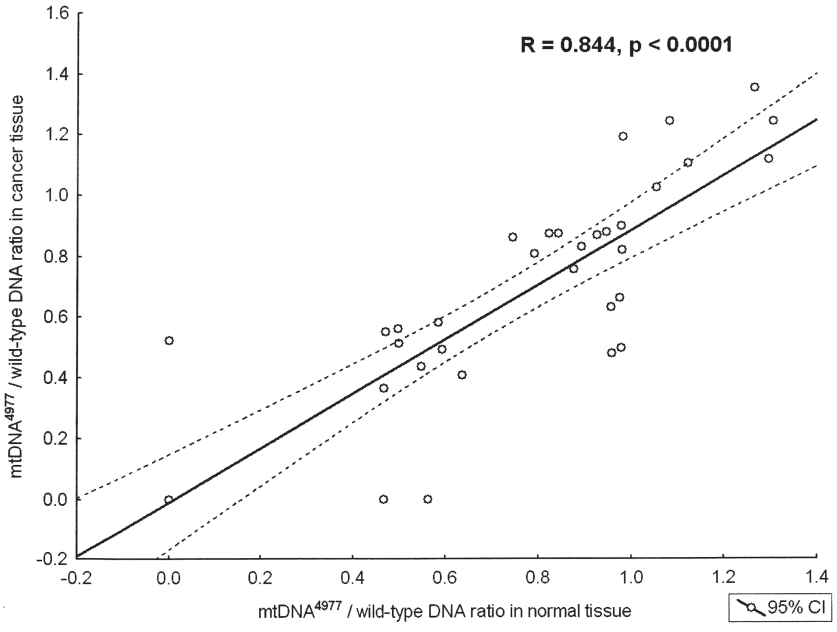

Figure 2. mtDNA^{4977} /wild-type mtDNA ratios in matched control verus endometrial cancer tissue calculated by Spearman's rank correlation test.

Table II. Mean, median and SD mtDNA^{4977}/wild-type mtDNA ratios in matched control and cancerous endometrial samples.

Samples	mtDNA^{4977}/wild-type mtDNA ratio		
	Mean (range)	Median	SD
Control	0.718 (0.0-1.306)	0.374	0.062
Cancerous endometrial	0.633 (0.0-1.349)	0.398	0.066

gels and processed for direct DNA sequencing at the DNA Sequencing and Oligonucleotide Synthesis Laboratory, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland. Sequences were compared using the BioEdit Sequence Alignment Editor v. 7.0.5.5 (20).

Statistical analysis. Statistical analyses were performed using the SPSS software package v. 14.0 PL for Windows. The groups were compared by the Mann-Whitney U test or Wilcoxon test when appropriate. mtDNA^{4977}/wild-type mtDNA ratios in matched control and cancerous human endometrial samples were correlated using the Spearman rank correlation test. P<0.05 was considered significant.

Results

The present study investigated 37 neoplastic endometrial and matched normal control samples for the prevalence of the mtDNA^{4977} deletion using the PCR-based methodology. The mtDNA^{4977} deletion was detected in 32 out of 37 (84%) matched control samples and in 30 out of 37 (81%) neoplastic endometrial tissues (Fig. 1). We noted a statistically significant correlation of mtDNA^{4977}/wild-type mtDNA ratios between normal control and cancerous endometrial samples (R=0.844, p<0.0001; Fig. 2). Table II summarizes the mean, median and SD for mtDNA^{4977}/wild-type mtDNA ratios. It is worth pointing out that the mean mtDNA^{4977}/wild-type mtDNA ratios was significantly higher in matched controls than in EC samples (p=0.021, Table II).

No correlation was reported between mtDNA^{4977}/wild-type mtDNA ratio and patient age (R=0.09, p=0.71, Spearman

Table III. Mean ± SD mtDNA[4977]/wild-type mtDNA ratio in control samples and cancerous endometrial tissues.

