Carcinogenesis vol.34 no.11 pp.2521–2524, 2013
doi:10.1093/carcin/bgt230
Advance Access publication June 26, 2013

Association of mitochondrial DNA copy number in peripheral blood leukocytes with risk of esophageal adenocarcinoma

Enping Xu[1,2,†], Wenjie Sun[1,†], Jian Gu[1], Wong-Ho Chow[1], Jaffer A.Ajani[3] and Xifeng Wu[1,*]

[1]Department of Epidemiology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA, [2]Department of Pathology, School of Medicine, Zhejiang University, Hangzhou 310058, China and [3]Department of Gastrointestinal Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA

*To whom correspondence should be addressed. Department of Epidemiology, Unit 1340, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030, USA.
Tel: +1 713 745 2485; Fax: +1 713 792 4657;
Email: xwu@mdanderson.org

Alterations of mitochondrial DNA (mtDNA) have been associated with the risk of a number of human cancers; however, the relationship between mtDNA copy number in peripheral blood leukocytes and the risk of esophageal adenocarcinoma (EAC) has not been reported. In this study, we determined relative mtDNA copy number in peripheral blood leukocytes of 218 EAC cases and 218 frequency-matched controls. We calculated odds ratios and 95% confidence intervals using unconditional logistic regression, adjusting for age, sex and smoking status. MtDNA copy number was significantly lower in cases than in controls (mean ± SD, 1.16 ± 0.30 versus 1.27 ± 0.43, *P* = 0.002). Dichotomized at the median value of mtDNA copy number in the controls, low mtDNA copy number was significantly associated with an increased risk of EAC (odds ratio: 1.55, 95% confidence interval: 1.05–2.29). A significant dose–response relationship was observed between mtDNA copy number and risk of EAC in quartile analysis. Our results suggest that low mtDNA copy number in peripheral blood leukocytes is associated with increased susceptibility to EAC.

Introduction

Esophageal cancer is among the leading causes of cancer death in the world. Although relatively rare in the USA, the incidence of esophageal cancer has been increasing during the past decade. In 2013, an estimated 17 990 new cases will be diagnosed and 15 210 patients will die from esophageal cancer (1). The two main types of esophageal cancer are esophageal squamous cell carcinoma, which typically occurs in the middle third of the esophagus, and esophageal adenocarcinoma (EAC), which predominately occurs in the lower third of the esophagus (2,3). The epidemiology of esophageal cancer varies significantly according to geographic location. In China and other countries in Asia, esophageal squamous cell carcinoma is the predominant cancer of the esophagus. In the USA and other Western countries, the incidence of EAC has increased at a rate exceeding any other cancers and has outpaced esophageal squamous cell carcinoma to become the predominant cancer of the esophagus (4–6). Gastroesophageal reflux is a major cause of EAC (4). Other established risk factors of EAC include obesity and smoking. However, not all those who have been exposed to these risk factors develop EAC, suggesting that host genetic susceptibility and possibly gene–environment interactions may contribute to individual EAC risk (3–5).

Mitochondria are specialized organelles within cells that play a critical role in cellular energy metabolism, free radical generation and apoptosis. Mitochondrial DNA (mtDNA) is a circular, maternally inherited, double-stranded extrachromosomal DNA that is 16.5 kb and contains 37 genes encoding polypeptides of the respiratory chain, transfer RNA and ribosomal RNA. MtDNA lacks introns and generally replicates at a high rate without an efficient DNA repair mechanism. Mutations in the mitochondrial genome or decreases in mtDNA copy number could lead to a deficiency in oxidative phosphorylation and an enhanced generation of adenosine triphosphate (ATP) by glycolysis (5). Decreased ATP generation by oxidative phosphorylation with concomitant enhanced glycolysis is often associated with cancer (6). Previous studies have reported that variations of mtDNA copy number in peripheral blood lymphocytes (PBLs) were associated with the risks of several cancers (7–18). There has been no such report in EAC. In this study, we used a case–control study to evaluate the association of mtDNA copy number in PBLs with the risk of EAC.

