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Correlation between important genes of mTOR pathway (*PI3K* and *KIT*) in Iranian women with sporadic breast cancer

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Abstract

Background: PI3K/Akt/mTOR pathway is a crucial pathway in the angiogenesis, tumour growth and cell differentiation of several cancers. The *PI3K* and *KIT* genes are key genes of this pathway. Previous studies have reported the importance of these genes in the development of gastrointestinal carcinoma, leukaemia, and melanomas. The role of mutations and overexpression of *PI3K* and *KIT* genes in breast cancer has been previously proved. This study investigates the correlation between *PI3K* and *KIT* gene mutations in sporadic breast cancer.

Methods: Multiplex Ligation-dependent Probe Amplification (MLPA) technique was used to determine the Copy Number Variation (CNV) of *PI3K* and *KIT* genes in 34 breast cancer tumours and PCR-sequencing was used to detect the mutation in *PI3K* exons 9 and 20.

Results: Our results reported that 27% of patients had CNV of the *KIT* gene; whereas, 20% and 17.5% of patients, had mutation and CNV in the *PI3K* gene, respectively. We did not found a significant correlation between the mutations of *PI3K* and *KIT* genes.

Conclusion: About two-tenth of the patients revealed CNV and lesser than two-tenth indicated mutation in the *PI3K* gene, whereas one-third of the patients demonstrated CNV in the *KIT* gene. Thus, administration of the *PI3K* and *KIT* gene inhibitor drugs might be proposed to suppress breast cancer in patients with mutation and CNV of each of these individual genes.

Keywords: mTOR pathway, *PI3K* gene, *KIT* gene, Breast Cancer

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Introduction

The majority of genes that undergo mutation in human cancers play a direct role in the cell cycle, and most of them participate in signal transduction. PI3K/Akt/mTOR pathway forms one of the most crucial signal transduction pathways in cancer development (1). This pathway is crucial in the cell processes such as cell survival, growth, division, and angiogenesis. *PI3K* and *KIT* genes are essential to initiate this pathway, although these genes are active in other pathways (2, 3). For this reason, cancers such as breast cancer probably could be suppressed through inhibiting these genes. (4).

Previous studies have reported that mutations and overexpression of *PI K3CA* gene are essential in the ovarian, endometrial, thyroid, nasopharyngeal and colorectal cancers (5-8).

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↑What is "already known" in this topic:
mTOR pathway genes is a key pathway for development of several cancers. There are some drugs for suppressing the mentioned oncogenes and their proteins to treat cancer.

→What this article adds:
There is a relationship between mutation and CNV of mTOR pathway genes in breast cancer but no significant correlation found between these genes.

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Alternatively, several studies have reported that mutations and overexpression of *KIT* gene can develop malignancies such as gastrointestinal carcinoma, leukaemia, and melanomas (9-14).

The Copy Number Variation (CNV) of *PIK3CA* and *KIT* genes accelerate cancer development and determines the prognosis and sensitivity to the anticancer drugs (15-16).

Oncogenic mutations, in particular, exons 9 and 20, and increased CNV in the *PI3K* pathway generally activate the phosphatidylinositol-4, 5-bisphosphate 3-kinase-catalytic subunit alpha (*PIK3CA*) mutation, which has been identified in several breast cancer subtypes. *PIK3CA* exons 9 and 20 are coding p110α domains and tyrosine kinase domain respectively. The G>A mutation in E542K and A>G mutation in H1047R are common mutations in exons 9 and 20 (2, 17, 18).

Furthermore, *KIT* genes is a kind of receptor tyrosine kinase performing in cell signal transduction. The stem cell factor (SCF) is bound to KIT and activated it. Phosphorylation cascade activation is followed by activation of various transcription factors (3). CNV and overexpression of the *KIT* gene are crucial in developing breast malignant tumours (19).

Further research is required to study the suppression of the malignant tumours such as breast cancer by using drugs which inhibit *PI3K* and *KIT* genes such as imatinib and sunitinib.

In this study, we investigated the CNV of *KIT* and *PIK3CA* genes, mutation of *PIK3CA* exons 9 and 20, and the relation between them in sporadic breast cancer.

