

Distribution of EGFR R521K Polymorphism in Different Iranian Ethnic Groups

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Abstract

Background: The R521K polymorphism of EGFR has attenuated function in ligand binding, tyrosine kinase activation, and growth stimulation.

Objectives: We initiated a study to examine R521K polymorphism in six Iranian ethnic groups.

Patients and Methods: This cohort study was designed on 300 Iranian healthy individuals and analyzed by PCR-RFLP protocol.

Results: The frequencies of A/A, G/A, G/G genotype were 7.7%, 53.6% and 38.7% respectively.

Conclusions: No subject with A/A genotype was detected in Kurds and Lures. The higher frequencies of A/A genotype in Arab and Caspian groups compared to Fars and Turks could be a key determinant of better response to anticancer drugs and favorable prognosis of CRC patients.

Keywords: Colorectal Cancer, Epidermal Growth Factor Receptor, R521K Polymorphism, Iranian Ethnic Groups

1. Background

Colorectal cancer (CRC) is one of the most frequent cause of cancer mortality in Iran [1, 2]. The relationship between dietary, environments factors, the age of over 50, genetic risk factor and CRC has been evaluated [3, 4].

The discovery of epidermal growth factor receptor (EGFR) in the process of cell proliferation has produced an exciting era in the treatment of CRC.

Epidermal growth factor receptor is a transmembrane glycoprotein of 170 KD encoded by a gene located in the short arm of chromosome 7 (chromosome 7 P 12.1-12.3) [5].

After binding a ligand the receptor dimerizes and that event leads to auto phosphorylation followed by recruitment and phosphorylation of many intracellular substrates [6]. This chain of phosphorylation leads to activation of several signaling pathway such as Ras/Raf/MAPK or phosphatidylinositol-3-kinase (PI3K) that are involved in cell growth, cell proliferation angiogenesis and inhibition of apoptosis [7, 8].

Blocking EGFR ligand binding with therapeutic monoclonal antibodies have been shown to be an effective treatment for CRC [9].

R521K is functional polymorphism in EGFR that arose from a G to A transition, resulting in an Arg to Lys substitution [10].

Compared with the wild type 521R allele, the lysine

allele variant has attenuated affinity in ligand binding, tyrosine kinase activation and induction of the proto-oncogenes Myc, Fos and Jun [11].

2. Objectives

This study aims to investigate allele frequencies of the polymorphism R521K in EGFR found in different ethnic groups of Iranian population that could be associated with a better outcome in CRC patients.

3. Patients and Methods

This cohort study was conducted in 300 healthy Iranian subjects and of the different ethnicity (169 Fars, 77 Turks, 13 Kurds, 17 Lures, 12 Arabs and 12 Caspian groups). Genomic DNA was extracted from peripheral blood lymphocyte using DNA purification kit (Tehran, Iran) extraction. The R521K (G < A) polymorphism in exon 13 of epidermal growth factor receptor (EGFR) gene was examined by as PCR-RFLP method as described previously [8]. The PCR primers were 5'-TGCTGTGACCCACTCTGTCT-3' (forward) and 5'-CCAGAAGGTTGCACTGT-3' (reverse). The PCR reactions were performed in a total volume of 25 μ M, containing 150 ng genomic DNA, 0.8 μ M of each primer 2.5 μ M PCR buffer, 0.5 μ M dNTPs, 0.8 μ M MgCl₂, 2.5 μ M DMSO

(Dimethyl sulfoxide) 1X Taq buffer and 1 unit of Taq DNA polymerase. The PCR cycle conditions of an initial denaturation at 94°C for 4 minutes, followed by 32 cycle of 94°C for 50 second, 59.4°C for 50 second, 72°C for 50 second and final extension at 72°C for 10 minutes. The 155 bp PCR product was digested overnight with MvaI restriction enzyme at 37°C and alleles were separated on polyacrylamide gels and visualized under ultraviolet (UV) light.