Abbreviations: ATP, adenosine triphosphate; CI, confidence interval; EAC, esophageal adenocarcinoma; mtDNA, mitochondrial DNA; OR, odds ratio; PBL, peripheral blood lymphocyte; RCC, renal cell carcinoma.

[†]These authors contributed equally to this work.

Materials and methods

Study design

The study design and specimen collection methods have been described previously (2). Briefly, the MD Anderson Cancer Center EAC case–control study was initiated in October 2004. Cases were identified by reviewing the pathology reports of all patients who reported to the Department of Gastrointestinal Medical Oncology for clinic visits. Only patients who were diagnosed within the past 12 months were enrolled. Participation was not restricted on the basis of age, sex or disease stage. Eligible controls were selected from a pool of control subjects in ongoing case–control studies during the same time period (past 12 months). Briefly, healthy controls were identified and recruited using random digit calling (19). Control subjects, who have had no prior history of cancer (except non-melanoma skin cancer), were frequency matched to the cases by age (±5 years), sex and race/ethnicity. The participation rate was 91.4% for cases. For controls, the overall response rate was ~51% and among those who agreed to participate, the response rate was ~88%. Written informed consent was obtained from all subjects, who were interviewed to elicit information on demographic characteristics, occupational history, tobacco and alcohol use, medical history and family cancer history. At the end of the interview, a 40 ml blood sample was obtained from each participant and delivered to the laboratory for processing. This study was approved by the institutional review board of MD Anderson Cancer Center.

Determination of mtDNA copy number via real-time PCR

Genomic DNA was extracted from whole-blood samples via QIAamp DNA Mini Kits (Qiagen, Valencia, CA). The mtDNA copy number was determined using a quantitative real-time PCR-based method as reported elsewhere with some modifications (20,21). Briefly, we used two pairs of primers in two steps of relative quantification of mtDNA content. One primer pair was used for the amplification of the *ND1* gene in mtDNA. The primer sequences were as follows: forward primer (ND1-F), 5′-CCCTAAAACCCG CCACATCT-3′; reverse primer (ND1-R), 5′-GAGCGATGGTGA GAGCTAAGGT-3′. Another primer pair was used for the amplification of the single-copy nuclear human globulin (*HGB*) gene. The primer sequences were as follows: forward primer (HGB-1), 5′-GTGCACCTGACTCCTGAGGAGA-3′; reverse primer (HGB-2), 5′-CCTTGATACCAACCTGCCCAG- 3′.

During the first step, the relative mtDNA copy number and *HGB* copy number were determined for each sample from standard curves. We then determined the ratio of mtDNA to *HGB* copy number, which was proportional to the mtDNA copy number in each cell. Each PCR in a total volume of 14 µl contained 1× SYBR Green Mastermix (Applied Biosystems; Foster City, CA), 215 nM ND1-F (or HGB-1) primer, 215 nM ND1-R (or HGB-2) primer and 5 ng of genomic DNA. The thermal cycling conditions for the mtDNA *ND1* gene amplification were 95°C for 10 min, followed by 40 cycles of 15 s at 95°C and 1 min at 60°C. The thermal cycling conditions for the *HGB* amplification were 95°C for 10 min, followed by 40 cycles of 15 s at 95°C and 1 min at 56°C. All samples were assayed in duplicates in a 384 well plate with an Applied Biosystems 7900 Sequence Detection System. The PCRs for mtDNA and *HGB* were always performed on separate 384 well plates with the same samples in the

same well positions to avoid possible position effect. A standard curve of a diluted reference DNA, one negative control and one calibrator DNA were included in each run. Each plate contained randomly selected samples to have equal representation of cases and controls. The ratio for each sample was normalized to a calibrator DNA to standardize different runs. The calibrator DNA was a genomic DNA sample from a healthy control subject that was used to compare the results from different independent assays. For each standard curve, one reference DNA sample was serially diluted 1:2 to produce a seven-point standard curve between 0.3125 and 20 ng of DNA. The R^2 for each standard curve was 0.99 or greater. Standard deviations for the cycle of threshold values were accepted at 0.25 or less. Otherwise, the sample was repeated. To assess intra-assay variation, we assayed nine blood DNA samples from healthy control subjects three times on the same days. To evaluate interassay variation, we evaluated the same blood DNA samples from nine control subjects on different days. The average intra-assay and interassay coefficient of variance was 4.5 and 5.5%, respectively (8). All laboratory personnel performing the experiments described above were blinded to the case–control status of all DNA samples.

