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TP53 Arg72Pro and *CCND1* A870G Polymorphisms and Esophageal Cancer Risk

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Abstract: The authors examined 184 residents from Kazakhstan to reveal an association of the *CCND1* gene A870G and *TP53* gene Arg72Pro polymorphisms with esophageal cancer risk. 86 of them were control group and 98 were patients with esophageal cancer. DNA samples were genotyped by direct sequencing method and TaqMan allelic discrimination method. Statistical analysis was performed using GraphPad InStat™ Software and "Case-Control Study Estimating Calculator" from TAPOTILI company. A significant association was revealed between *CCND1* homozygous genotype (A870A, OR=2.654) and *TP53* heterozygous (Arg72Pro, OR=1.417) and homozygous (Pro72Pro, OR=2.860) genotypes increased risk of esophageal cancer.

Key words: Cancer, esophageal, polymorphism, *CCND1*, *TP53*.

1. Introduction

In present time, the level of cancer cases in Kazakhstan is higher than in European region and one of the highest among Central Asian countries. The most common are tumors arising in permanently renewing tissues such as epithelium.

One of these epithelial tumors is esophageal cancer (EC). It is the fourth main cause of mortality from cancer diseases. The geographical distribution of EC is irregular. The highest indexes (3-5 times higher than average) were detected in China, Iran and countries of Central and Middle Asia region. In Kazakhstan and Turkmenistan the incidence of EC is high up to 23.7 – 28.3 cases per 100 thousands [1]. The male/female ratio for EC is about 3:1. Usually, EC is diagnosed at the late stages, therefore, the 5-year survival for this cancer is less than 5-10% [2].

Esophageal cancer is caused by a number of risk factors. In areas with low incidence of the disease, the main causes of EC are smoking and alcohol abuse. In areas with high EC incidence, the carcinogenic effect is linked to dietary habits such as drinking hot brewed tea, consumption of frozen meat, bony fish, etc. The poor nutrition with low vitamin contain also contribute to EC development. Other causes of esophageal cancer are different thermal, chemical and mechanical injuries

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of esophageal mucosa, achalasia of esophagus, gastroesophageal reflux disease (GERD), Barrett's esophagus, etc. [3].

The molecular base of any cancer is accumulation of mutations in certain genes playing a key role in such important processes as cell cycle regulation, apoptosis, DNA repair, xenobiotic biotransformation, etc.

Some of the mutations may persist in human populations for a long time providing a genetic polymorphism, i.e. existence of two and more allelic variants of a gene. These allelic variants may differ from wild type alleles by their functionality and may result in certain abnormalities and diseases.

In this investigation, the polymorphisms of two cell cycle regulatory genes – *CCND1* and *TP53* were studied in their association with esophageal cancer risk in population from Kazakhstan.

Cyclin Dl, encoded by *CCND1* gene (also known as *BCL1*, *PRAD1*) in chromosome 11 (11q13), plays an important part in regulation of cell cycle in response to growth factors during G1-S phase transition [4]. D-cyclins bind to and activate cyclin-dependent kinases CDK4 and CDK6. The cyclin-dependent kinases phosphorylate the Rb protein leading to release of the E2F transcription factors, which then results in proper G1/S transition. There are two variants of cyclin D1 transcripts: CD 1a and CD 1b. The CD lb transcript is coded by polymorphic allele of *CCND1* gene, which has G to A substitution in 870 codon. This alternatively spliced transcript encodes a protein lacking the ubiquitin destruction box, leading to increase nuclear half-life of the protein [5]. It results in increasing levels of nuclear CD1 even in the heterozygote state [6, 7].

TP53 is a key tumor suppressing gene located in chromosome 17 (17p13.1) and encoding the p53 protein. p53 is a transcriptional factor participating in a number of important pathways including cell cycle control, DNA repair, and apoptosis induction [8]. p53 is activated in case of UV radiation, protooncogene activation or DNA damage. Normally, this leads to activation of repair mechanisms, induction of cell cycle arrest, and the prevention of cancer growth via apoptosis. It has been shown that p53 can induce the expression of the bax [9] and cd95/fas [10] genes, both of which are promoters of apoptosis.

Mutations in *TP53* gene are found in over 50% of all human cancers [11]. Certain role in tumorogenesis may play different polymorphisms of *TP53* gene. One of such significant polymorphisms is guanine to cytosine substitution in 215 position causing Pro>Arg substitution in 72 codon of the protein. The Arg72Pro polymorphism is localized in the proline-rich domain, which is important to its apoptotic activity [12]. The Arg72 allelic variant is considered to be predominant and more effective in apoptosis initiation [13, 14].

