ELSEVIER

Cancer Genetics and Cytogenetics ■ (2009) ■

Cancer Genetics and Cytogenetics

Polymorphism (ALA16VAL) correlates with regional lymph node status in breast cancer

Claudia Giuliano Bica[a,*], Leonardo Leiria de Moura da Silva[a], Nadima Vieira Toscani[a], Cláudio Galleano Zettler[a], Maria Gabriela do Valle Gottlieb[b], Cláudio Osmar Pereira Alexandre[a], Márcia Silveira Graudenz[a], Ivana Beatrice Mânica da Cruz[b,c,*]

[a]*Programa de Pós-Graduação em Patologia, Universidade Federal de Ciências Médicas de Porto Alegre, Porto Alegre, Brazil*
[b]*Programa de Pós-Graduação em Ciências Biomédicas (Bioquímica Toxicológica)*
[c]*Programa de Pós-Graduação em Ciências Biomédicas (Farmacologia), Universidade Federal de Santa Maria, Av. Roraima 1000 Prédio 19—Santa Maria, Brazil 97.900-120*

Abstract We studied the possible association between Ala16Val manganese-dependent superoxide dismutase (MnSOD) gene genotypes and breast cancer lymph node status because previous investigations suggested an association between the AA genotype and breast cancer. We included 281 women (188 controls and 93 cases of invasive breast cancer with axillary lymph node metastasis (LN+) and without lymph node metastasis (LN−). DNA was extracted from paraffin-embedded tumor tissue or peripheral blood leukocytes, and MnSOD polymorphism was determined by polymerase chain reaction—restriction fragment length polymorphism techniques. In addition, the immunohistochemical profile (p53, Ki-67 and estrogen/progesterone receptors) was also compared between invasive breast cancer groups and different MnSOD genotypes. The frequency of the VV genotype was higher in the LN+ group than in the control and LN− groups ($\chi^2 = 5.081$, $P = 0.02$). Subjects with LN+ breast cancer (LN+ group) showed a higher incidence of VV genotype carriers associated with positive Ki-67 marker. Subjects with LN+ breast cancer (LN+ group) showed a higher incidence of VV genotype carriers associated with negative p53 marker. Despite the fact that the AA genotype is well established as being associated with an increased risk of breast cancer, the VV genotype may be associated with a higher metastatic potential, suggesting that MnSOD imbalance is the condition associated with carcinogenesis.

1. Introduction

It is well recognized that oxidative stress plays a role in different stages of carcinogenesis, and that the most important cellular protective mechanisms against oxidative stress involve antioxidant enzymes. Their action is based on the degradation of reactive oxygen species (ROS) and their transformation to H_2O_2. Manganese superoxide dismutase (MnSOD) is an enzyme with a major role in the mechanism of cellular defense against oxidative stress−inducing agents. This enzyme is critical in the management of oxidative stress by catalyzing the formation of hydrogen peroxide from two superoxide anions. Hydrogen peroxide is then converted to water by gluthathione peroxidase or catalase.

Some studies suggest that neoplastic cells in breast carcinomas retain their capacity to produce MnSOD and are thus protected from possible cellular damage due to reactive oxygen species [1,2]. In addition, MnSOD content varies according to the degree of differentiation of breast carcinoma [3]. Additionally, investigations in experimental models reported that superoxide dismutase derivatives can significantly inhibit both the production of ROS and metastatic tumor growth [4]. On the other hand, Connors et al. [5] tested the hypothesis that the MnSOD-dependent production of H_2O_2 can influence the invasive and migratory properties of tumor cell lines. The authors used both in vitro and in vivo methods to examine the role of MnSOD in invasion and migration, and found that the increase in mitochondrial H_2O_2 induced by MnSOD enhanced the invasive and migratory properties of tumor cells, whereas an efficient H_2O_2 detoxification could restrict the metastatic phenotype.

* Corresponding author. Tel.: +55-55-32208736; fax: +55-55-32208239.
E-mail address: ibmcruz@hotmail.com (I.B. Mânica da Cruz).

