

A 40-bp Insertion/Deletion Polymorphism of Murine Double Minute2 (MDM2) Increased the Risk of Breast Cancer in Zahedan, Southeast Iran

Mohammad Hashemi^{*1,2}, Mohsen Omrani², Ebrahim Eskandari-Nasab², Seyed-Shahaboddin Hasani², Mohammad Ali Mashhadi³ and Mohsen Taheri⁴

¹Cellular and Molecular Research Center, Zahedan University of Medical Sciences, Zahedan; ²Dept. of Clinical Biochemistry, School of Medicine, Zahedan University of Medical Sciences, Zahedan; ³Dept. of Internal Medicine, School of Medicine, Zahedan University of Medical Sciences, Zahedan; ⁴Genetics of Non-Communicable Disease Research Center, Zahedan University of Medical Sciences, Zahedan, Iran

Received 2 November 2013; revised 5 April 2014; accepted 8 April 2014

ABSTRACT

Background: MDM2 (Murine Double Minute2) is an oncoprotein that inhibits the P53 activity. Overexpression of *MDM2* gene has been reported in several human tumors. In the present study, we aimed to evaluate the impact of 40-bp insertion/deletion (ins/del) polymorphism on the promoter of *MDM2* and susceptibility to breast cancer in a sample of Iranian population. **Methods:** This case-control study was carried out on 236 patients with breast cancer and 203 healthy individuals. Genomic DNA was extracted from the whole blood by the salting-out method. The 40-bp ins/del polymorphism was determined by using polymerase chain reaction. **Results:** The findings indicated that *MDM2* ins/del variant increased the risk of breast cancer in co-dominant- (odds ratio [OR] = 2.09, 95% CI = 1.14-3.85, $P = 0.018$, del/del vs. ins/ins), dominant- (OR = 1.49, 95% CI = 1.02-2.18, $P = 0.038$, ins/del + del/del vs. ins/ins), and recessive- (OR = 1.86, 95% CI = 1.03-3.34, $P = 0.038$, del/del vs. ins/ins + ins/del) tested inheritance models. The del allele increased the risk of breast cancer (OR = 1.48, 95% CI = 1.11-1.98, $P = 0.008$) compared with ins allele. **Conclusions:** Our result revealed that 40-bp ins/del polymorphism in the promoter of *MDM2* increased the risk of breast cancer in an Iranian population. Further investigations with larger sample sizes and diverse ethnicities are needed to verify our findings. *Iran. Biomed. J. 18 (4): 245-249, 2014*

Keywords: Breast cancer, Murine Double Minute2 (*MDM2*), Polymorphism

INTRODUCTION

Breast cancer is one of the most common forms of cancers among Iranian women [1]. The exact cause of breast cancer is still unknown, but genetic factors are shown to play essential roles in the pathogenesis and progress of breast cancer [2]. The p53 transcription factor, encoded by the *p53* tumor suppressor gene, is an essential regulator of the cellular stress responses [3]. Among the genetic alterations, the tumor suppressor protein, P53, is a principal mediator of multiple cellular functions, including growth arrest, senescence, and apoptosis in response to cellular damage [4, 5]. The activity of P53 may either be inactivated or be attenuated in a vast majority of human cancers through mutations in the *P53* gene or aberrant expression of proteins acting in the P53 pathway, such as Murine Double

Minute2 (*MDM2*) [6].

MDM2, coded by the *MDM2* gene, is a key negative regulator of *P53*. Besides its directly inhibiting the transcriptional activity of *P53*, *MDM2* also functions as an E3 ubiquitin ligase responsible for the ubiquitination and proteolytic degradation of p53 [7]. Gene expression changes induced by p53 lead either to cell cycle arrest, which enables cells to repair DNA damage, or to apoptosis [8]. Overexpression of *MDM2* is observed both in epithelial cells of transgenic mice with induced mammary carcinomas [9] and in various human tumors, including breast cancer [10, 11]. Consequently, increased levels of p53 inhibitors in tumor cells resulted in the loss of p53 function. In response to many forms of stresses, the association between p53 and *MDM2* is disrupted, leading to p53 stabilization and activation [12].