2. Materials and Methods

EDTA-treated peripheral blood samples were obtained from 184 residents of Almaty city. 86 of them were normal control and 98 were patients with EC (91 with squamous cell carcinoma and seven with adenocarcinoma). The material was collected on the basis of Kazakh Research Institute of Oncology and Radiology by approbation of the patients. The clinical diagnosis verification was carried out cytologically and histologically on biopsy material.

DNA were extracted by standard phenol-chloroform method with modifications in lysis buffer composition (0.2 M sodium acetate and 1% sodium dodecylsulfate, pH 8.0).

The genotyping of *TP53* Arg72Pro and *CCND1* A870G polymorphisms was carried out by direct sequencing method and by TaqMan allelic discrimination method. The direct sequencing was performed using BigDye® Terminator v3.1 kit and Genetic Analysis System ABI PRISM® 3130 (Applied Biosystems). TaqMan allelic discrimination was performed using ABI PRISM® 7700 Sequence Detection System (Applied Biosystems).

The statistical analysis of the obtained data was performed using GraphPad InStat™ Software (V2.04. Ralf Stahlman, Purdue University) and "Case-Control

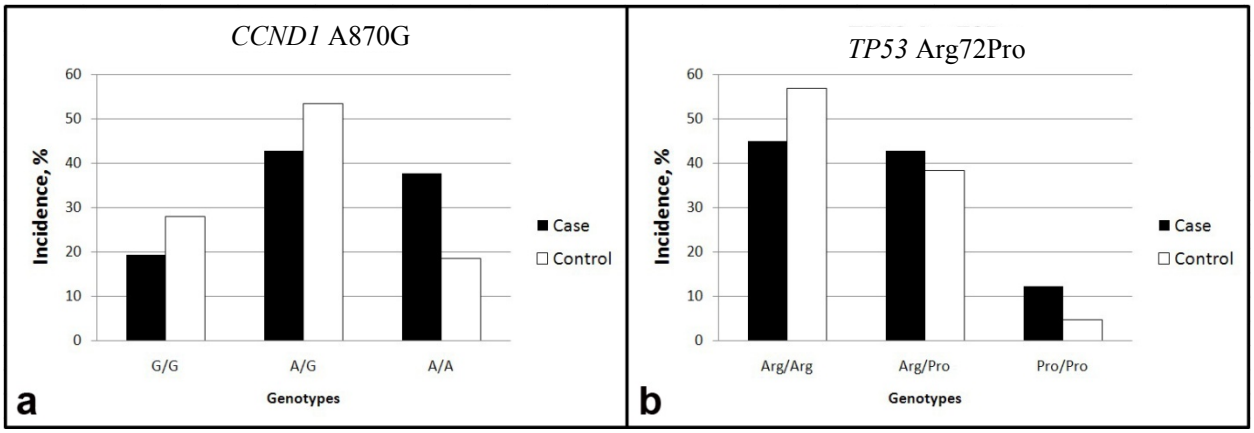

Fig. 1 The distribution of A870G *CCND1* (a) and Arg72Pro *TP53* (b) genotypes in case and control groups.

Table 1 The correspondence of the case and control groups by age, ethnicity and sex.

Group	Years of birth	Nationality, persons (%)		Sex, persons (%)		Total, persons
		Kazakh	Russian	Male	Female	
Case	1920-1977	88 (89.80)	10 (10.20)	45 (45.92)	53 (54.08)	98
Control	1921-1976	76 (88.37)	10 (11.63)	37 (43.02)	49 (56.98)	86

Study Estimating Calculator" from TAPOTILI company (Laboratory of Molecular Diagnostics and Genomic Dactiloscopy of "GosNII Genetika" State Scientific Centre of Russian Federation; http://www.tapotili.ru). The odd ratio calculation was carried out taking into account dominant and recessive model.

3. Results

The case-control study was conducted to find out association between the studied polymorphisms of the cell cycle and xenobiotic biotransformation genes and esophageal cancer risk. According to cytological and histological analysis of esophageal biopsy samples (Fig. 1) obtained from residents of Almaty city undergoing medical cure in Kazakh Research Institute of Oncology and Radiology, a group of patients was formed (98 individuals) at the age of 33-90 with diagnoses squamous cell carcinoma (SCC) and adenocarcinoma (AC) of esophagus. Among the patients with SCC, six high-differentiated, 34 moderately and 51 poorly differentiated cancers were diagnosed.

