

## Original Article

# Detection of Somatic mutation of codon 248 of p53 gene between Iranian women with breast cancer

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**Objective:** Detection of P53 gene mutation on its codon 248 between Iranian women with breast cancer by PCR-RFLP.

**Material and methods:** DNA extraction of tumor tissues was performed in 53 patients with primary breast cancer. Designing primer for the sub-studying codon was performed with NTI-Vector software and detection of codon 248 mutation was done by Msp I PCR\_RFLP.

**Results:** The somatic mutations of P53 on codon 248 were observed in 11.3% (6 of 53) of our cases, in which 9.4% were heterozygote mutation and the rest were homozygote mutation. This ratio is the highest reported percentage in the world.

**Conclusion:** This perusal indicates genotyping of P53 gene on codon 248 is important for Iranian women with breast cancer risk factors.

**Key words:** P53, mutation, codon 248, breast cancer

## Introduction

One in nine women in the U. S. will develop breast cancer in her lifetime.(1). Recently this disease has severely extended between Iranian women populations particularly for life style alterations and some specific carcinogenic agents known as risk factors for somatic mutation of the susceptible genes such as p53 (2, 3). Although major advances have been made in the early detection and treatment of breast cancer, little is understood regarding its etiology, biology, or the molecular events underlying its development (4, 5).

It is now accepted that the inactivation of p53 gene, as a result of mutation, is a key step in neoplastic transformation and progression (6, 7). It is estimated that about 50% of all human cancers contain a mutation in the p53 gene (5-9 28). However, it is not yet completely clear which of the many properties of p53 are particularly important in oncogenesis. In addition, the p53 activation system may be influenced by cell type and specific tissue effects. Mutations in the p53 gene and immunohistochemically detectable p53

protein are commonly observed in human breast tumors, although the reported frequency of these p53 abnormalities varies considerably (6,7).

The TP53 tumor suppressor gene is located at chromosome 17p13.1, consists of 11 Exons and encodes a 53 KD with 393 amino acid as a cell cycle regulatory nuclear phosphoprotein but normally expressed at a low level in cells (8,10,7,38). The wild-type p53 protein acts as a "guardian of the genome" (8, 9). This effect is usually related to the loss of p53 activated cell death, growth arrest, and/or control of genomic stability (6-12,38). The N-terminal part of the protein is involved in transcription control, the middle portion with a Zinc binding domain necessary for DNA binding and the C-terminal third of the protein facilitates the tetramerisation of the protein, which is claimed to be required for its function (10,7). The loss of p53 function allows propagation of genetically damaged cells, explaining why p53 inactivation is a common step in cancer development.

The p53 protein can be inactivated by nuclear exclusion and by interaction with either cellular

proteins (e.g. MDM2) or viral oncoproteins (e.g. adenovirus E1b, papilloma virus E6 protein, SV40 large T antigen). However, gene mutation is the most frequent cause of p53 inactivation (7,9,10,13). The inactivating mutations tend to cluster in a protein of the gene spanning codons 132 to 281 (Exons: 5,6,7,8 and 9) known to include four highly conserved sequence blocks (14,15). The result of several studies has been shown that codon 248 is one of the most important sites introduced as a hot-spot for p53 gene (3,16,17,38). We conducted this study to evaluate the presence of mutant codon 248 as a risk factor for breast cancer in Iranian women.

## MATERIALS AND METHODS

**Study Population.** Participants include 53 women 21–72 years of age residing in Cancer institute of Imam Khomeini hospital with malignant tumor in their breast tissue.

**Tumor Tissue Preparation and Histopathological Evaluation.** Tumors were sectioned and undergone standardized histopathological review. The invasive cancer area was selectively dissected by the study pathologist. DNA was derived from breast tissues according to the phenol-chloroform or a modified salt-out

method (18,19). We prepared and examined all samples by suitable methods for determining the individuals with somatic mutation p53 on codon 248.