The human *MDM2* is located on chromosome

*Corresponding Author; Tel.: (+98-541) 3235122; E-mail: mhd.hashemi@gmail.com or hashemim@zdmu.ac.ir

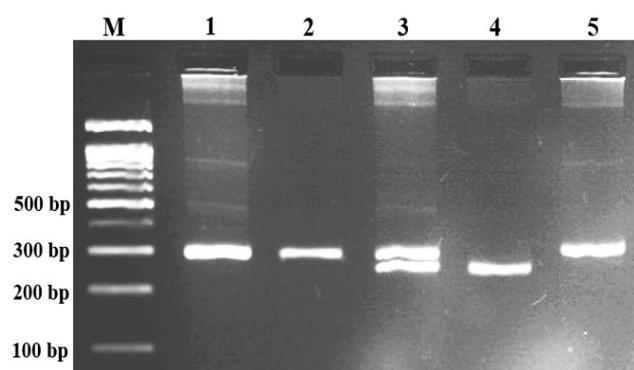


Fig. 1. Electrophoresis pattern of PCR product of 40-bp ins/del polymorphism of MDM2 resolved by 2% agarose gel electrophoresis. M, DNA marker; Lanes 1, 2, 5, ins/ins; lane 3, ins/del; lane 4, del/del.

12q14.3-15 and contains 11 exons. The *MDM2* gene has a basal promoter (P1) and an alternative promoter (P2) starting in the intron 1 [13]. The promoter P2 contains a p53-responsive element and has been shown to regulate MDM2 levels in stressed cells, while the promoter P1 functions principally in a non-stressed environment [13, 14]. Genetic variant rs2279744 (SNP309 T/G) within the intronic p53-responsive promoter of the *MDM2* has been shown to be associated with the increased affinity of the transcriptional activator Sp1, resulting in higher levels of *MDM2* mRNA and protein. This SNP has been shown to attenuate apoptotic activity and accelerate tumor formation [15, 16]. Several studies have reported the associations between rs2279744 variant and the risk of different types of cancer [17-19].

There is little and controversial data regarding the impact of 40-bp insertion/deletion (ins/del) polymorphism on the constitutive promoter of *MDM2* gene and cancer risk [20-22]. Therefore, the present study was aimed to find out the possible association between 40-bp ins/del polymorphism in the promoter region of *MDM2* and breast cancer in a sample of Iranian population.

MATERIALS AND METHODS

Patients. This case-control study was performed on 236 histopathologically confirmed breast cancer patients and 203 age-matched women with no history of cancer of any type (as the control group) in a Southeast Iranian population. The clinicopathologic characteristics of the patients have been described in detail previously [23]. Ethical approvals for recruitment were obtained from local Ethics Committee of Zahedan University of Medical Sciences (Iran), and an informed consent was obtained from all

patients and healthy individuals. Blood samples from patients and healthy controls were collected in EDTA tubes, and DNA were extracted using salting-out method as described previously [24].

Genotyping. Genotyping of 40-bp ins/del polymorphism of MDM2 was performed using forward 5'-GACCACTATGTTTAAGGAAG-3' and reverse 5'-TGACTCACCTACTTTCCAC-3' primers. PCR was performed using commercially available PCR premix (AccuPower PCR PreMix; Bioneer, Daejeon, South Korea) according to the manufacturer's recommended protocol. The PCR cycling conditions were initial denaturation at 95°C for 5 min, followed by 30 cycles of 30 s at 95°C, 25 s at 59°C, 30 s at 72°C, with a final extension of 72°C for 10 min. The product sizes for the heterozygous ins/del production were 287 and 247 bp, respectively. The PCR products were verified onto 2% agarose gels containing 0.5 µg/ml ethidium bromide, and observed under a UV light (Fig. 1). To ensure genotyping quality, we re-genotyped random samples (approximately 20% of total samples) and found no genotyping mistake.

Statistical analysis. Statistical analysis was calculated using statistical package SPSS 18 software. Data were analyzed by independent sample t-test and χ^2 test. The association between MDM2 ins/del variant and breast cancer was assessed by computing the odds ratio (OR) and 95% CI from logistic regression analyses. A *P* value less than 0.05 were considered statistically significant.

RESULTS

The study group consists of 236 breast cancer patients with an average age of 47.1 ± 12.3 years and 203 healthy women with a mean age of 45.3 ± 12.8 years. No significant difference was found between the groups concerning age (*P* = 0.136).

The genotype and allele frequencies of *MDM2* ins/del polymorphism in breast cancer patients and healthy subjects are shown in Table 1. The finding indicated that ins/del variant increased the risk of breast cancer in co-dominant- (OR = 2.09, 95% CI = 1.14-3.85, *P* = 0.018, del/del vs. ins/ins), dominant- (OR = 1.49, 95% CI = 1.02-2.18, *P* = 0.038, ins/del + del/del vs. ins/ins) and recessive- (OR = 1.86, 95% CI = 1.03-3.34, *P* = 0.038, del/del vs. ins/ins + ins/del) tested inheritance models.

