Tumor Biol. (2014) 35:923–927
DOI 10.1007/s13277-013-1120-5

RESEARCH ARTICLE

Association of XRCC5 VNTR polymorphism with the development of chronic myeloid leukemia

Manjula Gorre · Prajitha Edathara Mohandas · Sailaja Kagita · Sandhya Annamaneni · Raghunadharao Digumarti · Vishnupriya Satti

Received: 4 July 2013 / Accepted: 14 August 2013 / Published online: 28 August 2013

Abstract Double-strand breaks (DSBs) inducing agents influence the fidelity of DNA repair in both normal cells and leukemic cells, causing major genomic instability. In eukaryotic cells, non-homologous end joining pathway (NHEJ) is the major mechanism for DSB repair. Human X-ray repair cross-complementing 5 (XRCC5) gene encodes for the protein KU86, an important component of NHEJ pathway. Variable number of tandem repeats (VNTR) polymorphism (rs 6147172) in the promoter region of XRCC5 gene was shown to have effect on gene expression and was found to be associated with the development of several cancers. We analyzed VNTR polymorphism of XRCC5 gene in 461 chronic myeloid leukemia (CML) cases and 408 controls by polymerase chain reaction. Our results showed that frequency of 0R/0R genotype was significantly elevated in CML cases compared to that of controls ($p=0.05$). Significant difference in the genotype distribution was observed between cases and controls ($p=0.02$). The risk of CML development was found to be elevated for individuals carrying lower repeats (1R $p=0.03$; 0R $p=0.007$). Elevated 0R/0R genotype frequency was found to be significantly associated with early age at onset (≤30 years) and slightly elevated in chronic phase and poor hematologic response to imatinib mesylate. The influence of zero repeat on enhanced expression of XRCC5 might confer risk to error-prone repair leading to genomic instability and CML. Hence, the VNTR polymorphism in the promoter region of XRCC5 gene could serve as an important prognostic marker in CML development.

Keywords CML · NHEJ · XRCC5 · VNTR

M. Gorre
CSIR-SRF, Department of Genetics, Osmania University, Hyderabad, India
e-mail: manjula.gorre@gmail.com

P. E. Mohandas
CSIR-JRF, Department of Genetics, Osmania University, Hyderabad, India
e-mail: prajithadas06@gmail.com

S. Kagita
PDF, Department of Medical Oncology, Nizam's Institute of Medical Sciences, Hyderabad, India
e-mail: kagitasa@gmail.com

S. Annamaneni
Indian Statistical Institute, Hyderabad, India

R. Digumarti
Department of Medical Oncology, Nizam's Institute of Medical Sciences, Hyderabad, India
e-mail: rdigumarti@gmail.com

V. Satti (✉)
Department of Genetics, Osmania University, Hyderabad, India
e-mail: sattivishnupriya@gmail.com

Introduction

Chronic myeloid leukemia (CML), characterized by the presence of 9:22 chromosome translocation, is a triphasic disease progressing from initial chronic phase to accelerated and blast crisis. This transition is accompanied by an increased *BCR-ABL* expression in the leukemic cells due to additional chromosomal and genetic abnormalities causing genomic instability in Ph+ve cells [1]. The molecular mechanisms underlying instability have been extensively studied, and it was believed that the antiapoptotic effects exerted by the Bcr-Abl oncoprotein may favor the emergence of cell clones carrying additional genetic abnormalities.