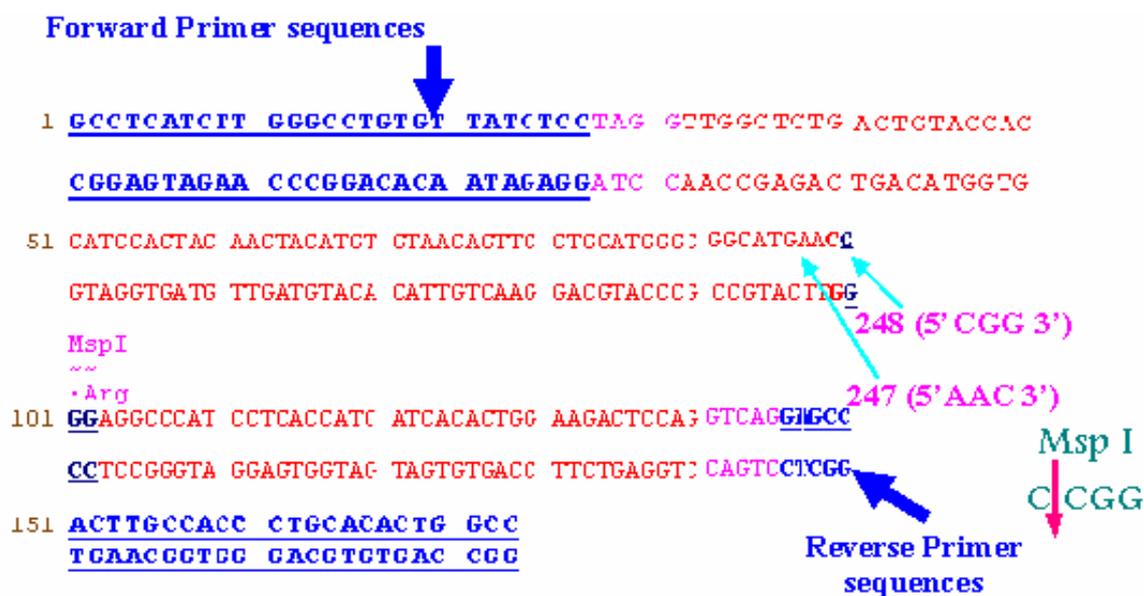
**p53 Mutation Screening.** Mutations of codon 248 (as a hotspot of the p53 gene) were evaluated by PCR-RFLP technique (51-53,55). The used primers (**FORWARD:** 5' GCC TCA TCT TGG GCC TGT GTT ATC TCC 3' ; **REVERSE :** 5' GGC CAG TGT GCA GGG TGG CAA GTG GCT C 3') and the suitable restriction enzyme (Msp I) were designed by NTI-Vector software (Fig 1).

### DNA Amplification, PCR Sequencing

Exon 7 of the p53 gene were separately amplified for tissue samples by 35 cycles of denaturing for 30s at 95°C, annealing for 90s at 58°C, extension for 30s at 72°C. After PCR amplification the PCR products were kept at 72°C for 8 min for completing the process. The amount of the requirement reagents for PCR included PCR buffer 2.5µL, Tag DNA polymerase 0.5 U, dNTP 200µM, MgCl<sub>2</sub> 1.5 mM, DNA concentration 20ng and for each primer 0.5µM(20,6,21).

**Restriction endonuclease Fragment Length Polymorphism (RFLP) step By MspI** (16,17,22-25)

**Fig 1-** Design of the suitable primers and restriction enzyme (Msp I) for Exon 7 of p53 including codon 248 by NTI-Vector software



We took advantage of the natural Msp I restriction site on codon 247/248 for evaluation of mutant p53 on codon 248 for every breast tissue sample. In practice, 3 IU Msp I was added to 10 µl PCR products and after incubation in 37°C for 24 hrs, the digested products have been taken for polyacrylamid 12%, then these obtained gels covered in Ethidium Bromide (10mg/ml) for 15 min and the bands were detected by Transluminator apparatus.

