Brief report

A prospective study of mitochondrial DNA copy number and risk of non-Hodgkin lymphoma

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Mitochondrial DNA (mtDNA) copy number is increased in patients with chronic lymphocytic leukemia (CLL), in Burkitt lymphoma and Epstein-Barr virus–transformed lymphoblastoid cell lines, and in T cells activated via the T-cell receptor. We hypothesized that having a higher mtDNA copy number in peripheral white blood cell DNA from healthy subjects would be associated with future risk of non-Hodgkin lymphoma (NHL). We analyzed mtDNA copy number in 104 incident male NHL cases and 104 matched controls within the prospective Alpha-Tocopherol, Beta-Carotene (ATBC) Cancer Prevention cohort. There was a dose-response relationship between tertiles of mtDNA copy number and risk of NHL (odds ratio [OR], 95% confidence interval [CI]: 1.0; 1.4 [0.7-2.8]; and 2.4 [1.0-5.5], respectively; P_{trend} = .046). The effect was most pronounced for the CLL/small lymphocytic lymphoma (SLL) subtype (OR: 1.0; 3.2 [0.7-15.7]; 14.1 [1.9-103.2]; P_{trend} = .009). These results suggest that mtDNA copy number could be associated with the risk of NHL, particularly CLL/SLL. (Blood. 2008;112:4247-4249)

Introduction

Mitochondria play a key role in production of cellular energy, free radical generation, and apoptosis, and are the major intracellular source and primary target of reactive oxygen species (ROS).[1,2] Human mitochondrial DNA (mtDNA) is present at high levels (10^3-10^4 copies per cell) with a steady state that can vary by tissue type. Compared with nuclear DNA, mtDNA lacks protective histones and has diminished DNA repair capacity, and therefore is particularly susceptible to ROS and other types of genotoxic damage.[3]

Various internal and external factors associated with synthesis of adenosine triphosphate (ATP) demand influence mtDNA copy number in nonneoplastic tissue, including cell growth and differentiation, hormone treatment, age, and reaction to oxidative damage, which are thought to play a role in lymphomagenesis.[4-6] The mtDNA copy number has also been shown to be increased in T cells activated via the T-cell receptor,[7] and in workers exposed to benzene, an established leukemogen and suspect lymphomagen.[8] Finally, it is noteworthy that mtDNA copy number is increased in cells from patients with chronic lymphocytic leukemia (CLL),[9] in cell lines derived from Burkitt lymphoma,[10] and in Epstein-Barr virus (EBV)–transformed lymphoblastoid cell lines.[10]

We therefore investigated mtDNA copy number measured in peripheral white blood cell (WBC) DNA as a possible biomarker for risk for non-Hodgkin lymphoma (NHL), as this biomarker is likely to reflect both exogenous and endogenous processes relevant for lymphomagenesis. We measured mtDNA copy number in NHL cases and matched controls from the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study.[11]

Methods

A detailed description of the study population has been reported previously.[12] Briefly, 29 133 eligible male smokers, aged 50 to 69 years, were recruited from southwest Finland and randomized in 1985-1988 to determine whether supplementation with α-tocopherol or β-carotene reduced the incidence of lung and other cancers. The study was approved by institutional review boards at the National Cancer Institute and the National Public Health Institute of Finland. Participants provided written informed consent in accordance with the Declaration of Helsinki.

All cases of NHL were identified using the Finnish Cancer Registry, which provides virtually 100% of case ascertainment in Finland.[13] The hospital records of the identified cases were reviewed by an experienced study oncologist for confirmation of the lymphoma diagnosis. Through April 30, 2002, 107 incident cases of NHL diagnosed after providing a whole blood sample in 1992 or 1993 were identified based on the histology information coded in the International Classification of Disease-Oncology, second edition (ICD-O-2: 9590-9595, 9670-9677, 9680-9688, 9690-9698, 9700-9717, 9760-9764, 9820-9828, 9940-9941).[14] Cases were further classified following the proposed adaptation[15] of the World Health Organization guidelines.[14] Controls were selected from the ATBC study participants who were alive, did not have cancer at the time of the case diagnosis, and were matched to cases on date of birth ($\pm$ 5 years).

DNA was extracted from the whole blood using the phenol-chloroform method. The fluorescence-based quantitative polymerase chain reaction (PCR) was used to determine the total mtDNA copy number by using the

The online version of this article contains a data supplement.

