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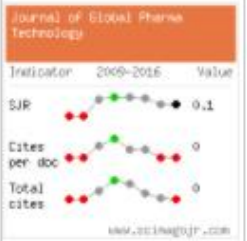

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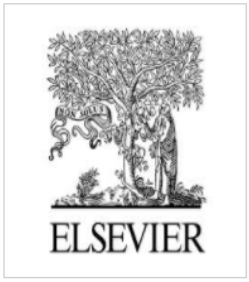

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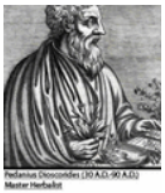

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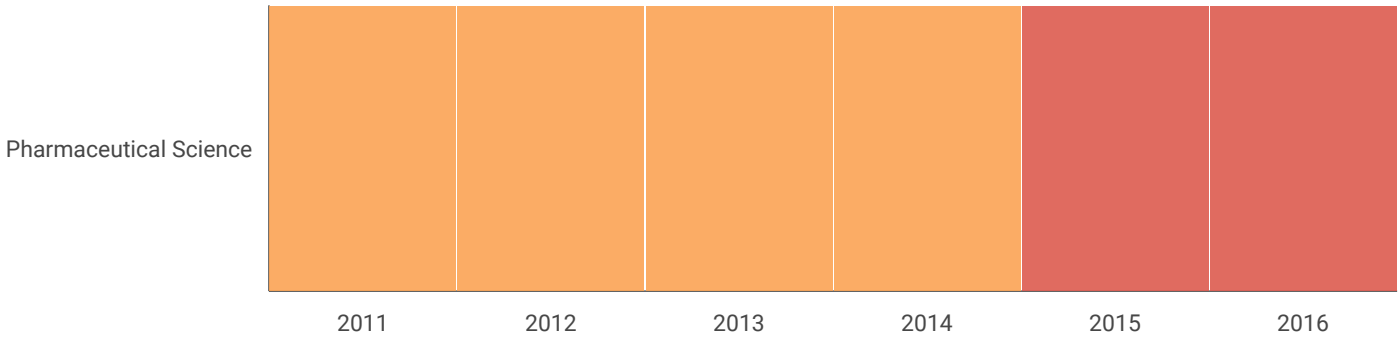

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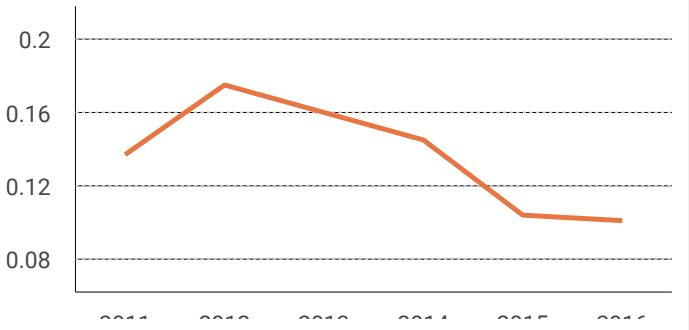

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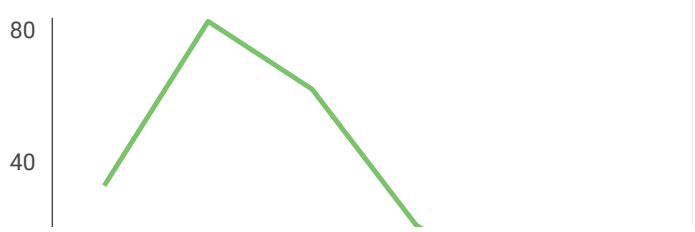