Statistical analysis

Statistical analysis was performed using STATA 10.0 software (Stata, College Station, TX). Differences in host characteristics between patients and control subjects were assessed via the chi-square test for categorical variables and Student's *t*-test for continuous variables. Multiple logistic regression modeling was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for EAC risk. Potential confounders, including age, sex and smoking, were adjusted in the model. The mtDNA copy number was categorized in two and four groups, using the median and quartiles among control subjects as cutoff points.

Never smokers were defined as those who had smoked fewer than 100 cigarettes during their lifetimes. Former smokers were defined as those who had smoked >100 cigarettes but had quit >1 year prior to diagnosis (patients) or interview (control subjects). Current smokers were defined as those who were currently smoking or had stopped smoking <1 year prior to diagnosis (patients) or interview (control subjects). Ever smokers included both former and current smokers.

Results

A total of 218 pairs of patients and controls were included in the analysis. The patients and controls were similar in distributions with regard to age, sex and race/ethnicity, as they were frequency matched on these variables. The proportion of ever smokers was significantly lower in controls than in patients; however, there were no statistically significant differences between the cases and controls in terms of pack-years of smoking. The relative mean mtDNA copy number was significantly lower in patients (1.16 ± 0.30) than in controls (1.27 ± 0.43). The median values among cases and controls were 1.13 (range = 0.35–2.27) and 1.22 (range = 0.46–4.24), respectively (Table I).

Table II shows, in general, that mtDNA copy numbers were higher among controls than among cases across all categories by sex, age, race/ethnicity and smoking status, although the difference in female, ever smokers and non white did not reach statistical significance. We also analyzed the correlation between mtDNA copy number and age among controls and did not find significant correlation ($r^2 = 0.001$, $P = 0.984$).

We next analyzed the association between mtDNA copy number and the risk of EAC (Table III). Using the median mtDNA copy number in controls as the cutoff point, we found that lower mtDNA copy number was associated with a significantly greater risk of EAC, with an OR of 1.55 (95% CI, 1.05–2.29). Analysis of the data by the quartile distribution of mtDNA copy number in controls revealed a dose–response association between mtDNA copy number and EAC risk. Compared with individuals within the highest quartile of mtDNA copy number, those in the higher mid, lower mid and lowest quartiles had an OR of 0.88 (95% CI, 0.49–1.59), 1.14 (95% CI, 0.65–2.02) and 1.77 (95% CI, 1.03–3.04), respectively (*P* for trend, 0.017). We also performed stratified analyses and observed that the association between lower mtDNA copy number and EAC risk reached significance in men, younger subjects and never smokers but not in women, older subjects and ever smokers (Table IV). However, there was no significant heterogeneity of the ORs across different strata of these variables in the test of heterogeneity ($P = 0.164$, 0.945 and 0.739 for sex, age and smoking strata, respectively).

Table I. Distribution of selected host characteristics

Variables	Cases ($n = 218$)	Controls ($n = 218$)	*P*
Age, mean (SD)	62.05 (10.32)	60.94 (10.44)	0.265
Sex, *n* (%)			
Male	173 (79.36)	173 (79.36)	
Female	45 (20.64)	45 (20.64)	1.00
Race, *n* (%)			
White	194 (88.99)	194 (88.99)	
Hispanic	14 (6.42)	14 (6.42)	
Black	7 (3.21)	7 (3.21)	
Other	3 (1.38)	3 (1.38)	1.00
Smoking, *n* (%)			
Never	68 (31.19)	96 (44.04)	
Former	96 (44.04)	84 (38.53)	
Current	54 (24.77)	38 (17.43)	0.015
Ever	150 (68.81)	122 (55.96)	0.006
Pack-year of smoking[a] (SD)	55.84 (68.01)	59.38 (69.93)	0.673
MtDNA copy number			
Mean (SD)	1.16 (0.30)	1.27 (0.43)	0.002
Median (range)	1.13 (035–2.27)	1.22 (0.46–4.24)	0.003

[a]Pack-year of smoking was assessed for ever smokers only.