In consideration of age, ethnicity, sex and social factor (urban residents), a control group (86 individuals) was formed from healthy Almaty residents. The normal state of esophageal epithelium of the control group representatives was confirmed by histological analysis.

The correspondence of the case and control groups is presented in Table 1.

The genotyping of A870G *CCND1* and Arg72Pro *TP53* polymorphisms in the studied groups showed the distribution presented in Fig. 1.

The distribution patterns of the allelic variants of the cell cycle regulatory genes *CCND1* and *TP53* among the studied groups satisfy to Hardy-Weinberg distribution (*TP53*: χ^2=4.30, *P*=0.04; *CCND1*: χ^2=7.03, *P*=0.008).

The *TP53* Arg72 allele frequency in the control group made up 0.762, and in the case group –0.663. The frequency of the second allele Pro72 in the control group was 0.238 and in the case group –0.337.

The frequency of *CCND1* G870 allele in the control group made up 0.547, while in the case group it was 0.408, correspondingly the A870 allelic variant occurred with the frequencies 0.453 in the control group and 0.592 in the case group.

Comparing the frequencies of polymorphic variants of *CCND1* and *TP53* genes in population of Kazakhstan with the NCBI SNP database, it could be noticed that in

Table 2 Odds ratios for esophageal cancer with *CCND1* A870G and *TP53* Arg72Pro genotypes.

Polymorphism	Genotype	Esophageal cancer, persons (%)	Control, persons (%)	OR	CI (95%)	χ^2	*P*
CCND1 A870G	G/G	19 (19.39)	24 (27.91)	0.342	0.148-0.793		
	G/A	42 (42.85)	46 (53.49)	0.395	0.192-0.811	6.76	0.00932
	A/A	37 (37.75)	16 (18.60)	2.654	1.345-5.236		
TP53 Arg72Pro	Arg/Arg	44 (44.90)	49 (56.98)	0.616	0.390-0.976		
	Arg/Pro	42 (42.86)	33 (38.37)	1.417	0.769-2.612	4.25	0.03929
	Pro/Pro	12 (12.24)	4 (4.65)	3.341	1.004-11.121		

control group of Almaty residents the frequency of the rare allele Pro72 (0.238) was more similar to European populations (0.233), and was considerably lower than frequencies in Asian population (0.409-0.511). The possible explanation of the fact may be distinction of Kazakh population from other Asian populations (Chinese, Japanese, Malaysian, etc.) by genotype, and affinity to European populations. The frequency of G870 allele of the *CCND1* gene in the control group (0.547) was more similar to Asian (0.456-0.656) than European (0.475-0.483) populations.

For estimation of significance of the studied polymorphisms in predisposition to esophageal cancer, a statistical analysis was carried out using χ^2 – test. The results of the estimation are presented in Table 2. Also two other variants of analysis were conducted. In the first variant the heterozygous genotypes were combined with the genotypes homozygous for the minor allele (dominant model); in the second variant the frequent allele carriers (homozygous and heterozygous for normal allele) were combined in one group and the genotypes homozygous for polymorphic allele represented the other group (recessive model).

4. Discussion

The obtained data indicate that there is a significant association between *CCND1* homozygous (A870A, OR=2.654) genotype and *TP53* heterozygous (Arg72Pro, OR=1.417) and homozygous (Pro72Pro, OR=2.860) genotypes and increased risk of esophageal cancer.

According to the dominant model, a significant association with esophageal cancer showed in *CCND1* G/A and A/A combined groups (OR=1.610; CI=0.809-3.201; *P*=0.17305) and *TP53* Arg/Pro and Pro/Pro combined groups (OR=1.625; CI=0.907-2.914; *P*=0.10204).

According to the recessive model, a significant association with esophageal cancer showed in *CCND1* A/A genotype (OR=2.654; CI=1.345-5.236; *P*=0.00421) and *TP53* Pro/Pro genotype (OR=2.860; CI=0.887- 9.229; *P*=0.06816).

Thus, in this work it was shown with great significance that the high risk of esophageal cancer in population of Kazakhstan residents could be associated with *CCND1* homozygous (A870A) genotype and *TP53* heterozygous (Arg72Pro) and homozygous (Pro72Pro) genotypes.

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