Double-strand breaks (DSBs) are the most lethal form of DNA damage, and non-homologous end-joining (NHEJ) is the dominant mechanism for their repair in eukaryotic cells [2]. Excessive DNA damage due to DSB-inducing agents may force the NHEJ components to process DSB aberrantly,

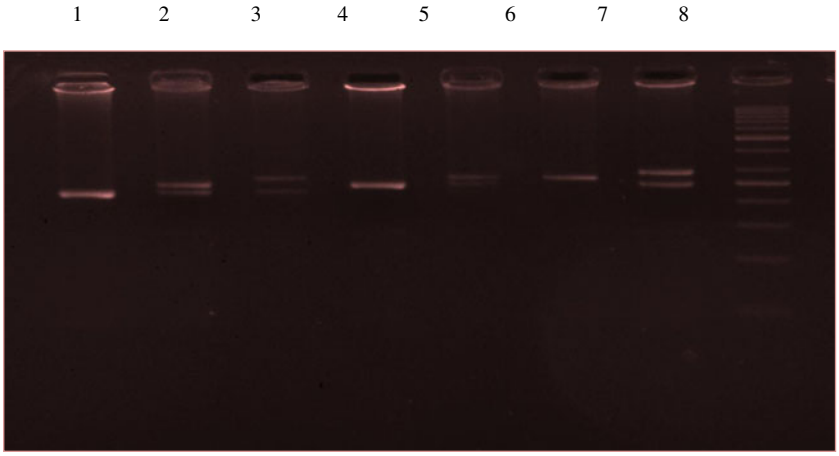

Fig. 1 VNTR polymorphism in *XRCC5* promoter region.Lane 1 224bp (0R/0R genotype), Lane 2 224bp, 245bp (0R/1R genotype), Lane 3 224bp, 266bp (0R/2R genotype), Lane 4 245bp (1R/1R genotype), Lane 5 245bp, 266bp (1R/2R genotype), Lane 6 266bp (2R/2R genotype), Lane 7 245bp, 287bp (1R/3R genotype), Lane 8 50bp ladder

resulting in chromosomal instability in myeloid leukemias; hence the NHEJ mechanism is regarded as error prone. The aberrant DNA repair dependent on the presence of NHEJ proteins (*KU70* and *KU86*) is crucial for the development and progression of malignancies including leukemia [3].

The human X-ray repair cross-complementing 5 (*XRCC5*) gene located on chromosome 2q35 encodes for the protein *KU86* of NHEJ component [4]. *XRCC5* is one of the three subunits of a DNA-dependent protein kinase which contributes to NHEJ repair. Loss of *XRCC5* function results in instability of the genome and increased risk for development of tumors. However, overexpression of *XRCC5* was also shown to be associated with the progression of bladder cancer, gastric cancer, and breast cancer [5, 6]. Genetic polymorphisms in the *XRCC5* gene had been reported to be involved in the origin of digestive system cancers [7].

The proximate promoter region of *XRCC5* contains seven copies of cis elements which are essential for basal expression of the gene. Variable number of tandem repeats (VNTR) polymorphism (rs 6147172) is located at −201 to −159 nucleotide sites, which includes the sites for *SP1* transcription factor binding [8]. This polymorphism contains three different alleles, which are two 21 nucleotides repeats (2R), one 21 nucleotides repeat (1R), and zero repeat unit (0R). The alleles 2R, 1R, and 0R possess seven, six, and five copies of cis regulatory elements, respectively.

The binding affinity of the transcription factor *Sp1* to the allele with two 21-bp repeats was found to be greater than that for the allele with one 21-bp repeat as determined with gel-shift and super-shift assays. However, plasmids containing zero or one repeat element were found to have higher transcriptional activities than plasmids containing two repeat elements as established with a reporter assay. Furthermore, fewer tandem repeats in the promoter of *XRCC5* was shown to be associated with enhanced levels of the *XRCC5* protein in bladder cancer patients [9].

A novel allele (3R) was introduced by Rajaei et al., (2012), which is capable of expanding the number of cis regulatory elements to eight. The 3R allele was shown to be associated with decrease in gene expression and was suggested to have protective role against the development of cancer [10–12]. The present study is planned to analyze the frequency distribution of different alleles of VNTR polymorphism in CML cases and controls in order to evaluate its role in development and progression of CML.