0165-4608/09/$ − see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.cancergencyto.2009.09.011

The MnSOD gene, located on human chromosome 6q25 and also known as SOD2, shows a polymorphism in the mitochondrial targeting sequence (a valine-to-alanine substitution, rs4880) that results in three different genotypes: AA, VV, and AV at amino acid position 16 [6]. Single amino acid polymorphism alanine (Ala) to valine (Val) at the 16th amino acid (16th amino acid from the beginning of the signal sequence or−9th amino acid from the first amino acid of the mature protein) of the signal sequence of MnSOD (Ala16Val) has been suggested to change the secondary structure of the premature protein and, therefore, the mitochondrial targeting of the enzyme. Recent investigations have used in vitro import of chimeric proteins composed of either one of the MnSOD/MTS fused to the mouse dihydrofolate reductase protein, and the import of the two human MnSOD precursor variants into rat liver mitochondria. The results showed that the Ala-MnSOD precursor generated 30−40% more of the active matricial, processed MnSOD homotetramer, than did the Val-MnSOD precursor. In this case, the Ala-MnSOD/MTS allows efficient MnSOD import into the mitochondrial matrix, while the Val variant causes partial arrest of the precursor within the inner membrane and decreased formation of the active MnSOD homotetramer in the mitochondrial matrix [7].

The Ala allele or genotype AA, thought to alter transport of the enzyme into mitochondria, has been associated with increased risk for breast cancer [8−14]. Additional studies show possible interaction between this polymorphism and antioxidant dietary pattern [15] and other lifestyle variables such as cigarette smoking [16] in breast cancer risk.

It is surprising that additional epidemiologic studies suggested that the V allele is not a ''good genetic variant'' because it has been associated with carotid atherosclerosis quantified as intima-media thickness by ultrasound [17], nonfamilial dilated cardiomyopathy in Japanese subjects [18], higher oxidized LDL levels in Brazilian subjects with synergic effect in diabetes type II patients, and obesity [19,20]. In addition, the A allele variant of the signal peptide increases mitochondrial MnSOD activity, protects macrophages against oxLDL-induced apoptosis, and reduces the risk of coronary artery disease and acute myocardial infarction [21].

These results suggest that a ''paradox'' exists in the MnSOD polymorphism because there is an imbalance in anti-oxidant modulation, mainly in homozygosis (AA and VV), which increases superoxide levels in VV subjects or hydrogen peroxide levels in AA subjects. In both cases, environmental interactions could increase or decrease risk of nontransmissible morbidities such as neoplasias and cardiovascular diseases.

In previous studies performed by our group, we found an association between the AA genotype of the Ala16Val MnSOD polymorphism with increased risk for prostate and breast cancer and immunosenescence profile, as well as DNA damage [22] and breast cancer in males . Taken together, these results suggest that the role of MnSOD in the initiation of carcinogenesis is strongly implicated in cellular damage in the etiology of several types of cancer because DNA mutation is a critical step in carcinogenesis [20]. DNA damage can result in either arrest or induction of transcription, induction of signal transduction pathways, replication errors, and genomic instability, all of which are associated with carcinogenesis. Therefore, the imbalance associated with the Ala allele of the Ala16Val MnSOD gene polymorphism could increase H_2O_2 in the cytosol, which could induce production of reactive oxygen species (ROS), cause DNA damage, and subsequently increase breast cancer risk.

On the other hand, there is cumulative evidence to support divergent effects on cell proliferation and death signaling for the two major intracellular reactive oxygen intermediates, O_2^- and H_2O_2. Under normal physiologic states, the intracellular ratio of O_2^- and H_2O_2 is maintained by a tight control imposed by cellular antioxidant enzyme systems, which helps in keeping a balance between the rates of cell proliferation and cell death, maintaining a homeostatic growth [23].

Based on these results, we hypothesized that Ala16Val MnSOD homozygous genotypes (AA and VV) represent a genetic MnSOD unbalanced state that is differentially associated with risk in different cancer stages. To analyze this hypothesis, we studied here the possible association between Ala16Val MnSOD gene genotypes and breast cancer lymph node status in women.