	N	Mean mtDNA[4977]/wild-type mtDNA ratio Mean ± SD		P[a]
		Control samples	Cancerous samples	
Patient age (years)				
50-60	17	0.655±0.379	0.570±0.404	0.048
FIGO stage				
II + III	12	0.938±0.159	0.768±0.251	0.012
Myometrial invasion				
<	13	0.788±0.389	0.705±0.367	0.033
Lymph node metastases				
Absent	24	0.769±0.369	0.629±0. 439	0.009
Infiltration of the neoplasm in the uterine cervix				
Present	11	0.934±0.166	0.793±0.248	0.021
Total	37	0.718±0.062	0.633±0.066	0.021

P[a], Wilcoxon test.

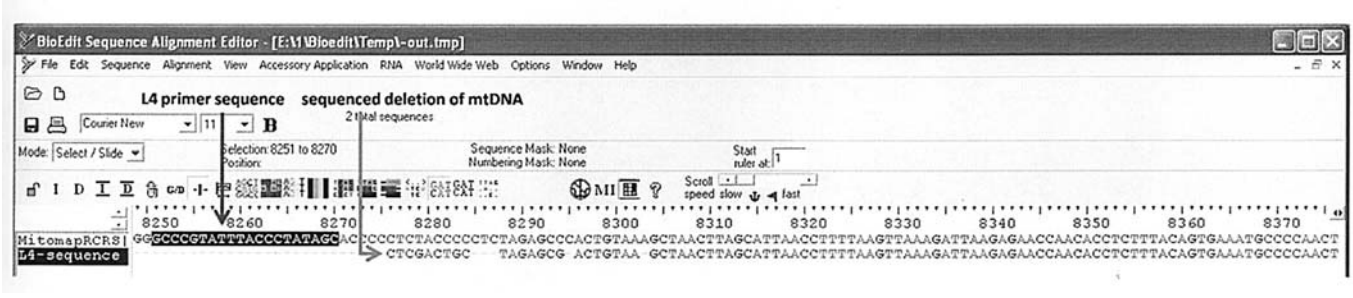

Figure 3. Sequence analysis of a 617-bp mtDNA fragment in EC sample number 238. Comparison with MITOMAP™ human mtDNA Cambridge sequence data (www.mitomap.org).

rank correlation test). Notably, there was a significant relationship between clinical stage of the disease (stage II versus stage III due to FIGO) and the amount of mtDNA[4977] in neoplastic endometria (p=0.048, Mann-Whitney U test). Table III summarizes data on the association between clinico-prognostic features of EC patients and the mean mtDNA[4977]/wild-type mtDNA ratios in normal and cancerous samples. There were significant differences in the mean mtDNA[4977]/wild-type mtDNA ratios in the groups of women who were 50-60 years old, with advanced clinical stage of the disease, cancer infiltration less than half of the myometrial wall, lack of lymph node metastases and tumors infiltrating the uterine cervix (Table III).

We randomly sequenced a 617-bp mitochondrial DNA fragment flanked by the second round of PCR-primers in two EC-positive cases in order to confirm the presence of mtDNA[4977] deletion. The sequence analysis confirmed that the amplified fragments originated from mtDNA and encompassed mtDNA[4977] deletion (Fig. 3).

Discussion

Several studies have evaluated the existence of a number of mtDNA alterations, including point mutations, multiple gene deletions, and MI in different human cancerous tissues and cell lines (4,6,7). In general, most of the mtDNA mutations are heteroplasmic in nature, and the cells contain thousands of mutated or deleted mtDNA molecules that coexist with wild-type mitochondrial DNA (21-23). Notably, mutations of mtDNA have been reported to occur twice as much as described for nuclear DNA, and the defect phenotype associated with a particular mitochondrial alteration is generally expressed when the proportion of mutated mtDNA overcomes a threshold (23,24).

Apart from investigations of various molecular genetic alterations during endometrial tumorigenesis (25-27), our group places emphasis on the evaluation of the prevalence of mtDNA alterations in various human malignancies, including EC (28,29). Homoplasmic MELAS A3243G mtDNA mutation

was previously detected, for the first time, in a colon tumor sample, and the additional changes in mtDNA were reported in coding regions of human ECs (28). Although somatic mtDNA mutations were reported either in early- or advanced-stage ECs, there was no significant relationship between the prevalence of mitochondrial alterations and clinicoprognostical variables of cancer (29). Data from the current study suggest that mtDNA 4977-bp deletion appears not to be specific to endometrial cancer tissues, being detected either in EC or in the matched control samples. It is of vital importance to emphasize that there was a significant correlation of mtDNA^{4977}/wild-type mtDNA ratios between normal control tissues and cancerous human endometrial samples (Fig. 2).