Materials and methods

Five milliliter of blood sample was collected from 461 CML cases diagnosed at Nizam's Institute of Medical Sciences, Hyderabad over a duration of 8 years (2005–2012). All primary Ph+ve CML cases reported with confirmed diagnosis during this period were included, and secondary or drug-induced cases were excluded in the study. Samples were

Table 1 Distribution of genotypes and alleles of XRCC5 (VNTR) among CML cases and controls

	Cases (n=461)		Controls (n=408)		p^a	p^b
	n	(%)	n	(%)		
Genotypes						
2R/2R	39	(8.46)	40	(9.80)	*0.02	1(ref)
2R/1R	175	(37.96)	133	(32.60)		0.236
2R/0R	18	(3.90)	34	(8.33)		0.097
1R/1R	153	(33.19)	143	(35.05)		0.714
1R/0R	48	(10.41)	45	(11.03)		0.769
0R/0R	28	(6.07)	13	(3.19)		*0.05
Alleles						
2R	271	(29.39)	247	(30.27)	0.916	1.000
1R	529	(57.38)	464	(56.86)		0.724
0R	122	(13.23)	105	(12.87)		0.719

p^a chi square test probability, p^b odds ratio probability, and *p significant

Table 2 Distribution of genotype classes and their association with CML development

Genotype classes	Cases		Controls		Unadjusted OR (95 % CI)	p	Adjusted OR (95 % CI)	P
	n	%	n	%				
Class I (2R/2R+2R/0R)	52	12.2	53	18.1	1.00	0.025*	1.00	0.025*
Class II (1R/1R+1R/2R+1R/0R)	353	82.5	231	79.1	1.56 (1.032–2.36)		1.55 (1.02–2.37)	
Class III (0R/0R)	23	5.4	8	2.7	2.93 (1.20–7.14)		3.00 (1.22–7.40)	

Odds ratios adjusted for age and gender

*p significant (chi-square test p value)

collected from the patients after obtaining written informed consent. Four hundred eight age and sex matched controls from the local population were also included. Epidemiological information of the patients was obtained through personnel interview using prepared questionnaire.

Clinical information such as phase of disease, hematological, and cytogenetic response of the patients to imatinib mesylate was noted down from tumor registry with the help of oncologist (co-author). CML is a triphasic disease comprising of chronic, accelerated, and blast crisis. The accelerated and blast crisis phases were clubbed into "advanced phase" in our study. Hematological "major" responders correspond to the patients who achieved normal leukocyte count within 3 months from the date of start of imatinib mesylate. Cytogenetic "major" responders correspond to the patients who achieved complete response to imatinib mesylate with regard to the blast percentage (number of cells carrying the Ph+ve chromosome). The study was approved by the ethical committee of the Department of Genetics, Osmania University and NIMS, Hyderabad.

Genomic DNA was isolated from EDTA-treated blood samples by nonenzymatic/salting out method [13]. The *XRCC5* VNTR polymorphism was analyzed by the polymerase chain reaction (PCR) using primers specific to the promoter region. Sequence of the primers (Bioserve Biotechnologies, India) is as follows: Forward: *5′-AGG CGG CTC AAA CAC CAC AC-3′*; *Reverse*: *5′-CAA GCG GCA GAT AGC GGA AAG-3′*.

PCR reaction was performed at an annealing temperature of 62 °C for 40 s [14] within a total volume of 10 μl containing 50 ng DNA, 25 mM dNTP mix, 25pM of each forward and reverse primer, and 0.25–0.5 U Taq polymerase (Bangalore Genei). PCR products were separated on 3.5 % agarose gel to identify the genotypes of VNTR polymorphism in *XRCC5* promoter region (Fig. 1). Allelic and genotypic frequencies were calculated for CML cases and controls. Interactive chi-square analysis was performed and odds ratios were calculated to study the distribution of different genotypes and alleles among CML cases and controls. Adjusted odds ratios were also calculated by adjusting age and sex using "SNP Stats" to study the association of genotype classes with the development of CML.

Results

In the case–control study of 461 CML cases and 408 controls, the mean age was found to be 36.94±12.48 years for cases and 33.10±14.74 years for controls. Then 65.84 and 34.16 % of cases and 60.70 and 39.30 % of controls were males and females, respectively (p =0.147).