The deletion allele increased the risk of breast cancer (OR = 1.48, 95% CI = 1.11-1.98, *P* = 0.008) in comparison with insertion allele. The genotype frequency of the MDM2 ins/del polymorphism was tested for Hardy-Weinberg equilibrium separately in

Table 1. Genotypic and allelic frequencies 40-bp ins/del polymorphism of MDM2 in breast cancer patients and control subjects

MDM2 40-bp ins/del	Breast cancer n (%)	Control n (%)	OR (95% CI)	P value
Co-dominant				
ins/ins	109 (46.2)	114 (56.1)	1.00	-
ins/del	89 (37.7)	70 (34.5)	1.33 (0.88-2.00)	0.178
del/del	38 (16.1)	19 (9.4)	2.09 (1.14-3.85)	0.018
Dominant				
ins/ins	109 (46.2)	114 (56.1)	1.00	-
ins/del + del/del	127 (53.8)	89 (43.8)	1.49 (1.02-2.18)	0.038
Recessive				
ins/ins + ins/del	198 (83.9)	184 (90.6)	1.00	-
del/del	38 (16.1)	19 (9.4)	1.86 (1.03-3.34)	0.038
Alleles				
ins	307 (67.2)	298 (73.4)	Ref.	-
del	165 (32.8)	108 (26.7)	1.48 (1.11-1.98)	0.008

cases and controls. The genotype in controls ($\chi^2 = 2.77$, $P = 0.100$) but not in cases ($\chi^2 = 6.88$, $P = 0.008$) was in Hardy-Weinberg equilibrium.

In breast cancer patients, the ins/del polymorphism was not associated with age, tumor grade, disease stage, estrogen/progesterone receptor, and HER2/neu status (data not shown).

DISCUSSION

In the present study, we investigated the impact of 40-bp ins/del polymorphism of *MDM2* on risk of breast cancer in a sample of Iranian population. The results showed that the del allele increased the risk of breast cancer in our population, and carriers of del allele were at 1.5-fold higher risk of breast cancer than those subjects with the Ins allele. Additionally, the del/del genotypes in the co-dominant and recessive models as well as the ins/del + del/del genotype in the dominant model were risk factors for developing breast cancer in our population. In contrast to our findings, Ma *et al.* [22] have found no association between *MDM2* 40-bp ins/del polymorphism and breast cancer. Hu *et al.* [21] have found that 40-bp ins/del polymorphism in the *MDM2* gene is associated with risk of lung cancer in Chinese population. Dong *et al.* [20] reported that *MDM2* ins/del polymorphism increases the risk of hepatocellular carcinoma in a Chinese population. With respect to the critical role of *MDM2* in tumorigenesis, it is expectable that individuals who carry the 40-bp deletion allele may change transcription factor binding site. Higher expression of *MDM2* increases the lifetime risk of developing breast cancer.

The *p53* tumor suppressor has a key role in

orchestrating cellular responses to numerous types of stresses, including DNA damage and oncogene activation with apoptosis, cell-cycle arrest, DNA repair, and cell metabolism [21, 25]. Dysfunction and mutations of *p53* have been established in most human cancers, leading to a deregulated *p53* activity that allows cells to proliferate and survive [26]. Many proteins regulate the activity of *p53*, and one of the most extensively studied regulators of *p53* is *MDM2* oncoprotein. The *p53* activity can be regulated by *MDM2* in different means, and even modest alterations of *MDM2* levels can affect the *p53* pathway [27]. Firstly, *MDM2* directly binds to the *p53* transactivation domain, consequently inhibiting its transcriptional activity. Secondly, *MDM2* promotes ubiquitination and degradation of *p53* by functioning as an E3 ubiquitin ligase [7, 28]. Finally, *MDM2* binds *p53* in the nucleus and shuttle it into the cytoplasm, promoting *p53* degradation. Notably, a negative feedback loop exists between *p53* and *MDM2*; *p53* stimulates the transcription of *MDM2*, and in turn the *MDM2* protein inhibits *p53* activity [29].

MDM2 overexpression is detected in a number of human cancers [30-32]. Since *MDM2* is a key component of the *p53*-mediated DNA-damage response, promoter polymorphism in this gene might influence this highly regulated pathway by modifying cellular *MDM2* protein levels [33].

It has been proposed that functional variants in promoter regions can lead to variable gene expression levels [34]. Polymorphisms in gene promoters, involved in DNA-damage responses and apoptosis, could have an impact on individual's vulnerability to cancer development [2, 23, 35].