The 4977-bp deletion is one of the most 'common' somatic mtDNA alterations, occurring between nucleotides 8,470 and 13,477 of the human mtDNA. The mtDNA^{4977} fragment encompasses genes involved in OXPHOS, including ATPase 6, ATPase 8, cytochrome oxidase III and NADH subunits (ND3, ND4, ND4L and ND5) (21). Although this deletion creates a mtDNA molecule that is smaller than the normal one, it may still replicate. It accumulates with age in post-mitotic cells, and prefers tissues with a high metabolic rate and a low proliferative index (30,31). This deletion first develops in the second and third decades of life in human muscles and liver tissues (32). It is worth pointing out that mtDNA^{4977} deletion is associated with sporadically developed human phenotypes, including Pearson syndrome, Kearns-Sayre syndrome and chronic progressive external ophtalmophlegia (1,33).

Making a comparison with adjacent tumor-free material, Dani and co-investigators (24) reported a lower amount of mtDNA^{4977} deletion in various human cancers (including breast, colorectal, gastric and head and neck tumors). Independently published reports dealing with sporadic breast (34), gastric (35) and oral (36) carcinomas revealed similar results. Although we did not find any statistically significant difference in the frequency of 'common' mtDNA^{4977} deletion between the tumoral and matched control samples studied herein, there are several explanations for this discrepancy. Firstly, the contamination of non-tumoral material (epithelial, vascular or connective tissues) in the cancerous samples collected at surgery may increase the amount of 4977-bp 'deleted' cells in neoplastic material. Moreover, the presence of mtDNA^{4977} deletion may be associated with metabolic and bioenergetic changes during the process of endometrial carcinogenesis. Therefore, the prevalence of this deletion in normal (proliferative and secretory endometria) and hyperplastic (with and without atypia) endometria should be evaluated and compared with the results presented above. These experiments are still in progress in our laboratory.

It is well-known that the frequency of 4977-bp deletion in human tissues increases with age and is associated with energy-demanding human tissues. Notably, the frequency of mtDNA^{4977} deletion in tissue samples of lung cancer patients >55 years of age was found to be significantly higher than in those <55 years of age (37). In the present study, most of the patients enrolled (30 out of 37, 81%) were >50 years of age. The mean mtDNA^{4977}/wild-type mtDNA ratio was significantly higher in the control group (0.655±0.379) than in the cancerous group (0.570±0.404, p=0.048, Table III) for women between 50 and 60 years of age (n=17). These data suggest that aging factors may play an important role in the accumulation of mtDNA deletions, particularly in peri-menopausal patients affected by EC.

It is worth citing the study conducted by Thayer *et al* (38), who identified this deletion in the leukocytes of all 71 individuals (ranging in age between 8 months to 99 years) of 21 maternal lines. All infants and children, who were free of any known mitochondrial disorders, displayed mtDNA^{4977} deletion. They also suggested the possibility of maternal transmission of 4977-bp mtDNA deletion in women without known mitochondrial disorders.

In conclusion, we suggest that mitochondrial 4977-bp deletion is not specific to EC tissues. The accumulation of mtDNA^{4977} is associated with aging processes, particularly in peri-menopausal women suffering from EC.

