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The novel allele (3R) of the VNTR polymorphism in the *XRCC5* promoter region dramatically decreases the gene expression

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ABSTRACT

Polymorphism of variable number of tandem repeats (VNTR) in the promoter region of X-ray repair cross-complementing 5 (MIM: 194364, *XRCC5*; rs6147172) was reported. The aim of the present study is to evaluate the influence of this polymorphism on *XRCC5* mRNA levels. Genotypes of *XRCC5* VNTR were determined by high resolution of melting analysis (HRMA). The quantitative *XRCC5* mRNA expression (compared to ß-actin expression) among 0R/1R, 1R/2R, and 1R/3R genotypes was investigated. There was a negative correlation between the overall number of tandem repeats and *XRCC5* expression ($r = -0.965$, df = 7, $P < 0.001$). The mRNA level of *XRCC5* decreased as function of number of tandem repeats. The 3R allele of the VNTR polymorphism in the *XRCC5* promoter region dramatically decreases the gene expression.

1. Introduction

Ku, a heterodimeric DNA-binding complex, is directly involved in repair of DNA double strand breaks (DSBs) as a member of the non-homologous end joining (NHEJ) pathway. Ku consists of two subunits, Ku70 and Ku80, which are encoded by the *XRCC6* and *XRCC5* genes, respectively [1].

Several genetic polymorphisms in the *XRCC5* (X-ray repair cross-complementing 5; MIM: 194364) have been reported. A variable number of tandem repeats of a 21 bp (VNTR, rs. 6147172) polymorphism in the promoter region of *XRCC5* has been reported [2]. This polymorphism has four alleles: 3R, 2R, 1R and 0R [3]. To determine if the VNTR polymorphism located in the promoter region affects the basal transcription activity of *XRCC5*, Wang et al., measured promoter activity with a Dual Luciferase Reporter Assay (Promega) and compared the activities of the 2R, 1R and 0R allele by transient transfection in HeLa, T24, and NIH3T3 cell. They concluded that fewer tandem repeats in the *XRCC5* promoter increase the activity of the *XRCC5* transcript [2]. Very recently a novel allele for this polymorphism (named 3R) was reported by our research group [3]. The aim of the present study was to evaluate the influence of the novel allele on *XRCC5* mRNA transcription levels in human normal peripheral blood cells.

2. Materials and methods

Peripheral blood samples were collected from individuals carrying different *XRCC5* VNTR genotypes (three individuals for each genotype) into heparinized blood collection tubes and immediately used for RNA extraction. Genomic DNA was isolated from EDTA treated blood samples. Genotyping for the *XRCC5* VNTR polymorphism was carried out using high resolution of melting analysis (HRMA) following a pre-amplification step by Rotor-Gene 6000 instrument (Corbett Life Science). In a recent report, we described the primers and conditions for HRMA [4]. Ethical approval for the current study was obtained from Shiraz University institutional review board.

Total RNA was isolated using RNX-plus solution (Cinnagen, Iran) according to the manufacturer's instructions. The concentration and purity of isolated RNA were determined using the S2100 Diode array spectrophotometer (WPA Biowave, Biochrom, Cambridge, UK). cDNA synthesis was performed at 37 °C for 15 min with 500 ng of extracted RNA, using PrimeScript RT Reagent kit (Takara Bio Inc.). Quantitative PCR was performed in 100 µl tubes using Rotor-Gene 6000 thermal cycler (Corbett Life Science). Primers used for the amplifications were 5′-TCC CAC CGA GGC ACA GTT GA-3′ (forward), and 5′-ACC TCA GCG GGA GGA TTC AGC A-3′ (reverse) for *XRCC5* and 5′-CGA GCA CAG AGC CTC GCC TT-3′ (forward) and 5′-ACA TGC CGG AGC CGT TGT CG-3′ (reverse) for ß-actin. The primers used were designed using NCBI Primer-BLAST tool. Each PCR reaction contained 10 µl SYBR Premix Ex Taq II master mix (Takara Bio Inc.), 2 µl of cDNA and 0.8 µl (0.4 µM) each of forward and reverse primers and 6.4 µl nuclease-free water.

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Real-time PCR was carried out through 40 cycles following initial 30 s enzyme activation at 95 °C. Each cycle consisted of denaturation for 10 s at 95 °C, annealing for 15 s at 60 °C and extension for 20 s at 72 °C. The SYBR green I fluorescence intensity was acquired at the end of extension step of each cycle. Comparative quantitation of *XRCC5* expression was calculated and normalized to ß-actin expression as calibrator using Rotor-Gene 6000 software (version 1.7). The mean efficiency of the takeoff point of cycling curves was used to calculate a fold change:

$$\text{Fold change} = \text{efficiency}^{\text{Ct(calibrator)}-\text{Ct(target gene)}}$$

To evaluate differences between XRCC5 mRNA levels and the genotypes of the VNTR polymorphism in *XRCC5* (rs. 6147172), one way analysis of variance (ANOVA) was used. Association between number of VNTR and mRNA levels was measured using Spearman correlation coefficient. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA; version 11.5).

3. Results and discussion

To investigate the functional impact of the allelic variants of VNTR polymorphism, the quantitative *XRCC5* mRNA expression (compared to ß-actin expression) among 0R/1R, 1R/2R, and 1R/3R genotypes was investigated (Table 1). Mean ± SD of the relative *XRCC5* expression were 11.41 ± 0.47, 1.19 ± 0.33, and 0.26 ± 0.08 for 0R/1R, 1R/2R, and 1R/3R genotypes, respectively. One way analysis of variance revealed a significant differences between the study genotypes ($F = 1025.8$; df = 2, 6; $P < 0.0001$). There was a negative correlation between the overall number of tandem repeats (number of *cis* regulatory elements) and *XRCC5* expression ($r = -0.965$, df = 7, $P < 0.001$).

The promoter region of *Ku80* contains several copies of Sp1 recognition *cis* regulatory elements (Ku promoter binding element). Very recently, we reported four alleles (0R, 1R, 2R, 3R) for VNTR of *XRCC5* [3]. The alleles 3R, 2R, 1R and 0R possess eight, seven, six and five copies of *cis* regulatory elements, respectively [3].

Table 1
Expression mRNA levels of *XRCC5* compared to ß-actin in nine individuals with different genotypes of *XRCC5* VNTR polymorphism (three individuals in each genotype).

Genotypes	Expression levels			*n*	Mean	SD
0R/1R	10.90	11.82	11.50	3	11.41	0.47
1R/2R	0.85	1.50	1.23	3	1.19	0.33
1R/3R	0.32	0.30	0.17	3	0.26	0.08

There is strong evidence that some transcription factors can bind VNTR sequences [5]. The VNTR polymorphism of *XRCC5* can alter the number of *cis* elements. Number copies of *cis* elements in the promoter region of XRCC5 regulate its expression [6]. The impact of VNTR polymorphism of *XRCC5* on gene expression, prompted us to investigate the *XRCC5* transcriptional activity in individuals carrying different genotypes of the *XRCC5* VNTR polymorphism. As we expected, the increase of the overall number of tandem repeats in the promoter region of *XRCC5*, down-regulates the gene expression. The 3R allele of the VNTR polymorphism in the *XRCC5* promoter region dramatically decreases the gene expression.

Over expression of the *XRCC5* in several types of cancers (such as gastric and colorectal cancers) has been reported [7–9]. Therefore, *XRCC5* may function as an oncogene in the development of cancers. It is hypothesized that the 3R allele compared to 0R allele may have protected role against development of cancer. Case-control studies are necessary to evaluate this hypothesis.

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