In summary, we have provided the evidence that the 40-bp ins/del polymorphism in the promoter of *MDM2*

gene increases the risk of breast cancer in a sample of Iranian population. However, larger sample sizes with different ethnicities are desired to validate our findings.

ACKNOWLEDGEMENTS

This work was supported by a dissertation grant (M.Sc. thesis of Mohsen Omrani) from Zahedan University of Medical Sciences (Iran).

REFERENCES

- Mousavi SM, Montazeri A, Mohagheghi MA, Jarrahi AM, Harirchi I, Najafi M, et al. Breast cancer in Iran: an epidemiological review. *Breast J.* 2007 Jul-Aug; 13(4):383-91.
- Hashemi M, Fazaeli A, Ghavami S, Eskandari-Nasab E, Arbabi F, Mashhadi MA, et al. Functional Polymorphisms of FAS and FASL Gene and Risk of Breast Cancer - Pilot Study of 134 Cases. *Plos one.* 2013; 8(1):e53075.
- Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. *Nature.* 2000 Nov; 408(6810):307-10.
- Levine AJ. p53, the cellular gatekeeper for growth and division. *Cell.* 1997 Feb; 88(3):323-31.
- Wu L, Levine AJ. Differential regulation of the p21/WAF-1 and mdm2 genes after high-dose UV irradiation: p53-dependent and p53-independent regulation of the mdm2 gene. *Mol Med.* 1997 Jul; 3(7):441-51.
- Michael D, Oren M. The p53 and Mdm2 families in cancer. *Curr Opin Genet Dev.* 2002 Feb; 12(1):53-9.
- Haupt Y, Maya R, Kazaz A, Oren M. Mdm2 promotes the rapid degradation of p53. *Nature.* 1997 May; 387(6630):296-9.
- Wade M, Li YC, Wahl GM. MDM2, MDMX and p53 in oncogenesis and cancer therapy. *Nat Rev Cancer.* 2013 Feb; 13(2):83-96.
- Lundgren K, Montes de Oca Luna R, McNeill YB, Emerick EP, Spencer B, Barfield CR, et al. Targeted expression of MDM2 uncouples S phase from mitosis and inhibits mammary gland development independent of p53. *Genes Dev.* 1997 Mar; 11(6):714-25.
- Bueso-Ramos CE, Manshoury T, Haidar MA, Yang Y, McCown P, Ordonez N, et al. Abnormal expression of MDM-2 in breast carcinomas. *Breast Cancer Res Treat.* 1996; 37(2):179-88.
- Baccouche S, Daoud J, Frikha M, Mokdad-Gargouri R, Gargouri A, Jliidi R. Immunohistochemical status of p53, MDM2, bcl2, bax, and ER in invasive ductal breast carcinoma in Tunisian patients. *Ann N Y Acad Sci.* 2003 Dec; 1010:752-63.
- Shieh SY, Ikeda M, Taya Y, Prives C. DNA damage-induced phosphorylation of p53 alleviates inhibition by MDM2. *Cell.* 1997 Oct; 91(3):325-34.
- Zauberman A, Flusberg D, Haupt Y, Barak Y, Oren M. A functional p53-responsive intronic promoter is contained within the human mdm2 gene. *Nucleic Acids Res.* 1995 Jul; 23(14):2584-92.
- Ries S, Biederer C, Woods D, Shifman O, Shirasawa S, Sasazuki T, et al. Opposing effects of Ras on p53: transcriptional activation of mdm2 and induction of p19ARF. *Cell.* 2000 Oct; 103(2):321-30.
- Bond GL, Hirshfield KM, Kirchhoff T, Alexe G, Bond EE, Robins H, et al. MDM2 SNP309 accelerates tumor formation in a gender-specific and hormone-dependent manner. *Cancer Res.* 2006 May; 66(10):5104-10.
- Bond GL, Hu W, Bond EE, Robins H, Lutzker SG, Arva NC, et al. A single nucleotide polymorphism in the MDM2 promoter attenuates the p53 tumor suppressor pathway and accelerates tumor formation in humans. *Cell.* 2004 Nov; 119(5):591-602.
- Dharel N, Kato N, Muroyama R, Moriyama M, Shao RX, Kawabe T, et al. MDM2 promoter SNP309 is associated with the risk of hepatocellular carcinoma in patients with chronic hepatitis C. *Clin Cancer Res.* 2006 Aug; 12(16):4867-71.
- Ohmiya N, Taguchi A, Mabuchi N, Itoh A, Hirooka Y, Niwa Y, et al. MDM2 promoter polymorphism is associated with both an increased susceptibility to gastric carcinoma and poor prognosis. *J Clin Oncol.* 2006 Sep; 24(27):4434-40.
- Ma Y, Bian J, Cao H. MDM2 SNP309 rs2279744 polymorphism and gastric cancer risk: a meta-analysis. *PLoS One.* 2013; 8(2):e56918.
- Dong D, Gao X, Zhu Z, Yu Q, Bian S, Gao Y. A 40-bp insertion/deletion polymorphism in the constitutive promoter of MDM2 confers risk for hepatocellular carcinoma in a Chinese population. *Gene.* 2012 Apr; 497(1):66-70.
- Hu Z, Ma H, Lu D, Qian J, Zhou J, Chen Y, et al. Genetic variants in the MDM2 promoter and lung cancer risk in a Chinese population. *Int J Cancer.* 2006 Mar; 118(5):1275-8.
- Ma H, Hu Z, Zhai X, Wang S, Wang X, Qin J, et al. Polymorphisms in the MDM2 promoter and risk of breast cancer: a case-control analysis in a Chinese population. *Cancer Lett.* 2006 Aug; 240(2):261-7.
- Hashemi M, Eskandari-Nasab E, Fazaeli A, Rezaei H, Mashhadi MA, Arbabi F, et al. Bi-directional PCR allele-specific amplification (bi-PASA) for detection of caspase-8 -652 6N ins/del promoter polymorphism (rs3834129) in breast cancer. *Gene.* 2012 Jun; 505(1):176-79.
- Hashemi M, Moazeni-Roodi AK, Fazaeli A, Sandoughi M, Bardestani GR, Kordi-Tamandani DM, et al. Lack of association between paraoxonase-1 Q192R polymorphism and rheumatoid arthritis in southeast Iran. *Genet Mol Res.* 2010; 9(1):333-9.
- Harris SL, Levine AJ. The p53 pathway: positive and negative feedback loops. *Oncogene.* 2005 Apr; 24(17):2899-908.