All of p53 mutations on codon 248 were analyzed for 53 breast cancer tissues by Msp I PCR-RFLP (39). Table 1

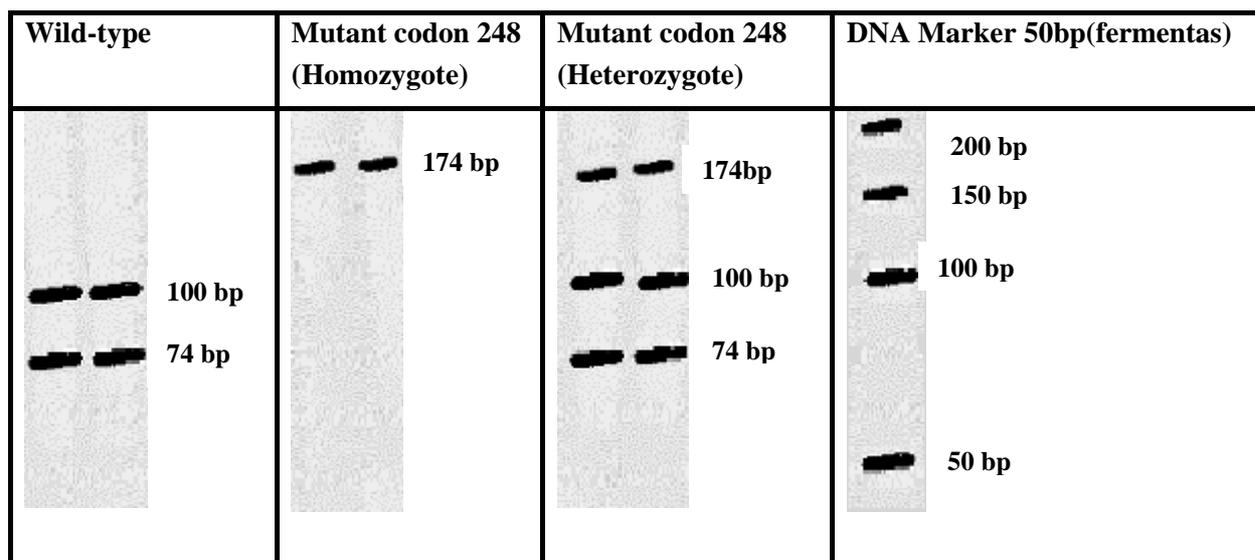
## Results

Mutations on codon 248 detected are as follows: 100 and 47-bp fragments represent the normal 248 codon; an additional 147-bp fragment indicates a heterozygous mutation in codon 248. The presence of only a 147-bp fragment indicates a homozygous mutation in codon 248 (Fig 2).

Our study specifications are summarized in Table 2, Examination of 53 breast tumors by Msp I PCR-RFLP indicated that 6 of them (11.3%) had somatic mutation and also in other evaluation, in addition, we detected 5 of 53 (9.4%) with somatic mutation heterozygote and 1 of 53 (1.9%) with somatic mutation homozygote on codon 248.

Mutation	Restriction Enzyme	Sizes of fragments (bp)	
		Normal	Mutant
Codon 248	<i>Map I</i>	100,74	174

**Table 1.** Restriction Enzymes, and Restriction Fragment Sizes of p53 codon 248



**Fig2 - P53 codon 248 genotyping on Exon 7 by Msp I**

	Mean age	Number of cases	%Heterozygote of mutant p53 on codon 248	%Homozygote of mutant p53 on codon 248	Somatic mutation %	Method
<b>Our study</b>	48	53	9.4	1.9	11.3	PCR_RFLP

**Table 2 - The evaluation of mutant p53 on codon 248 by Msp I PCR-RFLP technique.**

## Discussion

The high breast cancer incidence have prompted many researches to find its risk factors. After recognition of P53 alterations as a proliferation factor in many cancers about fifteen years ago, p53 genotyping and its protein determinations have been widely considered in breast cancer (14,18).

Correlation of cancer phenotypes, such as histological grade or proliferative rate, with precise molecular events, like alterations of p53 or other genes promise to expand our understanding of tumor behavior and perhaps foster new paradigms of risk assessment, prevention, early detection, prognosis, and antineoplastic therapies (26,10). It has been proved that tumors with p53 mutations often behave more aggressively, so the value of p53 as an independent prognostic factor in breast carcinomas has been addressed in several studies (10, 26, 27). Moreover, p53 mutation detection worth has been mentioned for carcinogenic agent determination in several studies (3, 28-30, 12).

Most of the abnormal p53 proteins have considerable longer half-life than wild-type form, resulting in accumulation of the mutant protein in the transfected or neoplastic cells (15).