Table 1. Comparison of baseline characteristics in non-Hodgkin lymphoma cases and individually matched controls

Characteristic	Cases (n = 104)	Controls (n = 104)
Age, y	58	57
Smoking		
Number of cigarettes/day	20	20
Years of smoking	39	39
Pack-years	36	35.5
Height, meters	1.75*	1.74
Body mass index (BMI), kg/m²	25.5	25.4
BMI < 25, %	38	40
BMI 25-29.9, %	45	48
≥30, %	17	12
Diastolic blood pressure, mm Hg	84	88
Systolic blood pressure, mm Hg	136*	140
Leisure-time physical activity, %		
Intensity		
Low	45	36
Moderate	48	52
Heavy	7	13
Frequency (times per week)		
< 1	46	47
1-2	32	28
≥ 3	22	25
Dietary intake†		
Alcohol, g/d	9.6	11.6
Multivitamin supplement use, %	5	5
Calories, kcal/day	2584	2782
Supplementation group		
Placebo, %	28	29
Alpha-tocopherol, %	24	24
Beta-carotene, %	25	25
Alpha-tocopherol/beta-carotene, %	23	22
Mitochondrial DNA copy number, median	120.6*	114.1
(interquartile range)	(105.6-155.9)	(99.6-130.7)

Data are medians unless otherwise noted.

*$P < .05$ for comparison of cases versus controls using Wilcoxon nonparametric test for continuous variables and using χ^2 test for categorical variables.

†Dietary intake data were available for 98 case subjects and 98 control subjects.

estimation of threshold cycle number of ND1, a mitochondrial gene, and that of the β-globin gene, *HBB*, a nuclear gene.[16] Cases and their matched controls were assayed consecutively within batches along with blinded quality control duplicate samples to evaluate assay reproducibility. The over-

all coefficient of variation for this assay was 13%. A total of 104 (24 diffuse large B-cell, 11 follicular, 34 CLL/small lymphocytic lymphoma [SLL], and 35 others) matched sets of cases and controls had mtDNA data for analysis (3 case-control pairs were dropped due to missing measurements). A detailed description of histologic subtypes for all 104 cases of NHL is presented in Table S1 (available on the *Blood* website; see the Supplemental Materials link at the top of the online article). Distributions of baseline characteristics for matched cases and controls were tested nonparametrically by the Wilcoxon signed rank test for continuous variables and the χ^2 test for categorical variables. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were estimated using conditional logistic regression models. The mtDNA copy number was categorized into tertiles based on the distribution among controls, and modeled as both a continuous and categorical variable. Tests for trends were calculated using the median value for each mtDNA copy number tertile. Variables that resulted in a 10% or greater change in the β-coefficient of the mtDNA variable in the base model that adjusted for matched age were considered confounders and included in the final, multivariable models. All *P* values are 2-sided.

Results and discussion

Case and control subjects were comparable in terms of age, body mass index (BMI), smoking history, physical activity, dietary intake, and vitamin supplementation group (Table 1). There were small differences in systolic blood pressure and height between cases and controls (Table 1). The mtDNA copy number was significantly higher in cases compared with controls (Table 1, $P = .013$), and the risk for developing NHL increased significantly with increasing mtDNA copy number (Table 2, $P_{trend} = .046$). The association was most pronounced for CLL/SLL, the most common histologic subtype in this case series (33%), with OR equal to 14.1 (95% CI, 1.9-103.2) for subjects in the highest tertile of mtDNA copy number compared with the lowest tertile. By contrast, risk estimates were unremarkable for subtypes of NHL other than CLL/SLL for which there were an adequate number of cases for subanalyses (Table 2). Adjustment in multivariable models for several demographic factors shown in Table 1 increased the OR substantially, although confidence intervals were wide due at least in part to the small sample size (Table 2).

To determine if the association might be driven in part by elevated mtDNA copy number among cases with undiagnosed, subclinical CLL/SLL at the time of blood sample collection, we excluded

Table 2. Odds ratio (OR) and 95% confidence interval for mtDNA copy number and non-Hodgkin lymphoma