26. Bennett WP, Hussain SP, Vahakangas KH, Khan MA, Shields PG, Harris CC. Molecular epidemiology of human cancer risk: gene-environment interactions and p53 mutation spectrum in human lung cancer. *J Pathol.* 1999 Jan; 187(1):8-18.
27. Bond GL, Hu W, Levine AJ. MDM2 is a central node in the p53 pathway: 12 years and counting. *Curr Cancer Drug Targets.* 2005 Feb; 5(1):3-8.
28. Kubbutat MH, Jones SN, Vousden KH. Regulation of p53 stability by Mdm2. *Nature.* 1997 May; 387(6630):299-303.
29. Momand J, Zambetti GP, Olson DC, George D, Levine AJ. The mdm-2 oncogene product forms a complex with the p53 protein and inhibits p53-mediated transactivation. *Cell.* 1992 Jun; 69(7):1237-45.
30. Meddeb M, Valent A, Danglot G, Nguyen VC, Duverger A, Fouquet F, et al. MDM2 amplification in a primary alveolar rhabdomyosarcoma displaying a t(2;13)(q35;q14). *Cytogenet Cell Genet.* 1996; 73(4):325-30.
31. Bueso-Ramos CE, Yang Y, deLeon E, McCown P, Stass SA, Albitar M. The human MDM-2 oncogene is overexpressed in leukemias. *Blood.* 1993 Nov; 82(9):2617-23.
32. Watanabe T, Hotta T, Ichikawa A, Kinoshita T, Nagai H, Uchida T, et al. The MDM2 oncogene overexpression in chronic lymphocytic leukemia and low-grade lymphoma of B-cell origin. *Blood.* 1994 Nov; 84(9):3158-65.
33. Bond GL, Hu W, Levine A. A single nucleotide polymorphism in the MDM2 gene: from a molecular and cellular explanation to clinical effect. *Cancer Res.* 2005 Jul; 65(13):5481-4.
34. Pastinen T, Sladek R, Gurd S, Sammak A, Ge B, Lepage P, et al. A survey of genetic and epigenetic variation affecting human gene expression. *Physiol Genomics.* 2004 Jan; 16(2):184-93.
35. Wagner K, Hemminki K, Grzybowska E, Klaes R, Burwinkel B, Bugert P, et al. Polymorphisms in genes involved in GH1 release and their association with breast cancer risk. *Carcinogenesis.* 2006 Sep; 27(9):1867-75.