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References

1. Simon DK and Johns DR: Mitochondrial disorders: clinical and genetic features. Ann Rev Med 50: 111-127, 1999.
2. Anderson S, Bankier AT, Barrell BG, Bruijn MH, Coulson AR, Drouin J, Eperon IC, Nierlich DP, Roe BA, Sanger F, Schreier PH, Smith AJ, Staden R and Young IG: Sequence and organization of the human mitochondrial genome. Nature 290: 457-465, 1981.
3. Andrews RM, Kubacka I, Chinnery PF, Ligtowlers RN, Turnbull DM and Howell N: Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. Nat Genet 23: 147, 1999.
4. Czarnecka AM, Golik P and Bartnik E: Mitochondrial DNA mutations in human neoplasia. J Appl Genet 47: 67-78, 2006.
5. Warburg O: On the origin of cancer cells. Science 123: 309-314, 1956.
6. Copeland WC, Wachsman JT, Johnson FM and Penta JS: Mitochondrial DNA alterations in cancer. Cancer Invest 20: 557-569, 2002.
7. Chatterjee A, Mambo E and Sidransky D: Mitochondrial DNA mutations in human cancer. Oncogene 25: 4663-4674, 2006.
8. Liu VWS, Yang HJ, Wang Y, Tsang PCK, Cheung ANY, Chiu PM, Ng TY, Wong LC, Nagley P and Ngan HYS: High frequency of mitochondrial genome instability in human endometrial carcinomas. Br J Cancer 89: 697-701, 2003.
9. Liu VWS, Wang Y, Yang H-J, Tsang PCK, Ng T-Y, Wong L-C, Nagley P and Ngan HYS: Mitochondrial DNA variant 16189T>C is associated with susceptibility to endometrial cancer. Hum Mutat 22: 173-174, 2003.
10. Pejovic T, Ladner D, Intengan M, Zheng K, Fairchild T, Dillon D, Easley S, Dillon D, Marchetti D, Schwartz P, Lele S, Costa J and Odunsi K: Somatic D-loop mitochondrial DNA mutations are frequent in uterine serous carcinoma. Eur J Cancer 40: 2519-2524, 2004.
11. Parrella P, Seripa D, Matera MG, Rabitti C, Rinaldi M, Mazzarelli P, Gravina C, Gallucci M, Altomare V, Flammia G, Casalino B, Benedetti-Panici PL and Fazio VM: Mutations of the D310 mitochondrial mononucleotide repeat in primary tumors and cytological specimens. Cancer Lett 190: 73-77, 2003.
12. Xu L, Hu Y, Chen B, Tang W, Han X, Yu H and Xiao C: Mitochondrial polymorphisms as risk factors for endometrial cancer in southwest China. Int J Gynecol Cancer 16: 1661-1667, 2006.