Frequencies of 2R/2R, 2R/1R, 2R/0R, 1R/1R, 1R/0R, and 0R/0R were 8.46, 37.96, 3.90, 33.19, 10.41, and 6.07 %, respectively, among the CML cases and 9.80, 32.60, 8.33, 35.05, 11.03, and 3.19 %, respectively, among controls. 0R/0R genotype was significantly elevated among CML cases

Table 3 XRCC5 VNTR and gender cross classification interaction (adjusted by age)

Genotype classes	Males				Females			
	Cases	Controls	Unadjusted OR (95 % CI)	Adjusted OR (95 % CI)	Cases	Controls	Unadjusted OR (95 % CI)	Adjusted OR (95 % CI)
Class I (2R/2R+2R/0R)	30	30	1.00	1.00	22	23	0.96 (0.44–2.07)	0.92 (0.42–2.02)
Class II (1R/1R+1R/2R+1R/0R)	235	140	1.68 (0.97–2.90)	1.71 (0.98–2.97)	118	91	1.30 (0.73–2.30)	1.25 (0.70–2.23)
Class III (0R/0R)	17	7	2.43 (0.88–6.70)	2.52 (0.90–7.02)	6	1	6.00 (0.68–52.90)	6.76 (0.76–60.38)

Interaction p value=0.52 (unadjusted OR)

Interaction p value=0.44 (adjusted OR)

Table 4 XRCC5 VNTR and age cross-classification interaction (adjusted by gender)

Genotype classes	≤30 years				>30 years			
	Cases	Controls	Unadjusted OR (95 % CI)	Adjusted OR (95 % CI)	Cases	Controls	Unadjusted OR (95 % CI)	Adjusted OR (95 % CI)
Class I (2R/2R+2R/0R)	18	25	1.00	1.00	34	28	1.69 (0.77–3.70)	1.71 (0.78–3.77)
Class II (1R/1R+1R/2R+1R/0R)	124	118	1.46 (0.76–2.81)	1.43 (0.74–2.76)	229	113	2.81 (1.47–5.37)	2.82 (1.48–5.39)
Class III (0R/0R)	12	3	5.56 (1.37–22.59)	5.40 (1.33–22.02)	11	5	3.06 (0.90–10.33)	2.91 (0.86–9.86)

Interaction p value=0.33 (unadjusted OR)

Interaction p value=0.30 (adjusted OR)

(6.07 %) compared to that of controls (3.19 %) ($p=0.05$), whereas the frequency of 2R/0R was elevated in controls (8.33 %) compared to the frequency in cases (3.90 %) ($p=0.097$). Frequency of 0R, 1R, and 2R alleles among the controls was 0.13, 0.57, and 0.30, respectively. No difference in the allele frequencies was observed between CML cases and controls (Table 1). We also detected the 3R allele in 1.91 % of cases and 2.64 % of controls.

The patients were grouped based on prevalence of VNTR genotypes in the study group into three classes. Class I: included patients with either 2R/2R or 2R/0R, Class II: patients with at least one 1R allele (1R/1R+1R/2R+1R/0R), and Class III: patients having only 0R allele (0R/0R). When these genotype groups were compared, there was significant difference between cases and controls ($p=0.025$). Odds ratios were also calculated by adjusting the age at onset and gender which showed significant association of Class III (0R/0R genotype) with the development of CML ($p=0.025$) (Table 2).

In addition, risk for CML development was found to be increased as the number of repeats decreased. When compared with Class I, Class II had a 1.55-fold increased risk of CML (95 % CI 1.02–2.37). Individuals in Class III had a 3.00-fold increased risk of CML compared to those carrying allele with two repeats after adjusting age and gender (95 % CI 1.22–7.40); chi-square test probability $p=0.025$ (Table 2).