Table II. mtDNA copy number by host characteristics of cases and controls

Variable	Cases		Controls		*P*
	N	Mean ± SD	*N*	Mean ± SD	
Sex					
Male	173	1.14 ± 0.30	173	1.26 ± 0.44	0.004
Female	45	1.22 ± 0.33	45	1.33 ± 0.41	0.164
Age					
Age ≤ 60	101	1.16 ± 0.33	108	1.29 ± 0.51	0.031
Age > 60	117	1.16 ± 0.28	110	1.26 ± 0.34	0.019
Race					
White	194	1.15 ± 0.29	194	1.27 ± 0.44	0.001
Other	24	1.24 ± 0.41	24	1.26 ± 0.33	0.825
Smoking					
Never	68	1.20 ± 0.29	96	1.35 ± 0.49	0.024
Ever	150	1.14 ± 0.31	122	1.21 ± 0.36	0.082

Table III. Risk of EAC as estimated by mtDNA copy number

MtDNA copy number	Cases, *N* (%)	Controls, *N* (%)	Adjusted OR[a]	*P*
By median				
≥1.22	83 (38.07)	109 (50.00)	1 (reference)	
<1.22	135 (61.93)	109 (50.00)	1.55 (1.05–2.29)	0.028
By quartile				
≥1.39	43 (19.72)	54 (24.77)	1 (reference)	
1.22–1.39	40 (18.35)	55 (25.23)	0.88 (0.49–1.59)	0.691
1.03–1.22	52 (23.85)	54 (24.77)	1.14 (0.65–2.02)	0.650
<1.03	83 (38.07)	55 (25.23)	1.77 (1.03–3.04)	0.038
P for trend				0.017

[a]Adjusted for age, sex, ethnicity and smoking status.

Discussion

In this case–control study, we found that lower mtDNA copy number in PBLs was associated with a significantly increased risk of EAC. To the best of our knowledge, this is the first molecular epidemiological study that has evaluated mtDNA copy number in PBLs as a susceptibility biomarker for EAC.

Table IV. Association of EAC with mtDNA copy number stratified by selected host characteristics

Host characteristics	Cases, *N* (%)	Controls, *N* (%)	Adjusted OR (95% CI)[a]	*P*
Sex				
Male				
>1.22	57 (32.95)	84 (48.55)	1 (reference)	
≤1.22	116 (67.05)	89 (51.45)	1.85 (1.19–2.88)	0.006
Female				
>1.22	26 (57.78)	25 (55.56)	1 (reference)	
≤1.22	19 (42.22)	20 (44.44)	0.86 (0.35–2.15)	0.750
Age, years				
Age ≤ 60				
>1.22	39 (38.61)	56 (51.85)	1 (reference)	
≤1.22	62 (61.39)	52 (48.15)	1.89 (1.06–3.38)	0.031
Age > 60				
>1.22	44 (37.61)	53 (48.18)	1 (reference)	
≤1.22	73 (62.39)	57 (51.82)	1.38 (0.79–2.39)	0.256
Smoking status				
Never smoking				
>1.22	30 (44.12)	56 (58.33)	1 (reference)	
≤1.22	38 (55.88)	40 (41.67)	1.90 (0.98–3.69)	0.058
Ever smoking				
>1.22	53 (35.33)	53 (43.44)	1 (reference)	
≤1.22	97 (64.67)	69 (56.56)	1.39 (0.85–2.28)	0.192

[a]Sex: adjusted for age, ethnicity and smoking status; age: adjusted for sex, ethnicity and smoking status; smoking status: adjusted for age, sex and ethnicity.