Methods

Patients

50 tissue samples of breast cancer were selected with the following criteria: female, primary, sporadic, no history of treatment regardless of age or histopathological sub-type from Mehrad Hospital (Tehran, Iran). All samples were collected from the tumour regions and DNA was extracted from them. The quality and quantity of DNAs were assessed by agarose gel electrophoresis and NanoDrop ND 2000 spectrophotometer. They were analysed for mutations in *PI3K* gene, 40 for CNV of *PI3K* and 44 for CNV of *KIT* gene. Informed consent was obtained from all individual participants included in the study. This study has had the approval of the ethics committee of "University of Social Welfare and Rehabilitation Sciences".

MLPA

We analysed CNV of *PI3K* and *KIT* gene using P173-A2 and P354-A2 kits (MRC-Holand) in 40 and 44 patients with sporadic breast cancer, respectively.

We used these kits to investigate CNV of the *KIT* gene and exons 2, 7 and 19 of the *PI3K* gene. Briefly, the DNA extracted from the dissected tumours and normal control samples were pre-heated to 98°C, and then the salt solution and probe mix were added to the DNA. After the ligation of annealed nucleotides, the target genes were amplified using polymerase chain reaction (PCR). PCR products were separated on an ABI3730-XL capillary sequencer (Applied Biosystems, Foster City, CA, USA). *PI3K* and *KIT* copy numbers were analysed using Coffalyser (ver. 140721.1958). The cut-off values between 0.7 and 1.3 were considered normal. Results below 0.7 or between 1.3 and 2 were interpreted as deletion and low-level amplification of gene, respectively; and values over 2 were referred to as high-level amplification.

PCR-Sequencing

Primers for exons 9 and 20 *of PI3K* gene were designed by primer 3 and UCSC genome browser (CinnaGen-Iran). Self-dimers, heterodimers and the melting temperature of the primers were checked by OligoAnalyzer (Table 1). DNA was amplified by the PCR technique for 50 samples followed by mutation analysis by direct DNA sequencing. The sequencing results were analysed using codon code aligner and Gene Runner software.

Statistical analysis

Data were analysed using SPSS 19.0 statistical package. Fisher's exact tests were used to analyse the association between the increase in copy number and mutations of *PI3K* gene, and CNV of *KIT*. A *p*-value less than 0.05 was considered as statistically significant.

Results

MLPA

MLPA analysis was performed successfully in all tumour samples using SALSA P354-A2 and P173-A2 kits. The increasing copy number of *PI3K* gene was observed in 4 of the 40 patients, whereas that of the *KIT* gene observed in 12 of the 44 patients.

PCR-Sequencing

Mutation in exons 9 and 20 of *PI3K* gene was detected in 20% of the patients. Two patients showed G>A mutation in E542K and nine with A>G mutation in H1047R (Table 2 and Fig. 1). Other cases were normal.

The present study indicates that the increase in copy number and hot spot mutations in *PI3K* gene are not significantly correlated with an increase in the copy number of the *KIT* gene (Table 3).

Table 1. Primer Sequences of 9 and 20 exons

Exon	Sequence(5'->3')	Product Size	Annealing temperature
9	Forward: GGCTAACTTCAGCAGTTACTATTCTGTGAC Reverse :GAAAAAGCATTTAATGTGCCAACTACC	616	58
20	Forward: TCATTTGCTCCAAACTGACCA Reverse: GGTCTTTGCCTGCTGAGAGT	388	60

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Table 2. The results of sequencing of 9 and 20 exons of *PI3K* gene

Case	Mutation	Nucleotide change
1	H1047R c:3140	A>G
2	H1047R c:3140	A>G
3	E542K c:1624	G>A
4	E542K c:1624	G>A
5	H1047R c:3140	A>G
6	H1047R c:3140	A>G
7	H1047R c:3140	A>G
8	H1047R c:3140	A>G
9	H1047R c:3140	A>G
10	H1047R c:3140	A>G
11	H1047R c:3140	A>G

Table 3. Comparisons between CNV of *KIT*, *PI3K*, and 9 and 20 exons of *PI3K* gene

		KIT	*PI3k*-CNV	*PI3K-9* and 20 exons
KIT	Pearson Correlation		0.107	0.069
	p-value		0.547	0.656
	N		34	44
PI3k-CNV	Pearson Correlation	0.107		0.238
	p-value	0.547		0.145
	N	34		39
PI3K-9 and 20 exons	Pearson Correlation	0.069	0.238	
	p-value	0.656	0.145	
	N	44	39	