2. Materials and methods

2.1. Design and subjects

In a previous case−control study performed by our group including 470 subjects in which we analyzed the association between Ala16Val MnSOD gene polymorphism in male and female breast cancer, we selected 93 patients with invasive breast cancer: 48 subjects with axillary lymph node metastasis (LN+) and 45 subjects without lymph node metastasis (LN−) plus 188 controls. Genotype and allelic frequencies of these subjects were compared to 188 control women. The groups were corrected for age and breast cancer biomarke (cell cycle and hormonal variables) profile detected by immunohistochemical staining in cancer tissue (p53, Ki-67, and estrogen/progesterone receptors). Clinical stages (I, II, and III) were compared between cancer groups and Ala16Val polymorphism carriers. As the study includes genetic variables, samples were recruited by random selection of Brazilians of European ancestry from the metropolitan area of Porto Alegre, Rio Grande do Sul State (RS). Compared with Brazil as a whole, the population of this state is composed mainly of people of European ancestry (82%). A previous study performed by Cruz et al. [24] in different ethnic populations showed different allele and genotype distributions, as well as different associations with diseases or biologic characteristics. For this reason, we just analyzed Caucasian subjects, who represent the main ethnic group [25,26].

European-descendent patients with breast cancer were consecutively recruited from the breast cancer unit of Complexo Hospitalar Santa Casa located in Porto Alegre, capital of the southernmost state of Brazil. Unlike Brazil as a whole, the population of this state is composed mainly of people of European ancestry (82%) [27]. Control women were recruited by random selection from the Health and Social Assistance Program of Gravataí, a city from the Porto Alegre Metropolitan area, Rio Grande do Sul State. All volunteers went for an outpatient visit at the research unit for laboratory tests, physical examination, and interviews. The Institutional Committee of Ethics approved the present study, and informed consent was obtained from all individuals whose information was prospectively collected.

2.2. MnSOD genotyping

Genomic DNA was isolated from peripheral blood leukocytes and from tissue samples using the DNA Mini Kit Purification (Mo Bio). The method used here to detect Ala16Val polymorphism is described in detail in Bica et al. The polymerase chain reaction product (10 μL) was digested with HaeIII (15 units, 37 °C, 6 hours; Gibco). Digested products [23 and 85 base pairs (bp)] were visualized on a 4% agarose gel (Amersham Biosciences Inc., Amersham, UK) stained with ethidium bromide. A mutation was introduced by a primer mismatch to create a restriction cut site for HaeIII in the −9 codon, and the following genotypes were observed: −9Ala/Ala (23 and 85 bp), −9Ala/Val (23, 85, and 110 bp), and −9Val/Val (110 bp).

2.3. Statistical analysis

We estimated allele frequencies were estimated by using the gene-counting method. Chi square analysis was used to estimate Hardy-Weinberg equilibrium. The allele and genotype frequencies were compared between the two patient groups (LN+ and LN−), using the chi-square test or Fisher's exact test. All levels of significance used were two-tailed. The odds ratio and 95% confidence intervals (95%CI) were determined for genotype/allele comparisons. To test intervening factors, we performed a multivariate analysis using forward Wald logistic regression. A computer statistics package (SPSS 11.0, Chicago, IL) was used for statistical analyses in this study.

3. Results

The demographics, breast cancer biomarkers (cell cycle and hormonal variables), and cancer clinical stages observed in the LN+ and LN− groups are shown in Table 1. Clinical stage comparison between the LN+ and LN− groups was not statistically significant.

The VV and AA + AV carriers were compared with regard to the percentage of compromised lymph nodes in the breast cancer group. VV subjects showed 27.92 ± 32.99 compromised lymph nodes, whereas the AA + AV carriers had only 13.47 ± 24.89. Despite the broad standard deviation, these means do differ statistically ($P = 0.04$).