Somatic mtDNA mutation and mtDNA copy number reduction are common phenomena in human cancers (22–26). Recently, many studies have investigated the association of constitutive mtDNA copy number in PBLs with the risk of cancers (7–18). Several studies reported that low mtDNA copy number in PBLs was significantly associated with an increased risk of cancer, including renal cell carcinoma (RCC) (8,14), hepatocellular carcinoma (17), soft tissue sarcoma (16) and Ewing's sarcoma (18), whereas others showing that high mtDNA copy number in PBLs was associated with increased risks of lung cancer (9), breast cancer (10), pancreatic cancer (12), colorectal cancer (13) and non-Hodgkin lymphoma (7). More interestingly, a recent prospective study reported a U-shaped association between mtDNA copy number in PBLs and colorectal cancer risk: both the lowest quartile and highest quartile conferred significantly increased cancer risks compared with the second quartile (15). These heterogeneous results are likely due to small sample sizes, different study designs and heterogeneous study populations. It is also possible that mtDNA copy number modulates cancer risks in a cancer type-specific manner.

This study is the first report of mtDNA copy number in PBLs with the risk of EAC. Our overall sample size was among the largest in all the above referred similar studies in other cancers. Biologically, it is plausible that reduced mtDNA copy number conferred an increased cancer risk due to the alteration of mitochondrial respiratory function. Injury to the respiratory machinery is a critical event in carcinogenesis. The key role of mitochondria is to generate cellular ATP through oxidative phosphorylation (27). A reduction in mtDNA copy number can alter mitochondrial gene expression and lead to a deficiency in oxidative phosphorylation and an increased generation of ATP by glycolysis (28). MtDNA copy number decrease may also promote cancer cells to become resistant to apoptosis (29). Once defective in apoptosis, normal cells would extend their original life span, accumulate DNA damage, deregulate cell proliferation machinery, increase cell mobility and exhibit other changes that play important roles in the initiation and promotion of cancers. In addition, Amuthan *et al*. (30) noted that partial loss of mtDNA induced an aggressive phenotype and overexpression of several tumor-specific markers. Finally, mtDNA depletion in cancer cells leads to epithelial-mesenchymal transition (31), which plays an important role in tumor formation and progression (32). Future studies are needed to elucidate the molecular mechanisms underlying our observed association between lower mtDNA copy number and an increased risk of EAC.

Our study had a few limitations. First, we did not repeat mtDNA copy number measurement in the same subject and a single measurement may not reflect mtDNA copy number over a lifetime. However, Thyagarajan *et al*. (15) conducted a small study and found mtDNA copy number is relatively stable over time. Second, although our sample size is sufficient for an overall association analysis, it is limited for stratified and interaction analyses. Large-scale validation is necessary to confirm and extend our observations. Third, we used the peripheral blood leukocytes to detect the relationship between mtDNA copy number and the risk of EAC. However, mtDNA copy number may be different among leukocyte subpopulations, such as neutrophils have low number of mtDNA copies (33). It is possible that the observed case–control differences may be partially due to systematic differences in leukocyte subpopulations. Future studies are needed to compare mtDNA copy number in different subpopulations of leukocytes between cases and controls. Finally, reverse causation is always a concern for retrospective case–control study evaluating an intermediate biomarker. Previous studies have shown examples of significant differences in cancer risk association with intermediate biomarkers by prospective versus retrospective studies (8,14,34–37). For example, the association of mtDNA copy number in PBLs with the risk of RCC was evaluated in three studies: two retrospective case–control studies showed that lower mtDNA copy number conferred an increased risk of RCC (8,14), whereas a prospective nested case–control study demonstrated a significant association of higher mtDNA copy number with increased risk of RCC (37). Prospective study is always the preferred design when evaluating intermediate biomarkers for cancer risk association. In our current study, we only included newly diagnosed EAC cases and collected blood samples before any treatment, which should reduce the impact treatment on mtDNA copy number. We did not observe significant relationship between tumor stage and grade and mtDNA copy number (data not shown), arguing against the effect of disease status on mtDNA copy number. A previous classic twin study estimated a genetic heritability of 65% for mtDNA copy number in PBLs (8), supporting its usage as a risk assessment biomarker. Nevertheless, future prospective validation is warranted to confirm our observations.