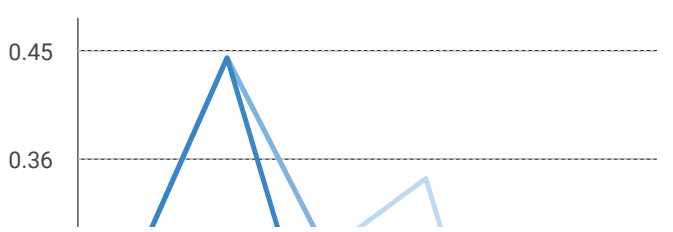

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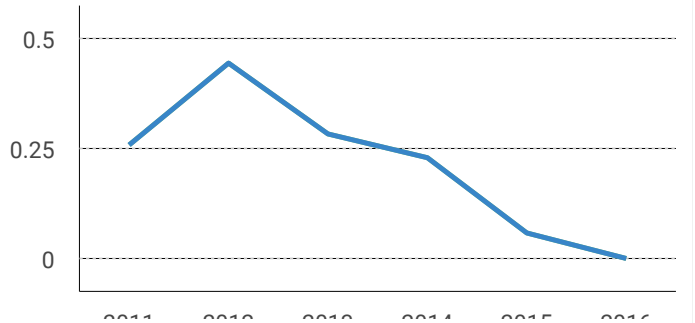

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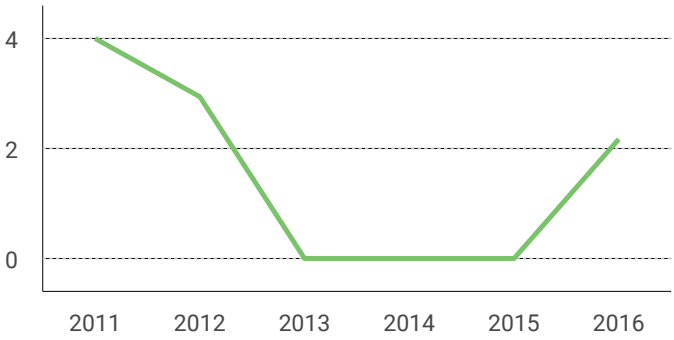

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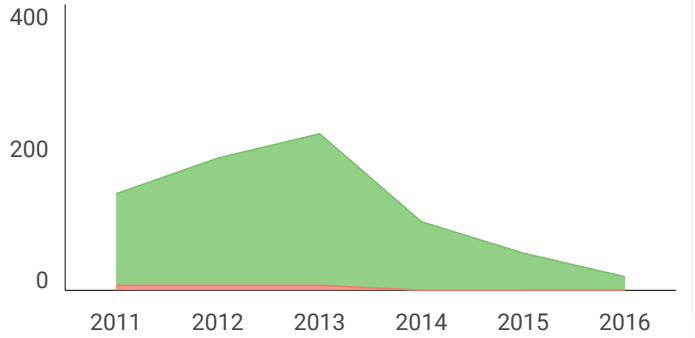

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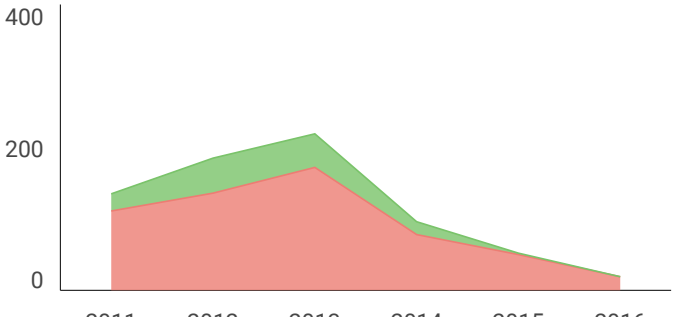

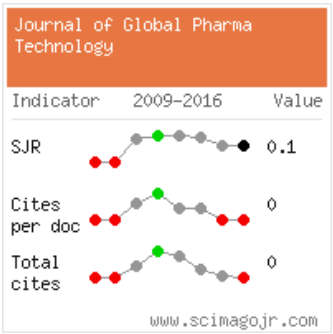

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RESEARCH PAPER

ND3 Mitochondrial Polimorphism G-10399-A and C-10401-T Significantly Associated with Elevated Serum 8-OhdG Concentration and Increased Risk of Malignant Behavior in Breast Cancer