The gene frequencies were all in Hardy-Weinberg equilibrium in the groups investigated here. Ala allele

Table 1
Demographic, cancer familial history, cell cycle and hormonal markers of the patients with lymph node (LN+) and without lymph node breast cancer (LN−)

Variables	LN+ ($n = 48$)	LN− ($n = 45$)	P[a]
Age [mean ± SD] (yr)	57.91 ± 12.14	58.15 ± 1.73	0.908
Breast cancer in first-degree relatives (%)	48.5	51.7	0.965
Age			
<50 years (%)	27.7	28.9	0.896
≥50 years (%)	72.3	71.1	
Tumor histologic type			
Lobular carcinoma (%)	12.8	14.0	0.689
Ductal carcinoma (%)	87.2	86.0	
ER/PR			
Positive	83.0	72.1	0.324
Negative	17.0	27.9	
Ki-67			
Positive	87.1	79.4	0.621
Negative	12.9	20.6	
p53			
Positive	35.3	25.7	0.547
Negative	64.7	74.3	
Clinical stages			
I	6.5	18.2	0.09
II	56.5	61.4	
III	37.0	20.5	

Abbreviation: SD, standard deviation; ER/PR, estrogen receptor/progesterone receptor.
[a] From the chi square test (categoric variables) or Student's *t*-test (continuous variables).

Table 2
MnSOD polymorphism genotype frequencies in control, LN+ and LN− groups

	MnSOD polymorphism genotypes		
Groups	VV	AA	AV
Control	43 (22.9)[a]	27 (14.4)	118 (62.8)
LN−	9 (20.0)[a]	7 (15.6)	29 (64.4)
LN+	20 (41.7)[b]	3 (6.3)	25 (52.1)

Groups with same letters did not present statistical differences in the chi square test.

frequency was 0.260 and Val allele frequency was 0.740 in the LN+ group. Ala allele frequency was 0.478 and Val allele frequency was 0.522 in the LN− group. As can be seen in Table 2, the frequency of the VV genotype was more frequent in the LN+ than in the LN− and the control groups ($\chi^2 = 5.081$, $P = 0.02$). Odds ratio to show the VV genotype was 2.857 (95%CI, 1.129-7.233) in the LN+ group when compared to LN− group.

Additional analysis comparing the odds ratio in the association between VV genotype and demographics, cell proliferation, and hormonal biomarkers was performed (Table 3). Subjects with LN+ breast cancer (LN+ group) showed a higher incidence of VV genotype carriers with positive Ki-67 marker. On the other hand, subjects with LN+ breast cancer (LN+ group) showed a higher incidence of VV genotype carriers with negative p53 marker. Multivariate analysis showed that the results obtained were independent of age, breast cancer in first-degree relatives, and clinical stages.

4. Discussion

In this study, we report an association between VV genotype and lymph node involvement in women with breast cancer. Several lines of evidence demonstrate that ROS are involved in carcinogenesis and cancer progression. In this process, the modulation of endogenous antioxidant defenses by antioxidant enzymes is crucial, mainly in the superoxide and hydrogen peroxide balance. As the antioxidant enzymes derive from the expression of nuclear genes, functional polymorphisms could affect this oxidative balance, as in the case of the Ala-16Val MnSOD gene polymorphism. Previous case−control studies suggested an association between the AlaAla genotype and breast cancer risk. Most of these investigations, however, were limited to an association between the polymorphism and cancer initiation (carcinogenesis) without analyzing the relationship with cancer progression. In this paper, we performed these analyses comparing two female breast cancer groups (positive and negative lymph nodes). In the first instance, the results described here that showed an association between positive lymph nodes and VV genotype could be paradoxical, since previous studies found an association between the AA genotype and breast cancer risk, including two studies performed by our research team [12].

Our results could be biologically explained, considering that the functional enzyme from the VV genotype is less capable of lowering cellular levels of oxidative damage compared to the AA genotype.

This differential capacity is related to the A allele, which confers a 40% higher MnSOD activity than does the V allele after import into isolated mitochondria in vitro. A recent study analyzed the functional consequences in whole cells of the Ala-16-Val polymorphism using HuH7 human hepatoma cells transfected with vectors encoding the A and V MnSOD-variants. The Ala variant resulted in fourfold higher levels of the mature exogenous protein and MnSOD activity compared to the Val-variant. The study showed that slowly imported Val-MnSOD is degraded by proteasomes in cells and may be associated with decreased mRNA stability, possibly due to impaired cotranslational import [28].