13. Wang Y, Liu VW, Xue WC, Tsang PC, Cheung AN and Ngan HY: The increase of mitochondrial DNA content in endometrial adenocarcinoma cells: a quantitative study using laser-captured microdissected tissues. Gynecol Oncol 98: 104-110, 2005.
14. Wang Y, Xue WC, Liu VWS and Ngan HYS: Detection of mosaic pattern of mitochondrial DNA alterations in different populations of cells from the same endometrial tumor. Mitochondrion 7: 171-175, 2007.
15. Mikuta JJ: Preoperative evaluation and staging of endometrial cancer. Cancer 76: 2041-2043, 1995.
16. World Health Organisation Classification of Tumours, Pathology and Genetics of Tumours of the Breast and Female Genital Organs. Tavassoli FA and Devilee P (eds). IACR Press, Lyon, pp218-226, 2003.
17. Miturski R, Postawski K, Semczuk A, Bogusiewicz M, Baranowski W, Jakowicki JA and Keith G: Global DNA methylation in relation to hMLH1 and hMSH2 protein immunoreactivity in sporadic human endometrial carcinomas. Int J Mol Med 11: 569-574, 2003.
18. Shao J-Y, Gao H-Y, Li Y-H, Zhang Y, Lu Y-Y and Zeng Y-X: Quantitative detection of common deletion of mitochondrial DNA in hepatocellular carcinoma and hepatocellular nodular hyperplasia. World J Gastroenterol 10: 1560-1564, 2004.
19. Kao S-H, Chao H-T and Wei Y-H: Mitochondrial deoxyribonucleic acid 4977-bp deletion is associated with diminished fertility and motility of human sperm. Biol Reprod 52: 729-736, 1995.
20. Hall TA: BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl Acids Symp Ser 41: 95-98, 1999.
21. Porteous WK, James AM, Sheard PW, Porteous CM, Packer MA, Hyslop SJ, Melton JV, Pang C-Y, Wei Y-H and Murphy MP: Bioenergetic consequences of accumulating the common 4977-bp mitochondrial DNA deletion. Eur J Biochem 257: 192-201, 1998.
22. Shenkar R, Navidi W, Tavare S, Dang HM, Chomyn GA, Hardi GA, Cortopassi G and Arnheim N: The mutation rate of the human mtDNA^{4977} deletion. Am J Hum Genet 59: 772-780, 1996.
23. Dani SU, Dani MAC and Simpson AJG: The common mitochondrial DNA deletion ΔmtDNA^{4977}: shedding new light to the concept of a tumor suppressor mutation. Med Hypotheses 61: 60-63, 2003.
24. Dani MAC, Dani SU, Lima SPG, Martinez A, Rossi BM, Soares F, Zago MA and Simpson AJG: Less ΔmtDNA^{4977} than normal in various types of tumors suggests that cancer cells are essentially free of this mutation. Genet Mol Res 3: 395-409, 2004.
25. Semczuk A and Jakowicki JA: Alterations of pRb1-cyclin D1-cdk4/6-p16(INK4A) pathway in endometrial carcinogenesis. Cancer Lett 203: 1-12, 2004.
26. Semczuk A, Marzec B, Skomra D, Roessner A, Cybulski M, Rechberger T and Schneider-Stock R: Allelic loss at TP53 is not related to p53 protein overexpression in primary human endometrial carcinomas. Oncology 69: 317-325, 2005.
27. Jeczen R, Skomra D, Cybulski M, Schneider-Stock R, Szewczuk W, Reossner A, Rechberger T and Semczuk A: P53/MDM2 overexpression in metastatic endometrial cancer: correlation with clinicopathological features and patient outcome. Clin Exp Metastasis 24: 503-511, 2007.
28. Lorenc A, Bryk J, Golik P, Kupryjanczyk J, Ostrowski J, Pronicki M, Semczuk A, Szolkowska M and Bartnik E: Homoplasmic MELAS A3243G mtDNA mutation in a colon cancer sample. Mitochondrion 3: 119-124, 2003.
29. Semczuk A, Lorenc A, Putowski L, Futyma K, Bryk J, Miotla P and Bartnik E: Clinicoprognostical features of endometrial cancer patients with somatic mtDNA mutations. Oncol Rep 16: 1041-1045, 2006.
30. Cortopassi GA, Shibata D, Soong N-W and Arnheim N: A pattern of accumulation of a somatic deletion of mitochondria DNA in aging human tissues. Proc Natl Acad Sci USA 89: 7370-7374, 1992.
31. Bogliolo M, Izzotti A, Flora S, Carli C, Abbondandolo A and Degan P: Detection of the '4977-bp' mitochondrial DNA deletion in human atherosclerotic lesions. Mutagenesis 14: 77-82, 1999.
32. Lee HC, Pang CY, Hsu HS and Wei YH: Differential accumulation of 4,977 bp deletion in mitochondrial DNA of various tissues in human ageing. Biochim Biophys Acta 1226: 37-43, 1994.
33. Holt IJ, Harding AE, Cooper JM, Schapira AH, Toscano A, Clark JB and Morgan-Hughes JA: Mitochondrial myopathies: clinical and biochemical features of 30 patients with major deletions of muscle mitochondrial DNA. Ann Neurol 26: 699-708, 1989.
34. Ye C, Shu X-O, Wen W, Pierce L, Courtney R, Gao Y-T, Zheng W and Cai Q: Quantitative analysis of mitochondrial DNA 4977-bp deletion in sporadic breast cancer and benign breast diseases. Breast Cancer Res Treat 108: 427-434, 2008.
35. Kamalidehghan B, Houshmand M, Ismail P, Panahi MSS and Akbari MHH: ΔmtDNA^{4977} is more common in non-tumoral cells from gastric cancer samples. Arch Med Res 37: 730-735, 2006.
36. Shieh DB, Chou WP, Wei YH, Wong TY and Jin YT: Mitochondrial DNA 4977-bp deletion in paired oral cancer and precancerous lesions revealed by laser microdissection and real-time quantitative PCR. Ann NY Acad Sci 1011: 154-167, 2004.
37. Dai JG, Xiao YB, Min JX, Zhang GQ, Yao K and Zhou RJ: Mitochondrial DNA 4977 bp deletion mutations in lung carcinoma. Indian J Cancer 43: 20-25, 2006.
38. Thayer RE, Wittock R, Parr R, Zullo S and Birch-Machin MA: A maternal line study investigating the 4977-bp mitochondrial DNA deletion. Exp Gerontol 38: 567-571, 2003.