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Abstract

Introduction: ND3¬ gene polymorphisms had been associated with the risk of breast cancer. However, it association with cancer cell behavior and progression is yet to be evaluated and thus this research evaluate the relationship between ND3 gene polymorphisms with 8-OHdG concentration that represent oxidative DNA damage and with the risk of malignant cancer behavior. Method: A case-control study was conducted in Faculty of Medicine Udayana University and Sanglah General Hospital from January to December 2016. The case group was defined as sample with lymph vascular invasion and histological grade III. The blood samples were obtained to evaluate the polymorphism and serum 8-OHdG concentration. The polymorphisms were detected using PCR and sequencing while serum 8-OHdG concentration was determined using ELISA. Result: 70 subjects were enrolled in this study with 35 samples for each group. Increased serum 8-OHdG were associated with the presence of ND3 polymorphism [375.37±203.56ng/mL in wild type vs 610.65±271.9ng/mL (G-10399-A) and 636.18±287.75ng/mL (C-10401-T); $p<0.05$] and the number of polymorphism [391.45±210.97ng/mL for no polymorphism vs 487.52±227.18ng/mL in one polymorphism and 861.36±253.27ng/mL for two polymorphism; $p<0.05$]. Finally, the presence of polymorphism increased the risk of more malignant cancer behavior (OR: 4.792; 95%CI: 1.741 – 13.188). Conclusion: G-10399-A and C-10401-T ND3 polymorphism clearly increased oxidative DNA damage and the risk of malignant cancer behavior in breast cancer patients.

Keywords: *ND3 gene polymorphism G-10399-A and C-10401-T, 8-OHdG, malignant behavior, breast cancer*

Introduction

Breast cancer is the second most prevalent disease in women after cervical cancer [1]. It also associated with significant amount of mortality annually surpassed only by cervical cancer. Despite sharp increase in the survivability rate over the last two decade, the cumulative prevalence of breast cancer increased and would potentially increase the burden of the disease[1,2].

Polymorphisms in mitochondrial several mitochondrial genes had been proved to be associated with the occurrence of breast cancer[3]. The genes that encode the protein involved in the electron transport chain are particularly important because the defect in this process could result in increased production of ROS; one of the principal cause of cancer [4]. One of such gene is ND3 gene that encodes NADH dehydrogenase 3 of the complex-1 of the electron transport chain [4, 6].

ND3 gene had been associated with several kinds of solid cancer namely bladder, prostate, and thyroid cancer[7]. However, its association with breast cancer is still controversial. The presence of the polymorphism especially A10389G clearly result in the alteration of mitochondrial

complex-1 function. However, several studies that evaluate its association in the population showed non-conclusive findings. Meta-analysis conducted by Mao et.al found that A10389G polymorphism may not be the risk factor of breast cancer while Grzybowska- Szatkowska et.al found that the polymorphism is associated with breast cancer in Polish population [5, 8].

Nevertheless, the polymorphism has a potential to affect the progression and alter the properties of breast cancer itself. Free radical has been proved to not only take part in the carcinogenesis but also in the establishment of cancer microenvironment [9]. It induces the differentiation of normal fibroblast into cancer associated fibroblast (CAF) that plays crucial role in supporting cancer progression [10, 11].

It also induces local inflammation that attracts macrophages and neutrophils that ultimately support cancer progression and increase the invasiveness of cancer mass [12].However, the role of ND3 polymorphism in breast cancer progression is not yet evaluated. Considering the important role of ROS in cancer progression and the already-establish association of ND3 polymorphism with increased free radical formation, it is important to evaluate the true nature of the association of ND3 polymorphism with cancer progression.

Material and Method

Subject Selection and Grouping

An analytic cross-sectional study was conducted in Faculty of Medicine Udayana University and Sanglah General Hospital from January to December 2016. Patients with invasive breast cancer with clinical stadium II and III were included in this study. Those who had cancer other than breast cancer (e.g phyllodes cancer) or proved to be benign and refuse to take part in the study were excluded. The subjects were subsequently divided into control and case group.