In this way, the conversion rate of superoxide into hydrogen peroxide associated with the A allele is more effective, but excessive superoxide dismutation to H_2O_2, which is an important oxidative molecule, could stimulate cancer

Table 3
Odds ratio to age, cell cycle and hormonal markers variables LN+ and LN- groups with VV genotype

	LN+ group		LN− group	
Variables	OR to VV	95%CI	OR to VV	95%CI
Age				
<50 years	3.000	0.737−12.209	0.636	0.366−1.007
≥ 50 years	1.882	0.873−4.058	0.753	0.538−1.053
Ki-67				
Positive	3.250	1.213−8.708	0.609	0.410−0.904
Negative	1.750	0.265−20.325	0.428	0.628−4.880
p53				
Positive	2.250	0.585−8.652	0.643	0.331−1.250
Negative	2.836	1.182−6.806	0.563	0.343−0.923
ER/PR				
Positive	1.987	0.874−4.516	2.500	0.818−7.642
Negative	2.500	0.818−7.642	0.500	0.193−1.296

OR, odds ratio; 95%CI, 95% confidence interval.

progression. These results have a biologic plausibility if we consider studies that investigated MnSOD expression modulation associated with breast cancer progression. Some studies have suggested that MnSOD suppresses cell growth in several tumor cells in vivo and in vitro [29,30]. Although the molecular mechanism of this repressive effect in tumor cells is not fully understood, MnSOD expression has been reported to be more frequent in tumor cells of invasive breast carcinomas than in situ carcinomas or non-neoplastic breast epithelial cells . Kim and colleagues described the potential role of manganese superoxide dismutase enzyme in the inhibition of tumor cell growth [31]. The study showed an apparent decrease in MnSOD levels in numerous tumor cell lines when compared to their non-malignant counterparts. These data led to the proposal that MnSOD could exert a tumor suppressive effect in estrogen-dependent human breast cancer cells. Kattan and colleagues investigated the role of manganese superoxide dismutase on the growth and invasive properties of human estrogen-independent breast cancer cells. The authors also concluded that MnSOD plays a role in regulating tumor cell growth and invasive properties of estrogen-independent metastatic breast cancer cells [32].

Although these results are not universal, based on this study, we suggest that Ala16Val MnSOD gene polymorphism is associated with breast cancer metastatic potential beause the VV genotype was found to be more frequent in LN+ breast cancer.

Therefore, despite the methodologic limitation of our study, we suggest here the possible association between VV genotype and breast cancer progression, Ki-67, and 53 cell cycle markers. Based on these results and previous studies, we suggest that the MnSOD imbalance that occurs in the Ala16Val MnSOD homozygous states (AA and VV) confers a differential role in breast cancer initiation and progression. In this case, tumor initiation, the AA genotypes potentially increase breast cancer risk, whereas in tumor progression, the VV genotype seems to be the risk genotype. This MnSOD imbalance, however, can be positively or negatively influenced by other intervening variables such as diet, physical activity patterns, smoking addiction, alcohol consumption, and genes, and needs to be considered in further investigations.

Acknowledgments

The authors thank Professor João Carlos Prolla, Márcia Wink, Maira Caleffi, and Silvana de Almeida for their helpful assistance and suggestions, and Maristela Taufer and Graziela Oliveira for help in molecular genetics analysis. Dr. A. Leyva provided English editing of the manuscript.

References

[1] Chung-man Ho J, Zheng S, Comhair SA, Farver C, Erzurum SC. Differential expression of manganese superoxide dismutase and catalase in lung cancer. Cancer Res 2001;61:8578−85.

[2] Skrzydlewska E, Kozuszko B, Sulkowska M, Bogdan Z, Kozlowski M, Snarska J, Puchalski Z, Sulkowski S, Skrzydlewski Z. Antioxidant potential in esophageal, stomach and colorectal cancers. Hepatogastroenterology 2003;50:126−31.

[3] Tsanou E, Loachim E, Briasoulis E, Damala K, Charchanti A, Karavasilis V, Pavlidis N, Agnantis NJ. Immunohistochemical expression of superoxide dismutase (MnSOD) anti-oxidant enzyme in invasive breast carcinoma. Histol Histopathol 2004;19:807−13.