In conclusion, to our knowledge, our case–control study offers the first evidence that low mtDNA copy number in PBLs is associated with an increased risk of EAC with a significant dose–response effect. Our findings support an important role for mitochondria dysfunction in esophageal carcinogenesis.

Funding

National Institutes of Health (CA111922); MD Anderson Cancer Center Research Trust; University of Texas MD Anderson Cancer Center institutional support for the Center for Translational and Public Health Genomics.

Conflict of Interest Statement: None declared.

References

1. Siegel,R. *et al.* (2013) Cancer statistics, 2013. *CA. Cancer J. Clin.*, **63**, 11–30.
2. Pan,J. *et al.* (2009) Genetic susceptibility to esophageal cancer: the role of the nucleotide excision repair pathway. *Carcinogenesis*, **30**, 785–792.
3. Yang,P.C. *et al.* (1988) Incidence of cancer of the esophagus in the US by histologic type. *Cancer*, **61**, 612–617.
4. Shaheen,N. *et al.* (2002) Gastroesophageal reflux, barrett esophagus, and esophageal cancer: scientific review. *JAMA*, **287**, 1972–1981.
5. Shadel,G.S. (2008) Expression and maintenance of mitochondrial DNA: new insights into human disease pathology. *Am. J. Pathol.*, **172**, 1445–1456.
6. Qian,W. *et al.* (2010) Alterations in bioenergetics due to changes in mitochondrial DNA copy number. *Methods*, **51**, 452–457.
7. Lan,Q. *et al.* (2008) A prospective study of mitochondrial DNA copy number and risk of non-Hodgkin lymphoma. *Blood*, **112**, 4247–4249.
8. Xing,J. *et al.* (2008) Mitochondrial DNA content: its genetic heritability and association with renal cell carcinoma. *J. Natl Cancer Inst.*, **100**, 1104–1112.