The cross-classification interaction studies of VNTR polymorphism within age by adjusting gender showed that the frequency of Class III (0R/0R genotype) had a 5.40-fold (95 % CI 1.33–22.02) increased risk for the individuals with age <30 years to develop CML (Tables 3 and 4). Polymorphism is not associated with the gender of the CML patient (Table 3). Genotype frequencies of VNTR polymorphism was also compared with respect to clinical variables such as phase of CML, hematological response, and cytogenetic response to imatinib mesylate. The frequency of 0R/0R genotype was found to be 6.35 % in chronic phase and 1.63 % in advanced phase. Interestingly, genotype elevation was observed in the frequency of 0R/0R in hematological poor responders (7.46 %) compared to major responders (3.82 %) (Table 5).

Table 5 Distribution of XRCC5 (VNTR) polymorphism among clinical variables

Clinical variable	2R/2R+2R/0R		1R/1R+1R/2R+1R/0R		0R/0R		P
	N	(%)	N	(%)	N	(%)	
Phase of CML							
Chronic ($n=298$)	41	(13.75)	238	(80.95)	19	(6.35)	0.081
Advanced ($n=61$)	4	(6.55)	56	(91.80)	1	(1.63)	
Hematological response							
Major ($n=235$)	29	(12.34)	197	(83.82)	9	(3.82)	0.459
Poor ($n=67$)	8	(11.94)	54	(80.59)	5	(7.46)	
Cytogenetic response							
Major ($n=178$)	24	(13.48)	146	(82.02)	8	(4.49)	0.824
Poor ($n=92$)	10	(10.86)	78	(84.78)	4	(4.34)	

p chi-square test probability

Odds ratios were calculated and the probabilities were found to be insignificant

Discussion

Significant difference in the genotype distribution of XRCC5 VNTR polymorphism was observed among cases and controls ($\chi^2=13.403$, df=5; $p=0.02$) (Table 1). Previously, significant difference in distribution was reported for bladder cancer [9] and AML [14] in Chinese population. Frequencies of 0R, 1R, and 2R alleles reported in the present study were found to be far from the frequencies reported from Chinese population [9] and nearer to that of Iranian population [10]. Prevalence of 1R allele was higher, similar to the findings of Iranian population, and 0R allele frequency was lower than the frequencies reported for China.

Frequency of 3R allele in our population (0.03) was found to be nearer to that of Iranian population and lower than that of European population [12]. Slight elevation of 3R allele frequency in controls support the study of Iranians that 3R allele, which decrease the *XRCC5* gene expression, might have protective role against the development of cancer [11].

The genotype classes were also compared with respect to clinical variables such as phase of CML, hematologic response, and cytogenetic response to imatinib mesylate among CML cases. Although there was no significant difference with respect to these variables, frequency of 0R/0R genotype was found to be elevated in cases with chronic phase and the patients with poor hematologic response (Table 5).

In our study, the risk for CML development was found to be elevated for the individuals carrying lower repeats (genotype class II (1R/1R+1R/2R+1R/0R); $p=0.03$ and genotype class III (0R/0R); $p=0.007$). Earlier study by Wang et al. (2009) also reported that the genotypes 2R/0R, 1R/1R, 1R/0R, and 0R/0R were found to be associated with increased risk of AML compared to the 2R/2R and 2R/1R genotypes in a Chinese population [14].

Results of our study suggest that risk of CML development was found to increase as the number of repeats in the VNTR polymorphism of *XRCC5* gene promoter decrease. 0R/0R genotype was found to be significantly associated with development of CML. Zero repeat was also found to be significantly associated with early age at onset of disease. *XRCC5* gene expression was found to be elevated in relation to zero repeat of VNTR in the promoter region of the gene. Elevated *XRCC5* gene expression leads to error prone repair of DNA causing genomic instability.

