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Short communication

The effects of MSH2 deficiency on spontaneous and radiation-induced mutation rates in the mouse germline

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Abstract

Mutation rates at two expanded simple tandem repeat (ESTR) loci were studied in the germline of mismatch repair deficient *Msh2* knock-out mice. Spontaneous mutation rates in homozygous $Msh2^{-/-}$ males were significantly higher than those in isogenic wild-type ($Msh2^{+/+}$) and heterozygous ($Msh2^{+/-}$) mice. In contrast, the irradiated $Msh2^{-/-}$ mice did not show any detectable increases in their mutation rate, whereas significant ESTR mutation induction was observed in the irradiated $Msh2^{+/+}$ and $Msh2^{+/-}$ animals. Considering these data and the results of other publications, we propose that the *Msh2*-deficient mice possess a mutator phenotype in their germline and somatic tissues while the loss of a single *Msh2* allele does not affect the stability of heterozygotes.

Keywords: Mismatch repair; MSH2; Germline mutation; Expanded simple tandem repeat loci; Knock-out mouse; Ionising radiation

1. Introduction

Mismatch repair (MMR) is essential in the avoidance of mutations and maintenance of genome stability. Given that MMR is involved in the removal of a variety of mismatched DNA pairs arising during replication and recombination, as well as those caused by oxidative stress and some mutagens, mutations inactivating this pathway often result in genomic instability and cancer predisposition [1]. For example, heterozygous carriers of mutations affecting several MMR genes are at risk for a wide range of malignancies, mostly colorectal cancers, and the majority of them display microsatellite instability in tumours [2]. To gain further insights into the in vivo effects of MMR deficiencies, a number of mouse knockout (KO) mutants affecting this pathway have been generated [3]. The results of several studies have shown that, in line with the human data, some of these KO mice have a mutator phenotype in somatic tissues [4–8]. However, it should be stressed that the majority of the data were obtained by analysing the frequency of mutations in transgenic animals carrying either bacterial or human reporters, and to date little is known about the effects of MMR deficiency on mutation rate at endogenous loci. In our previous studies, we have analysed the germline effects of several DNA repair deficiencies on spontaneous and radiation-induced mutation rates at endogenous expanded simple tandem repeat (ESTR) DNA loci [9–11]. Using the

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same approach, here we have analysed the effects of MMR deficiency on ESTR mutation rates in the germline of *Msh2* KO male mice. Given that MMR is involved in the repair of endogenous and exogenous DNA damage, ESTR mutation rates were therefore established in the germline of non-exposed and irradiated mice.

2. Materials and methods

2.1. Mice

Msh2 KO mice on (129/Sv × C57BL/6) mixed background, generated by de Wind et al. [4], were used in this study. Isogenic wild-type ($Msh2^{+/+}$), heterozygous ($Msh2^{+/-}$) and homozygous ($Msh2^{-/-}$) males were generated by mating the $Msh2^{+/-}$ KO parents. To obtain control offspring, non-exposed $Msh2^{+/+}$, $Msh2^{+/-}$ and $Msh2^{-/-}$ males were mated with BALB/c females. $Msh2^{+/+}$, $Msh2^{+/-}$ and $Msh2^{-/-}$ males were given whole-body acute irradiation of 1 Gy X-rays delivered at 0.6 Gy min^{-1} (Andrex SMART 225 machine) and mated to untreated BALB/c females 10 weeks post-irradiation, ensuring that the litters generated were conceived with sperm derived from irradiated A_s spermatogonia [12]. The animal procedures were carried out under guidance issued by the 'National regulations for experimental organisms' of The Netherlands.

2.2. DNA isolation and ESTR typing

Genomic DNA was extracted from tails using a standard phenol-chloroform technique and digested to completion with *Alu*I. All parents and offspring were profiled using two mouse-specific hypervariable single-locus ESTR probes Ms6-hm and Hm-2, as described previously [13]. Briefly, DNA samples were electrophoresed through a 40 cm long 0.8% agarose gel (SeaKem type LE, FMC) in 1 × TBE buffer (89 mM Tris–borate, pH 8.3, 2 mM EDTA) and transferred to a nylon membrane (MAGMA, Osmonics). Following Southern blot hybridisation, autoradiographs were scored by two independent observers. DNA fragment sizes were estimated by the method of Southern [14], with a 1 kb DNA ladder (Invitrogen) included on all gels.

The maternal BALB/c inbred strain was selected because of the non-overlapping size range of alleles for two known ESTR loci in the wild-type and *Msh2* KO male mice. The mean progenitor allele sizes in MSH2-deficient-strain were ~6.5 and 21 kb for *Ms6-hm* and *Hm-2*, respectively, whereas in the BALB/c strain they were 2.5 and 3.5 kb. This substantially facilitated the scoring of mutations and allowed unambiguous establishment of the parental origin of mutant bands identified by gel electrophoresis. ESTR mutants were identified as novel DNA fragments present in offspring, which cannot be ascribed to either parent (Fig. 1A and B). Only bands showing a shift of at least 1 mm relative to the progenitor allele were scored as mutants.

3. Results and discussion

Table 1 presents a summary of ESTR mutation data. ESTR mutation rates per locus in the germline of males were estimated by dividing the total number of mutations scored in the offspring by the total number of ESTR alleles. The spontaneous ESTR mutation rate in wild-type $Msh2^{+/+}$ and heterozygous $Msh2^{+/-}$ males were similar. In contrast, ESTR mutation rate in the germline of non-exposed $Msh2^{-/-}$ males was three-fold higher than in the isogenic wild-type animals. These data are in line with the results of previous studies showing elevated mutation rates in somatic tissues of non-exposed $Msh2^{-/-}$ KO mice [4–8] and the lack of measurable increases in $Msh2^{+/-}$ heterozygotes [4–6]. In these studies, mutation rates were analysed at transgenic bacterial reporter genes and endogenous loci. Considering these results and the data presented here, we conclude that the MSH2-deficient homozygous mice possess a mutator phenotype in their germline and somatic tissues and that the loss of a single *Msh2* allele does not affect the stability of heterozygotes.

A comparison of ESTR mutation rates in the germline of irradiated males (Table 1) revealed that exposure to ionising radiation resulted in a similar 2.5-fold increase in mutation rates in the germline of wild-type and heterozygous males. However, the irradiated $Msh2^{-/-}$ males did not show any detectable increases in their mutation rate. In this respect, the pattern of ESTR mutation induction in the *Msh2*-deficient mice is similar to that in severe combined immunodeficiency (*scid*) and poly(ADP-ribose) polymerase deficient ($PARP\text{-}1^{-/-}$) strains (Fig. 1C). In all three DNA-repair deficient strains the spontaneous ESTR mutation rate significantly exceeds that in the isogenic wild-type mice, whereas the irradiated males do not show any measurable increases in mutation rates. Meanwhile, the loss of p53 function does not affect ESTR mutation rate in the mouse germline. We have previously hypothesised that the lack of ESTR mutation induction in irradiated *scid* and $PARP\text{-}1^{-/-}$ can be explained by the high cell killing effects of irradiation on their germline [9]. Our current data further strengthen this notion. It should be noted that the data showing the increased survival of irradiated embryonic stem (ES) cells deficient in MSH2 [15] cannot directly be compared with the results of our study. The authors studied the effects of very low dose-rate exposure, whereas here $Msh2^{-/-}$ males were acutely irradiated.

The germline length change was defined for 100 de novo paternal ESTR mutations found in the offspring of all control and exposed animals (Table 1). Within each genotype, the incidence of ESTR mutations involving