[4] Hyoudou K, Nishikawa M, Kobayashi Y, Ikemura M, Yamashita F, Hashida M. SOD derivatives prevent metastatic tumor growth aggravated by tumor removal. Clin Exp Metastasis 2008;25:531−6.

[5] Connor KM, Hempel N, Nelson KK, Dabiri G, Gamarra A, Belarmino J, Van De Water L, Mian BM, Melendez JA. Manganese superoxide dismutase enhances the invasive and migratory activity of tumor cells. Cancer Res 2007;67:10260−7.

[6] Shimoda-Matsubayashi S, Matsumine H, Kobayashi T, Nakagawa-Hattori Y, Shimizu Y, Mizuno Y. Structural dimorphism in the mitochondrial targeting sequence in the human manganese superoxide dismutase gene. A predictive evidence for conformational change to influence mitochondrial transport and a study of allelic association in Parkinson's disease. Biochem Biophys Res Commun 1996;226:561−5.

[7] Sutton A, Khoury H, Prip-Buus C, Cepanec C, Pessayre D, Degoul F. The Ala16Val genetic dimorphism modulates the import of human manganese superoxide dismutase into rat liver mitochondria. Pharmacogenet 2003;13:145−57.

[8] Mitrunen K, Sillanpää P, Kataja V, Eskelinen M, Kosma VM, Benhamou S, Uusitupa M, Hirvonen A. Association between manganese superoxide dismutase (MnSOD) gene polymorphism and breast cancer risk. Carcinogenesis 2001;22:827−9.

[9] Millikan RC, Player J, de Cotret AR, Moorman P, Pittman G, Vannappagari V, Tse CK, Keku T. Manganese superoxide dismutase Ala-9Val polymorphism and risk of breast cancer in a population-based case-control study of African Americans and whites. Breast Cancer Res 2004;6:264−74.

[10] Cai Q, Shu XO, Wen W, Cheng JR, Dai Q, Gao YT, Zheng W. Genetic polymorphism in the manganese superoxide dismutase gene, antioxidant intake, and breast cancer risk: results from the Shanghai Breast Cancer Study. Breast Cancer Res 2004;6:647−55.

[11] Bergman M, Ahnström M, Palmebäck Wegman P, Wingren S. Polymorphism in the manganese superoxide dismutase (MnSOD) gene and risk of breast cancer in young women. J Cancer Res Clin Oncol 2005;131:439−44.

[12] Slanger TE, Chang-Claude J, Wang-Gohrke S. Manganese superoxide dismutase Ala-9Val polymorphism, environmental modifiers, and risk of breast cancer in a German population. Cancer Causes Control 2006;17:1025−31.

[13] Cox DG, Tamimi RM, Hunter DJ. Gene x gene interaction between MnSOD and GPX-1 and breast cancer risk: a nested case-control study. BMC Cancer 2006;6:217.

[14] Bica CG, de Moura da Silva LL, Toscani NV, da Cruz IB, Sá G, Graudenz MS, Zettler CG. MnSOD gene polymorphism association with steroid-dependent cancer. Pathol Oncol Res 2009;15:19−24.

[15] Ambrosone CB, Freudenheim JL, Thompson PA, Bowman E, Vena JE, Marshall JR, Graham S, Laughlin R, Nemoto T, Shields PG. Manganese superoxide dismutase (MnSOD) genetic polymorphisms, dietary antioxidants, and risk of breast cancer. Cancer Res 1999;59:602−6.

[16] Tamimi RM, Hankinson SE, Spiegelman D, Colditz GA, Hunter DJ. Manganese superoxide dismutase polymorphism, plasma antioxidants, cigarette smoking, and risk of breast cancer. Cancer Epidemiol Biomarkers Prev 2004;13:989−96.

[17] Kakko S, Päivänsalo M, Koistinen P, Kesäniemi YA, Kinnula VL, Savolainen MJ. The signal sequence polymorphism of the MnSOD gene is associated with the degree of carotid atherosclerosis. Atherosclerosis 2003;168:147−52.