Conclusion

This is the first study of *XRCC5* VNTR polymorphism in CML patients from Indian population, where the risk of CML was found to be increased in persons carrying fewer tandem repeat alleles. Zero repeat (0R) allele was found to confer risk for early onset (≤30 years) of disease and poor hematologic response to imatinib mesylate. Zero repeat was found to be associated with elevated gene expression and error prone DNA repair. Hence, *XRCC5* gene plays an important role not only in NHEJ pathway of DNA repair but also in conferring risk to CML development. In our study, we observed an elevation of 0R/0R genotype frequency in hematological poor responders. However, several factors (BCR/ABL dependent or BCR/ABL independent mechanisms) were found to be involved in the progression of CML, hence further functional studies on XRCC5 gene are needed to confirm its role in CML progression.

Acknowledgement We are thankful to the Council of Scientific and Industrial Research (CSIR) and Osmania University–Department of Science and Technology (OU-DST-PURSE) program for their financial assistance.

Conflicts of interest None.

References

1. Faderl S, Talpaz M, Estrov Z, OBrien S, Kurzrock R, Kantarjian HM. The biology of chronic myeloid leukemia. N Engl J Med. 1999;341:164–72.
2. Khanna KK, Jackson SP. DNA double strand breaks: signaling, repair, and the cancer connection. Nat Genet. 2001;27:247–54.
3. Nicola B, Terry JG, Manyee C, Ghulam JM, Feyruz VR. Increased error-prone NHEJ activity in myeloid leukemias is associated with DNA damage at sites that recruit key non homologous end-joining proteins. Cancer Res. 2003;63:1798–805.
4. Cai QQ, Plet A, Imbert J, Lafage-Pochitaloff M, Cerdan C, Blanchard JM. Chromosomal location and expression of the genes coding for Ku p70 and p80 in human cell lines and normal tissues. Cytogenet Cell Genet. 1994;65:221–7.
5. Stronati L, Gensabella G, Lamberti C, Barattini P, Frasca D, Tanzarella C, et al. Expression and DNA binding activity of the Ku heterodimer in bladder carcinoma. Cancer. 2001;92:2484–92.
6. Pucci S, Mazzarelli P, Rabitti C, Giai M, Gallucci M, Flammia G, et al. Tumor specific modulation of KU70/80 DNA binding activity in breast and bladder human tumor biopsies. Oncogene. 2001;20:739–47.
7. Yang MD, Tsai CW, Chang WS, Tsou YA, Wu CN, Bau DT. Predictive role of XRCC5/XRCC6 genotypes in digestive system cancers. World J Gastrointest Oncol. 2011;3(12):175–81.
8. Ludwig DL, Chen F, Peterson SR, Nussenzweig A, Li GC, Chen DJ. Ku80 gene expression is Sp1-dependent and sensitive to CpG methylation within a novel cis element. Gene. 1997;199:181–94.
9. Wang S, Wang M, Yin S, Fu G, Li C, Chen R, et al. A novel variable number of tandem repeats (VNTR) polymorphism containing Sp1 binding elements in the promoter of XRCC5 is a risk factor for human bladder cancer. Mutat Res. 2008;638:26–36.
10. Rajaei M, Saadat I, Saadat M. High-resolution melting analysis for detection of variable number of tandem repeats polymorphism of XRCC5. Biochem Biophys Res Commun. 2012;425:398–400.
11. Rajaei M, Saadat I, Saadat I. The novel allele (3R) of the VNTR polymorphism in the *XRCC5* promoter region dramatically decreases the gene expression. Biochem Biophys Res Commun. 2013;430:640–1.
12. Rajaei M, Saadat I, Saadat M. Introducing a novel allele for the polymorphism of variable number of tandem repeats in the promoter region of XRCC5. Biochem Biophys Res Commun. 2012;427:503–5.
13. Lahiri DK, Nurnberger Jr JI. A rapid nonenzymatic method for the preparation of HMW DNA from blood for RFLP studies. Nucleic Acids Res. 1991;19(19):5444.
14. Wang G, Wang S, Shen Q, Yin S, Li C, Li A, et al. Polymorphisms inXRCC5, XRCC6, XRCC7 genes are involved in DNA double-strand breaks (DSBs) repair associated with the risk of acute myeloid leukemia (AML) in Chinese population. J Nanjing Med Univ. 2009;23(2):93–9.