The expected restriction fragments were:

G/G = 38 bp + 50 bp + 67 bp

G/A = 38 bp + 50 bp + 67 bp + 117 bp

A/A = 38 bp + 117 bp

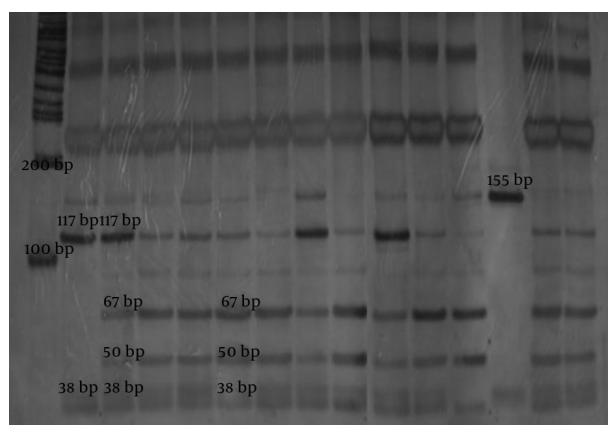
All data analyses were carried out using SPSS-13 statistical software. P values < 0.05 were considered statistically significant.

4. Results

The genotype distributions were in Hardy-Weinberg equilibrium. A total 300 subjects, 38.6% were homozygous or Arg/Arg variant (normal genotype), 53.6% were heterozygous that having both the alleles (Arg/Lys) and 7.6% were homozygous for Lys/Lys genotype (mutant genotype).

The results of R521K genotype distribution in different ethnic groups of Iranian population are summarized in Table 1. In this study, we found that G/G and G/A genotype were detected in all six ethnic groups, we did not find any A/A genotype (mutant type) in Kurdes and Lures.

Figure 1. PCR Products After Being Digested by MvaI Were Separated by Polyacrylamide Gel Electrophoresis



Lane 1 in 100 bp DNA ladder; Lane 2 is homozygous genotype Lys/Lys with two bands 38, 117 bp; Lane 3, 8 are heterozygous genotype Arg/Lys showing four bands 38, 50, 67, 118; Lane 4, 5, 6, 7 are homozygous genotype Arg/Arg showing three bands 38, 50, 67 bp.

5. Discussion

We examined six Iranian ethnic groups for the prevalence of R521K polymorphism in 300 healthy subjects, including Fars, Turks, Kurds, Lures, Arabs and Caspian groups. Heterozygous (G/A) is the most common genotype in five ethnic groups, although GG genotype is predominant allele and occurred in high frequencies in Arab ethnic groups. From this study, we conclude that GA and GG genotype found in all six ethnic groups. AA was not detected in Kurds and Lures but was found among the Fars, Turk, Arabs and Caspian groups. The frequency of AA allelic variant appeared to be lower in Fars and Turks compared to Arabs and Caspian groups.

A polymorphic variant EGFR arising from single nucleotide change (G > A) leading to arginine (R) to lysine (K) substitution in codon 521 in the extracellular domain of EGFR has been identified.

EGFR + 521A/A genotype confers an attenuated function in EGFR ligand binding, growth stimulation, tyrosine kinase activation and induction of proto-oncogenes. Moreover, A/A genotype was associated with poor clinical outcome and shorter PFS (Progression free survival) compared with other genotypes [11, 12].

The R521K EGFR polymorphism correlates with a decrease in EGFR phosphorylation, decrease invasion, lower nodal involvement, reduce subsequent metastasis and longer disease-free and overall survival in stage II/III colorectal carcinoma patients who have received curative surgery [13, 14].

According to SNP database, A/A genotype (mutant type) frequency was less when compared with East Asian, but remarkably higher than that African-American and Sub-Saharan Africans population. We not observed significant differences with the most European populations.

Based on finding that A/A genotype of R521K EGFR polymorphism influence the efficacy of anticancer drugs and its associated with increased response to monoclonal antibodies by reducing its activation and consequential down regulation of its target gene, our results suggest that Kurds and Lures may predict sub-optimal rates of clinical response to monoclonal antibodies. The higher frequencies of A/A genotype in Arabs and Caspian groups compared with Fars and Turks, it appears that the use of monoclonal antibodies might produce good outcome and favorable prognosis in these groups of Iranian CRC patients.

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Table 1. R521K Genotype Distribution in Different Ethnic Groups of Iranian Population^a

Ethnicity	A/A	G/A	G/G
Fars	8.3	51.5	40.2
Turk	6.5	58.44	35.06
Kurd	0	53.85	46.15
Lure	0	70.59	29.41
Arab	16.67	33.33	50
Caspian	16.67	50	33.33

^aValues are expressed as percentage.

Footnotes

Authors' Contribution: Asal Shahrokhshahi, gathering data, review and writing; Massoud Houshmand, review and editing.

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