Those with histological grade III and positive lymph vascular invasion were included in the case group. A blood sample was taken from each subject for DNA isolation and the clinic pathological data were obtained from medical record.

DNA Isolation and Sequencing

The DNA was isolated using Promega Blood DNA Isolation Kit and following the manufacturer instruction. The isolated DNA was subsequently amplified by Polymerase Chain Reaction (PCR) which produces 201-bp fragment product. The amplified gene product was sent to Eijkman Institute for sequencing process.

Examination of Serum 8-OHdG Concentration Blood 8-OHdG concentration was examined by Enzyme link Immunosorbent Assay (ELISA) technique using Abcam 8-hydroxy 2 deoxyguanosine ELISA Kit following the manufacturer instruction.

Statistical Analysis

All of the data obtained were analyzed descriptively to obtain the proportion of each variable in each group and the mean of 8-OHdG. Then, analytical study were conducted using independent sample T-test to evaluate the difference concentration of 8-OHdG between case and control group. Risk assessment was conducted using chi-square test by classifying the 8-OHdG concentration into high and low and assess whether the presence of ND3 polymorphism increased the risk of invasive morphology in breast cancer.

Results

70 subjects were enrolled in this study with each 35 samples in each group. The mean age was 40.93±9.204 years old for all subjects. Meanwhile, it is 34.31±5.88 years old in control group and 47.54±6.874 years in case group. After sequencing, it appear that 30 subjects (42.9%) had wild type ND3 gene, 25 had (35.7%) had G-10399-A variant and 16 (22.9%) had the C-10401-T variant. 7 subjects had two variants polymorphism while 26 had only one variant.

The extent of oxidative damage was evaluated using 8-OHdG concentration in the blood serum. For overall subjects, the mean concentration of 8-OHdG was 474.129±253.27 ng/mL. For control group, it was 393.357±198.875 ng/mL while in the case group it was 554.9±277.839 ng/mL. The descriptive analysis of the data is described in Table 1.

Table 1 Descriptive analysis of samples data

Variables	Mean
Age	Overall : 40.93±9.204 Control : 34.31±5.88 Case : 47.54±6.874
8-OHdG	Overall : 474.129±253.27 ng/mL Control : 393.357±198.875 ng/mL Case : 554.9±277.839 ng/mL
ND_3 Polymorphism	Wild Type C-10399-C : 30 (42.9%) G-10399-A : 25 (35.7%) C-10401-T : 16 (22.9%)
Number of Polymorphism	One Polymorphism : 26 (37.1%) Two Polymorphisms: 7 (10%)

Bivariate analysis clearly showed that subject with wild type ND3 gene tend to had lower level of 8-OHdG. The other 2 polymorphism tend to had significantly higher level of 8-OHdG with the highest mean concentration observed in C-10401-T variant. Because some subjects had both C-10401-T and G-10399-A variant, we also analyzed whether the presence more than one variant of ND3 gene would affect the concentration of 8-OHdG. Kruskal-Wallis analysis clearly revealed that the difference concentration between the groups were statistically significant with increasing number of polymorphism accompanied with increase concentration of 8-OHdG (Table 2).

Table2: Bivariate analysis between ND3 gene polymorphism variant and number of polymorphism with serum 8-OHdG concentration

Comparison Variables	Serum 8-OHdG Concentration	Statistical Analysis
Polymorphism:		
Wild Type	375.37±203.56 ng/mL	P < 0.05
G-10399-A	610.65±271.9 ng/mL	
C-10401-T	636.18±287.75 ng/mL	
Number of Polymorphism:		
No Polymorphism	391.45±210.97 ng/mL	P < 0.05
One Polymorphism	487.52±227.18 ng/mL	
Two Polymorphism	861.36±253.27 ng/mL	

Risk analysis revealed that the presence of polymorphism significantly increase the risk of malignant characteristic of breast cancer (Table 3).