[18] Hiroi S, Harada H, Nishi H, Satoh M, Nagai R, Kimura A. Polymorphisms in the SOD2 and HLA-DRB1 genes are associated with

nonfamilial idiopathic dilated cardiomyopathy in Japanese. Biochem Biophys Res Commun 1999;261:332−9.

[19] Gottlieb MG, Schwanke CH, Santos AF, Jobim PF, Müssel DP, da Cruz IB. Association among oxidized LDL levels, MnSOD, apolipoprotein E polymorphisms, and cardiovascular risk factors in a south Brazilian region population. Genet Mol Res 2005;4: 691−703.

[20] Montano MA, Barrio Lera JP, Gottlieb MG, Schwanke CH, da Rocha MI, Manica-Cattani MF, Dos Santos GF, da Cruz IB. Association between manganese superoxide dismutase (MnSOD) gene polymorphism and elderly obesity. Mol Cell Biochem 2009;. [Epub ahead of print].

[21] Fujimoto H, Taguchi JI, Imai Y, Ayabe S, Hashimoto H, Kobayashi H, Ogasawara K, Aizawa T, Yamakado M, Nagai R, Ohno M. Manganese superoxide dismutase polymorphism affects the oxidized low-density lipoprotein-induced apoptosis of macrophages and coronary artery disease. Eur Heart J 2008;29: 1267−74.

[22] Taufer M, Peres A, de Andrade VM, de Oliveira G, Sá G, do Canto ME, dos Santos AR, Bauer ME, da Cruz IB. Is the Val16Ala manganese superoxide dismutase polymorphism associated with the aging process? J Gerontol A Biol Sci Med Sci 2005;60:432−8.

[23] Agnoletto MH, Guecheva TN, Donde F, de Oliveira AF, Franke F, Cassini C, Salvador M, Henriques JA, Saffi J. Association of low repair efficiency with high hormone receptor expression and SOD activity in breast cancer patients. Clin Biochem 2007;40:1252−8.

[24] Da Cruz IB, Oliveira G, Taufer M, Leal NF, Schwanke CH, Glock L, Moriguchi Y, Moriguchi EH. Angiotensin I-converting enzyme gene polymorphism in two ethnic groups living in Brazil's southern region: association with age. J Gerontol A Biol Sci Med Sci 2003; 58:M851−6.

[25] Parra FC, Amado RC, Lambertucci JR. Color and genomic ancestry in Brazilians. Proc Natl Acad Sci USA 2003;100:177−82.

[26] Mattevi VS, Zembrzuski VM, Hutz MH. Association analysis of genes involved in the leptin-signaling pathway with obesity in Brazil. Int J Obesity 2002;26:1979−85.

[27] Marrero AR, Das Neves Leite FP, De Almeida Carvalho B, Peres LM, Kommers TC, Da Cruz IM, Salzano FM, Ruiz-Linares A, Da Silva Júnior WA, Bortolini MC. Heterogeneity of the genome ancestry of individuals classified as White in the state of Rio Grande do Sul, Brazil. Am J Hum Biol 2005;17:496−506.

[28] Sutton A, Imbert A, Igoudjil A, Descatoire V, Cazanave S, Pessayre D, Degoul F. The manganese superoxide dismutase Ala16Val dimorphism modulates both mitochondrial import and mRNA stability. Pharmacogenet Genomics 2005;15:311−9.

[29] Soini Y, Vakkala M, Kahlos K, Pääkkö P, Kinnula V. MnSOD expression is less frequent in tumor cells of invasive breast carcinomas than in in situ carcinomas or nonneoplastic breast epithelial cells. J Pathol 2001;195:156−62.

[30] Oberley LW. Mechanism of the tumor suppressive effect of MnSOD overexpression. Biomed Pharmacother 2005;59:143−8.

[31] Kim KH, Rodriguez AM, Carrico PM, Melendez JA. Potential mechanisms for the inhibition of tumor cell growth by manganese superoxide dismutase. Antioxid Redox Signal 2001;3:361−73.

[32] Kattan Z, Minig V, Leroy P, Dauça M, Becuwe P. Role of manganese superoxide dismutase on growth and invasive properties of human estrogen-independent breast cancer cells. Breast Cancer Res Treat 2008;108:203−15.