DNA Repair 40 (2016) 27–34

Contents lists available at ScienceDirect

DNA Repair

journal homepage: www.elsevier.com/locate/dnarepair

Polymorphisms in double strand break repair related genes influence radiosensitivity phenotype in lymphocytes from healthy individuals

Kamalesh Dattaram Mumbrekar[a], Hassan Venkatesh Goutham[a], Bejadi Manjunath Vadhiraja[b], Satish Rao Bola Sadashiva[a,*]

[a] Department of Radiation Biology & Toxicology, School of Life Sciences, Manipal University, Manipal, Karnataka, India
[b] Department of Radiation Oncology, Manipal Hospital, Bangalore, Karnataka, India

ARTICLE INFO

Article history:
Received 28 October 2015
Received in revised form 28 February 2016
Accepted 29 February 2016
Available online 4 March 2016

Keywords:
Double strand break
DNA repair
γ-H2AX
Radiosensitivity
SNP

ABSTRACT

Background: A range of individual radiosensitivity observed in humans can influence individual's susceptibility toward cancer risk and radiotherapy outcome. Therefore, it is important to measure the variation in radiosensitivity and to identify the genetic factors influencing it.
Methods: By adopting a pathway specific genotype-phenotype design, we established the variability in cellular radiosensitivity by performing γ-H2AX foci assay in healthy individuals. Further, we genotyped ten selected SNPs in candidate genes *XRCC3* (rs861539), *XRCC4* (rs1805377), *XRCC5* (rs3835), *XRCC6* (rs2267437), *ATM* (rs3218698, rs1800057), *LIG4* (rs1805388), *NBN* (rs1805794), *RAD51* (rs1801320) and *PRKDC* (rs7003908), and analysed their influence on observed variation in radiosensitivity.
Results: The rs2267437 polymorphisms in *XRCC6* was associated ($P = 0.0326$) with increased DSB induction while rs1805388 in *LIG4* ($P = 0.0240$) was associated with increased radioresistance. Further, multiple risk alleles decreased the DSB repair capacity in an additive manner. Polymorphisms in candidate DSB repair genes can act individually or in combination to the efficacy of DSB repair process, resulting in variation of cellular radiosensitivity.
Conclusions: Current study suggests that γ-H2AX assay may fulfil the role of a rapid and sensitive biomarker that can be used for epidemiological studies to measure variations in radiosensitivity. DSB repair gene polymorphisms can impact the formation and repair of DSBs.
Impact: γ-H2AX foci analysis as well as DSBs repair gene polymorphisms can be used to assess cellular radiosensitivity, which will be useful in population risk assessment, disease prediction, individualization of radiotherapy and also in setting the radiation protection standards.

1. Introduction

Human exposure to ionizing radiations can occur in different situations such as during clinical diagnostic and treatment procedures involving the use of radiation, or nuclear accidents or during space explorations. Response to radiation is non-homogeneous not only in a population but also at individual level [1]. A large range of response to radiation (radiosensitivity) has been observed in apparently normal individuals [2]. It is perhaps best illustrated by normal tissue radioresponse as adverse effects after radiotherapy [3]. To a great extent, an individual's radiosensitivity depends on the biological factors related to genetic profile of the individual [4–6].

Cellular radiosensitivity can be evaluated by quantification of double strand break (DSB) damage and repair [7]. It has been observed that among different types of DNA damages, DSBs are repaired with slower repair kinetics and are the most lethal. Hence, they are useful in explaining clinical radiosensitivity than the other types of damages that have fast repair kinetics [8]. Gamma H2AX foci (γ-H2AX) assay is deemed to be a highly sensitive method for analyzing DSBs at physiological and therapeutically relevant radiation dose range [9]. With an intra-individual variation of less than 4% [10], the γ-H2AX foci assay is more sensitive compared to other methods that rely on detection of physical breaks in DNA. Further, it has shown excellent potential in evaluating therapeutic progress

Abbreviations: CI, confidence interval; CV, coefficient of variation; DSB, double strand break; DSBR, double strand break repair; HR, homologous recombination; NHEJ, non-homologous end-joining; OR, odds ratio; PBL, peripheral blood lymphocytes; PCR-RFLP, polymerase chain reaction —restriction fragment length polymorphisms; SNP, single nucleotide polymorphisms.

* Corresponding author. Department of Radiation Biology & Toxicology, School of Life Sciences, Manipal University, Manipal 576104, Karnataka, India.
E-mail address: satishraomlsc@gmail.com (S.R. Bola Sadashiva).

http://dx.doi.org/10.1016/j.dnarep.2016.02.006
1568-7864/

Table 1
Candidate gene and SNPs tested for the association with DSB damage and repair.

Chromosome	Gene	rs ID	Nucleotide; aa change	Location	FS score
2	*XRCC5*	rs3835	G|A	intron	0.065
5	*XRCC4*	rs1805377	G|A	intron	1
8	*NBN*	rs1805794	C|G; Glu > Gln	exon	0.899
8	*PRKDC*	rs7003908	G|T	intron	0.5
11	*ATM*	rs1800057	C|G; Pro > Arg	exon	0.602
11	*ATM*	rs1799757	–|T	intron	–
13	*LIG4*	rs1805388	C|T; Thr > Ile	exon	0.849
14	*XRCC3*	rs861539	G|A; Thr > Met	exon	0.5
19	*RAD51*	rs1801320	G|C	5′ UTR	0.242
22	*XRCC6*	rs2267437	C|G	5′ upstream	–

aa, amino acid; UTR, untranslated region; FS score, functional significance score.