Diagnosis	mtDNA copy number				
	Continuous (/15 increment)	Tertile 1 (60.7-102.6)	Tertile 2 (102.7-126.5)	Tertile 3 (126.6-295.9)	P_{trend}
NHL					
n	104/104	23/33	36/36	45/35	
Unadjusted OR (95% CI)	1.2 (1.1-1.5)	1.0 (ref)	1.4 (0.7-2.8)	2.4 (1.0-5.5)	.046
Multivariable OR (95% CI)	1.3 (1.1-1.6)	1.0 (ref)	1.7 (0.8-3.5)	3.9 (1.4-11.0)	.010
Diffuse large B-cell NHL					
N	24/24	6/7	10 /8	8/9	
Unadjusted OR (95% CI)	1.1 (0.8-1.4)	1.0 (ref)	1.7 (0.3-9.0)	1.0 (0.2-5.9)	.88
Multivariable OR (95% CI)	1.4 (0.8-2.4)	1.0 (ref)	3.7 (0.4-37.0)	0.7 (0.1-8.2)	.82
CLL/SLL					
n	34/34	3/11	12/14	19/9	
Unadjusted OR (95% CI)	1.5 (1.1-2.2)	1.0 (ref)	3.2 (0.7-15.7)	14.1 (1.9-103.2)	.009
Multivariable OR (95% CI)	1.7 (1.1-2.7)	1.0 (ref)	1.2 (0.1-10.6)	27.7 (1.4-563.6)	.020
NHL excluding CLL/SLL					
n	70/70	20/22	24/22	26/26	
Unadjusted OR (95% CI)	1.1 (1.0-1.4)	1.0 (ref)	1.2 (0.5-2.6)	1.1 (0.4-3.1)	.84
Multivariable OR (95% CI)	1.2 (1.0-1.5)	1.0 (ref)	1.4 (0.6-3.5)	2.0 (0.5-7.0)	.29

Multivariable model adjusted for pack-years, height, systolic blood pressure, and leisure-time physical activity (5 categories, combining intensity and frequency). NHL indicates non-Hodgkin lymphoma; CLL/SLL, chronic lymphocytic leukemia/small lymphocytic leukemia.

the 5 cases (15% of total cases) diagnosed within the first year of follow-up after blood sample collection, and found that results were essentially unchanged (OR 2.7, 95% CI 0.5-13.7 and OR 12.3, 95% CI 1.7-89.2 for higher tertiles, respectively; $P_{trend} = .01$). In addition, to evaluate the potential effects of the trial vitamin supplementation on the relationship between mtDNA copy number and risk of NHL, we carried out further analyses stratified by α-tocopherol versus no α-tocopherol supplementation, and β-carotene versus no β-carotene supplementation, and found risks to be similar in each group with nonsignificant tests for interactions (data not shown).

To the best of our knowledge, this is the first study of the relationship between mtDNA copy number measured in peripheral WBC DNA and risk of NHL. A particular strength of this study is that blood samples were collected prospectively from study subjects when they were healthy.

There are likely to be multiple determinants of mtDNA copy number in a particular cell type, with evidence that elevated mtDNA copy number per cell is associated with some forms of oxidative stress, age, activation of T cells, and benzene exposure.[8,17-19] It has been shown that cells under mild oxidative stress may increase mitochondria and mtDNA production through a pathway that bypasses cell-cycle control. In cells treated with cell cycle–arresting drugs or H_2O_2, even though overall cell division was under arrest, mitochondria continued to proliferate as if the cells were going to divide.[18] During the process of oxidative phosphorylation involving ROS generated by both endogenous and exogenous processes, accumulation of mutated mtDNA may occur.[20] Certain mutated mtDNAs may obtain a replicative advantage[20] that can generate increased superoxide and nitric oxide and lead to aberrant mitochondrial biogenesis, which has been associated with deficient or defective apoptosis.[9]

As noted, increased mtDNA copy number has also been detected in cells from patients with CLL,[9] in Burkitt lymphoma cell lines,[10] and in lymphoblastoid cell lines.[10] As mtDNA copy number is decreased in several other tumors,[21-23] it is clear that mtDNA copy number is not increased simply as a function of enhanced cellular proliferation in neoplastic cells, and has some degree of specificity for particular tumor types.

In summary, we observed that mtDNA copy number was associated with increased risk of NHL and CLL/SLL in particular. Because this study is relatively small, replication is necessary, preferably in other prospective cohort studies with a larger number of NHL cases and longer follow-up to avoid potential disease bias.

Acknowledgments

Authorship

Contribution: Q.L., N.R., D.A., M.R.B., and C.-S.L. initiated the mtDNA project; D.A. and J.V. initiated and carried out the prospective Alpha-Tocopherol, Beta-Carotene (ATBC) Cancer Prevention cohort; Q.L., N.R., S.W., and D.A. designed the nested case-control study study; C.-S.L. analyzed mtDNA copy number; U.L., Q.L., N.R., and D.A. carried out statistical analysis; Q.L., U.L., C.-S.L., S.W., S.C., M.R.B., J.V., D.A., and N.R. drafted and revised the manuscript; and all authors reviewed and approved the manuscript.

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