Table 3: Risk estimation analysis between study groups with the presence of ND3 Polymorphism

		Group		Statistical Analysis
		Control	Case	
Presence of ND_3 Polymorphism	-	25	12	P < 0.05 OR: 4.792; 95%CI: 1.741 – 13.188
	+	10	23	
Total		35	35	

Discussion

Oxidative stress is undeniably plays important role in initiating carcinogenesis and cancer progression [13]. Inside the cell, mitochondria are the primary site where most free radical form especially when there is an alteration in the electron transport chain process [4]. Normally, the free radicals produced would be neutralized by endogenous antioxidant systems (superoxide dismutase, Glutathione Peroxides, and Catalase) [14]. However, in some instances, the rate of ROS formation overwhelm the antioxidant capacity which result in many health problems, in which, carcinogenesis is one of them [13].

One of possible factor that alter the electron transport chain and speed up ROS formation is the polymorphism or mutation within the elements of electron transport chain. ND3 is one of them since it is integrated within the complex I of electron transport chain [15]. Polymorphisms within ND3 gene could affect the efficiency of electron transport chain by creating an uncouple electron transport which leads to increase rate of internal ROS formation [7].

The polymorphisms of ND3 gene had been associated with increased risk of breast cancers. Czarneka et.al reported that the ND3 polymorphism A10398G was significantly associated with sporadic breast cancer in Poland [5]. Then, Canter et.al found that the same polymorphism might also contribute to the risk of breast cancer in African-American women (OR: 1.6; 95%CI: 1.10-2.31) [16].

Finally, Jiang et.al also supported those findings, stated that the A10398G ND3 polymorphism also contributed significantly to the breast cancer risk among Chinese Han women (OR: 1.49; 95%CI: 1.05-2.11) [6]. However, meta-analysis conducted by Mao et.al showed that this polymorphism may not be the risk factor of breast cancer [8].

However, this study evaluated different sets of ND3 polymorphism namely G10399A and C10401T. Despite the role of ND3 polymorphism in carcinogenesis of breast cancer, we investigated its role in cancer progression. According to our study, it clearly appear that G10399A and C10401T ND3 polymorphism significantly increased the amount of DNA damage by ROS as represented by the concentration of 8-OHdG. Furthermore, the two polymorphisms clearly increase the risk of more malignant phenotype of breast cancer, significantly associated with lymph vascular invasion and high histological grade. Our finding could be considered as unique because despite the controversies in its role in increasing breast cancer risk, the presence of ND3 polymorphism could enhance the invasiveness and malignant phenotype of breast cancer.

Recent advances in cancer researches explain the findings in our study. Free radicals are not just important in initiating carcinogenesis where they because DNA damage that eventually lead to inactivation of tumor suppressor genes or gain of function mutation of proto-oncogenes. Martinez-Outschoorn et.al found that tumor cell could induce stromal evolution and differentiation of CAF by overproducing ROS [17]. The presence of CAF within tumor stromal could significantly increase its malignant properties and eventually enhance metastasis process [18]. CAF also support cancer cell metabolism by supplying cancer cell with constant supply of lactate that then be used as carbon backbone or energy production by cancer cell [11].

CAF also secrete TGF-β that, despite its cancer inhibitory properties in early stage cancer, would enhance epithelial-to-mesenchymal transition (EMT) in the advanced cancer [19]. Since EMT enhances cell mobility and invasiveness, it would also lead to distant metastasis [20]. Furthermore, CAF also secretes many immune-modulating cytokines and VEGF family which contribute to immune-evasiveness of the tumor cells and angiogenesis [19].

Regarding of our findings and the evidences that support it, it is clear that ND3 polymorphisms could act as a biomarker to predict the behavior of breast cancer and, thus, patient's prognosis. However, further research is needed to confirm this finding in controlled laboratory research so the true association between G10399A and C10401T with breast cancer cell behavior could be elucidated.

Conclusion

G10399A and C10401T ND3 gene polymorphism were associated with increased oxidative damage of the DNA by ROS and increased the risk of more malignant phenotype of breast cancer.

However, the findings need to be validated with further research to elucidate the true association and the mechanism of malignancy enhancement caused by these two polymorphisms.

Acknowledgement

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