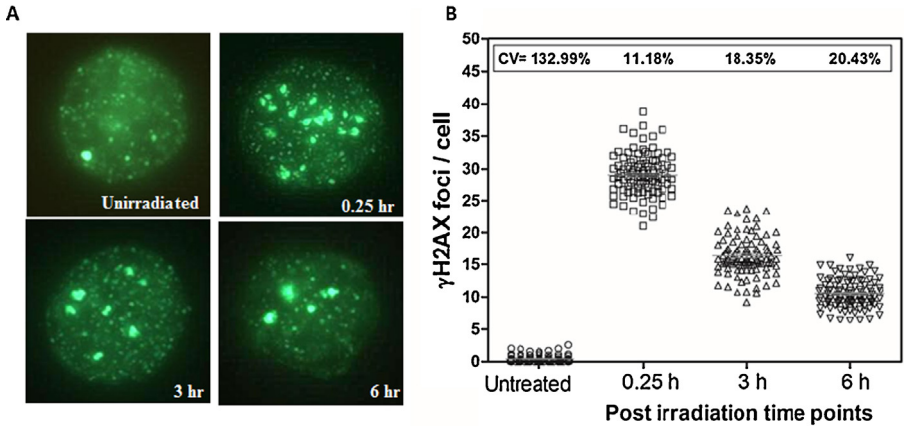

Fig. 1. Double strand breaks and its repair in 2 Gy irradiated lymphocytes of healthy donors measured by γ-H2AX assay, (A) Representative images, (B) Double strand breaks and repair data for 100 individuals.

Table 2
Details of the genotyping by PCR- RFLP for the selected SNPs tested in this association study.

Gene/rs ID	Forward and Reverse primer	PCR product size (in base pairs)	Enzyme	Dominant genotype	Mutant genotype
XRCC5/rs3835	F-ATGATGAGGAGTGATATGTGGAAGAG R-AGTGCTAAGTATCGTCTGCAACTGAT	151	*Alu I*	78, 73	151
XRCC4/rs1805377	F-TCATTTCACTTATGTGTCTCTTCATT R-TGTTTCTCAGAGTTTCTAAAGACATG	170	*Tsp509 I*	170	88, 82
NBN/rs1805794	F-CGTCCAATTGTAAAGCCAGAA R-TCCTGAAACAAGCATTAAAGAGG	174	*Hinf I*	125, 49	174
PRKDC/rs7003908	F-CGAACTCACGAATTGCCTAAGAGTC R-GCTGTTTTTCATATAGAGTTAACAG	241	*Pvu II*	241	141,100
ATM/rs1800057	F-AGCACAGAAAGACATATTGGAAG R-ACTATGTAAGACATTCTACTGCC	494	*AlwI*	305, 189	494
ATM/rs1799757	F-ACTAAGCTGCTGGTCTGAAC R-GTCCTGGAACAATCTTAAAGC	200	*FnuH I*	200	176, 24
LIG4/rs1805388	F-TCTGTATTCGTTCTAAAGTTGAACA R-TGCTTTACTAGTTAAACGAGAAGAT	121	*HpyCH4 III*	65, 56	121
XRCC3/rs861539	F-GCTCGCCTGGTGGTCATCGACTCG R-AAGAGCACAGTCCAGGTCAGCTG	336	*Nla III*	336	231,105
RAD51/rs1801320	F-TGGGAACTGCAACTCATCTGG R-GCTCCGACTTCACCCCGCCGG	131	*NgoM IV*	71, 60	131
XRCC6/rs2267437	F-AACGTGAGGATGGTATCTGCG R-AGGAGGGCGGGAGCC	178	*Nar I*	178	147,31

and cancer progression, occupational genotoxicity screening and as cancer biomarker [11].

Radiosensitivity is greatly influenced by genetic factors; for example in a study on twins, radiosensitivity was explained by 62.5% genetic heritability [12]. It is now well accepted that genetic alterations in proteins participating in DNA damage-repair are responsible for inter-individual differences in radioresponse [13]. Earlier studies have shown the association between SNPs in genes related to non-homologous end joining (NHEJ) and homologous recombination (HR) pathways involved in DSB repair (DSBR) and the risk of *de novo* cancers [14,15], secondary cancers [16], immunodeficiency disorders [17] as well as increased normal tissue toxicity in cancer patients undergoing radiotherapy [18,19]. But the underlying genetic factors that precisely contributes to the inter-individual differences in cellular radiosensitivity are not known [20]. Further, there is no predictive laboratory test available to estimate the degree of radiosensitivity of each individual, necessitating the investigation of cellular and molecular parameters that can be used for laboratory testing [21]. Understanding the cellular and genetic basis of radiosensitivity and identification of