Dr. Pezeshki et al. (2001) determined p53 protein expression and p53 gene mutations by immunohistochemical analysis and single-strand conformation polymorphism, respectively, in order to assess the role of p53 in infiltrative ductal breast carcinoma among Iranian patients. In his study, no significant correlation was observed between p53 gene mutations and p53 protein expression (31).

These findings imply the p53 genotyping investigations advantage to protein determination in precise detection of many p53 abnormalities.

P53 mutation is present in up to 50% of human tumors, with the highest incidence in lung and gastrointestinal tumors and a significant number of breast cancers. Breast tumors with p53 mutations have been shown to be more aggressive and present at a more advanced stage (6, 18, 32). P53 activity has been associated with prognosis and prediction of tumor response to various therapies and deserves further investigations with the perspective of developing more targeted treatments (18). All these findings emphasize the

importance of TP53 genotyping determination (8,9).

Several studies have evaluated the mutations of p53 gene but their results are inconsistent. These varieties involve both the reported mutant codons and their frequencies (33, 34 )The aim of this study is mutant p53 determination on codon 248; thus we investigated its incidence among residing 53 cases in Cancer Institute of Imam Khomeini hospital by PCR-RFLP technique. This method is able to detect wild-type and mutant p53 on codon 248 without determining the type of mutations.

Codon 248 encodes Arg on P53 Binding Site (PBS) in the central domain of p53 protein during P53 gene expression. Mutant p53 gene on codon 248 breaks main contact with DNA in minor groove (19 ) Msp I enables to digest wild type p53 gene on codon 248 of Exon 7 by our applied PCR-RFLP technique.

Several studies performed p53 genotyping in breast cancer and reported codon 248 as a hotspot for p53 mutation (34,26 ,35).

We reported the highest mutation frequencies (11.3%) for this codon in comparison with other studies. This high statistic may have several explanations. First, this amount may involve mutant codon 247 for the defect of our applied technique. On the other hand, the last nucleotide (C) of codon 247 (5'AAC3') is necessary for detecting by Msp I (C\*CGG of Msp I recognition site). Thus, it may be expected that some of our mutation reports may belong to mutant codon 247. However, according to many reports, codon 247 does not belong to the most frequent mutant codons in p53 (18). Taken together, we can be sure that all of our reported mutations belong to codon 248. It's recommended to do direct sequencing technique of Exon 7 for more assurance.

Second, the relationship between somatic mutations of some genes such as p53 and cancer depends on tissue specifications as some tissues like breast, colorectal, skin are more sensitive for proliferation.

Third, p53 gene mutation pattern varies in different cancers e.g. p53 mutations are mostly G:C to T:A transversions in lung cancer (33,28) and C:T Transitions at dipyrimidine sites in skin cancer (36), whereas the pattern of p53 mutations involve a wide spectrum such as Transition, Transversion and Frame shift in breast cancer. For

instance, the presence of frame shift mutations is specific between Caucasian population (36), of course it should be mentioned that these differences may return to sensitivity of applied method.

Forth, difference between reported diverse results of somatic point mutation of p53 about both mutant codons and their frequencies among different studies can imply the presence of specific environmental carcinogenesis factors (29).

Our applied technique is the easiest and most precise for homozygote and heterozygote detection of mutant p53 on codon 248 in comparison with the others.

the important functions of p53 and high frequency p53 mutations have resulted in significant interest in exploiting the p53 pathway for novel cancer therapies. In addition, the type of mutation and its biological function may be considered in the analysis of the predictive value of Tp53(12,37). By the way, if p53 mutation is applied as a routine molecular prognostic marker, it is important to determine whether its mutations all confer similar properties, or whether some are associated with a more aggressive phenotype than others. Results from in vitro studies suggest that mutations affecting different sites in the p53 gene may result in different effects on the proteins' activity, notably its transactivational and apoptotic functions (26, 10).

At the end, our reported high frequency of mutant p53 on codon 248 necessitates validating these data in further large scale studies. we believe that p53 mutation determination may be noted as a diagnostic and prognostic marker among Iranian women population particularly in those with family history of cancer.

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