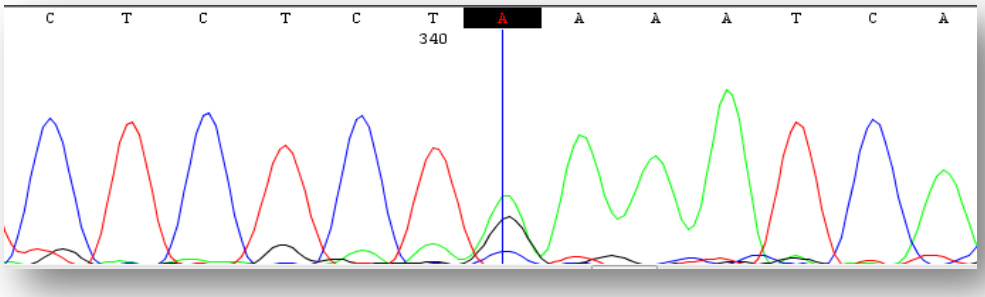

Fig. 1. PI3K gene mutation (E542K c: 1624 G>A) in the breast cancer tissues

Discussion

This is the first study in Iranian population which focused on the relationship between *PI3K* and *KIT* genes for PI3K/Akt/mTOR pathway. Although *KIT* and *PI3K* genes are activated and participate in several common pathways, we did not observe any significant correlation between them; one of the possible reasons might be the activation of the *PI3K* and *KIT* genes separately by other pathways and genes.

In this study, two-tenth of the patients reported mutations in exons 9 and 20, lesser than two-tenth patients revealed an increased copy number in the *PI3K* gene.

PI3K gene could be crucial in cancer development, with an increased risk of developing breast cancer. Administering tyrosine kinase inhibitor drugs could suppress breast cancer.

In previous studies, the expression level of this gene and CNV was different. This can be related to the genetic diversity of the population (18, 20).

Our investigation, like other studies, showed mutations of G>A in E542K and A>G in H1047R are the most prevalent mutations in *PI3K* gene in patients with sporadic breast cancer.

Similar to our study, other studies such as findings of Ian J. Majewski, reported that 23% patients with breast cancer had a mutation in the PI3K gene or the study of SibylleLoibl that 21.4% patients had a mutation in this gene (2, 22). Alternatively, we confirmed results of Irina Palimarul and Mohammad Firoozinia (18, 23).

Our findings revealed that almost one-third of the patients have increased copy number gene in KIT; we investigated CNV of KIT gene using MLPA (P354-A2 kit, personal communication with MRC Holland). This kit was initially designed and routinely used for piebaldism disease. Since the *KIT* gene is crucial in the key pathways such as angiogenesis, tumour growth and cell differentiation, we used it to investigate the CNV of *KIT* gene in patients with sporadic breast cancer. We confirmed the results of previous studies that have reported maximum mutation of the *KIT* gene in breast cancer, unlike other cancers, is an increase in CNV.

In most cases, increase in CNV could be lead to gene overexpression.

Alternatively, in correlation to other studies, reported overexpression of the KIT gene as a crucial factor in de-

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veloping breast cancer, and no correlation between an increase in the copy number and overexpression of the *KIT* gene (19, 24-29).

We presume that other mechanisms are also involved. These include epigenetic variation and translational, post-transcriptional and protein degradation regulations causing gene overexpression without altering these genes; however, the small sample size could also contribute to this discordance.

In the present study, *KIT* and *PI3K* genes revealed an increase in the copy number in sporadic breast cancer. Alternatively, several genes that can change the structure and function of a protein are essential for administering tyrosine kinase inhibitor drugs, in particular for gastrointestinal tumours, as they are resistant to these drugs (30). Therefore, although no significant correlation between these genes was established, each gene on its own can probably be used for targeted therapy with tyrosine kinase inhibitors.

Tyrosine kinase inhibitors are essential drugs, which have been successfully administrated in various cancers such as gastrointestinal cancer and leukaemia (31, 32); however, further studies are warranted to verify these initial findings.

Conclusion

CNV of PI3K gene was seen in 20% of 34 cases with sporadic breast cancer, while mutation in the same gene in less than 20%. In 30% of cases CNV of KIT gene was evident and no correlation was detected between PI3K and KIT CNVs. Since these are two important genes in development cancer, maybe one or both could be used as target in tyrosine kinase inhibitor drugs in sporadic breast cancer.

Conflict of interest

All authors declare that they have no conflict of interests.

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