9. Bonner,M.R. *et al.* (2009) Mitochondrial DNA content and lung cancer risk in Xuan Wei, China. *Lung Cancer*, **63**, 331–334.
10. Shen,J. *et al.* (2010) Mitochondrial copy number and risk of breast cancer: a pilot study. *Mitochondrion*, **10**, 62–68.
11. Liao,L.M. *et al.* (2011) Mitochondrial DNA copy number and risk of gastric cancer: a report from the Shanghai Women's Health Study. *Cancer Epidemiol. Biomarkers Prev*., **20**, 1944–1949.
12. Lynch,S.M. *et al.* (2011) Mitochondrial DNA copy number and pancreatic cancer in the alpha-tocopherol beta-carotene cancer prevention study. *Cancer Prev. Res. (Phila)*., **4**, 1912–1919.
13. Qu,F. *et al.* (2011) Association between mitochondrial DNA content in leukocytes and colorectal cancer risk: a case-control analysis. *Cancer*, **117**, 3148–3155.
14. Purdue,M.P. *et al.* (2012) A case-control study of peripheral blood mitochondrial DNA copy number and risk of renal cell carcinoma. *PLoS One*, **7**, e43149.
15. Thyagarajan,B. *et al.* (2012) Mitochondrial copy number is associated with colorectal cancer risk. *Cancer Epidemiol. Biomarkers Prev*., **21**, 1574–1581.
16. Xie,H. *et al.* (2013) Reduced mitochondrial DNA copy number in peripheral blood leukocytes increases the risk of soft tissue sarcoma. *Carcinogenesis*, **34**, 1039–1043.
17. Zhao,S. *et al.* (2011) Association of mitochondrial DNA content in peripheral blood leukocyte with hepatitis B virus-related hepatocellular carcinoma in a Chinese Han population. *Cancer Sci*., **102**, 1553–1558.
18. Yu,M. *et al.* (2012) Cell-free circulating mitochondrial DNA in the serum: a potential non-invasive biomarker for Ewing's sarcoma. *Arch. Med. Res*., **43**, 389–394.
19. Olson,S.H. *et al.* (1992) Evaluation of random digit dialing as a method of control selection in case-control studies. *Am J. Epidemiol*., **135**, 210–222.
20. Kaaman,M. *et al.* (2007) Strong association between mitochondrial DNA copy number and lipogenesis in human white adipose tissue. *Diabetologia*, **50**, 2526–2533.
21. Santos,T.A. *et al.* (2006) Mitochondrial content reflects oocyte variability and fertilization outcome. *Fertil. Steril*., **85**, 584–591.
22. Lee,H.C. *et al.* (2004) Somatic mutations in the D-loop and decrease in the copy number of mitochondrial DNA in human hepatocellular carcinoma. *Mutat. Res*., **547**, 71–78.
23. Lee,H.C. *et al.* (2005) Mitochondrial genome instability and mtDNA depletion in human cancers. *Ann. N. Y. Acad. Sci*., **1042**, 109–122.
24. Tseng,L.M. *et al.* (2006) Mitochondrial DNA mutations and mitochondrial DNA depletion in breast cancer. *Gene Chromosome Canc.* **45**, 629–638.
25. Yamada,S. *et al.* (2006) Correlation between copy number of mitochondrial DNA and clinico-pathologic parameters of hepatocellular carcinoma. *Eur. J. Surg. Oncol*., **32**, 303–307.
26. Kose,K. *et al.* (2005) Somatic mutations of mitochondrial DNA in digestive tract cancers. *J. Gastroenterol. Hepatol*., **20**, 1679–1684.
27. Hatefi,Y. (1985) The mitochondrial electron transport and oxidative phosphorylation system. *Annu. Rev. Biochem*., **54**, 1015–1069.
28. Warburg,O. (1956) On respiratory impairment in cancer cells. *Science*, **124**, 269–270.
29. Chandel,N.S. *et al.* (1999) Cells depleted of mitochondrial DNA (rho0) yield insight into physiological mechanisms. *FEBS Lett*., **454**, 173–176.
30. Amuthan,G. *et al.* (2001) Mitochondria-to-nucleus stress signaling induces phenotypic changes, tumor progression and cell invasion. *EMBO J*., **20**, 1910–1920.
31. Naito,A. *et al.* (2008) Progressive tumor features accompany epithelial-mesenchymal transition induced in mitochondrial DNA-depleted cells. *Cancer Sci*., **99**, 1584–1588.
32. Huber,M.A. *et al.* (2005) Molecular requirements for epithelial-mesenchymal transition during tumor progression. *Curr. Opin. Cell Biol*., **17**, 548–558.
33. Maianski,N.A. *et al.* (2004) Functional characterization of mitochondria in neutrophils: a role restricted to apoptosis. *Cell Death Differ*., **11**, 143–153.
34. Pooley,K.A. *et al.* (2010) Telomere length in prospective and retrospective cancer case-control studies. *Cancer Res*., **70**, 3170–3176.
35. Wentzensen,I.M. *et al.* (2011) The association of telomere length and cancer: a meta-analysis. *Cancer Epidemiol. Biomarkers Prev*., **20**, 1238–1250.
36. Weischer,M. *et al.* (2013) Short telomere length, cancer survival, and cancer risk in 47102 individuals. *J. Natl Cancer Inst*., **105**, 459–468.
37. Hofmann,J.N., *et al.* (2012) A prospective study of blood mitochondrial DNA copy number and risk of renal cell carcinoma [abstract]. *Proceedings of the 103rd Annual Meeting of the American Association for Cancer Research*; 31 March–4 April 2012; Chicago, IL. AACR, Philadelphia, PA; *Cancer Res*., **72**: A4461. doi:1538–7445.AM2012-4461

Received February 20, 2013; revised June 19, 2013; accepted June 24, 2013