

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

Karen L-A. Burr^{a,c}, Annemarie van Duyn-Goedhart^b, Peter Hickenbotham^a, Karen Monger^a, Paul P. W. van Buul^b, and Yuri E. Dubrova^{a,*}

^a Department of Genetics, University of Leicester, Leicester LE1 7RH, United Kingdom

^b Department of Toxicogenetics, Leiden University Medical Center, 2300 RC Leiden, The Netherlands

^c Current address: University of Dundee, Department of Molecular and Cellular Pathology, Ninewells Hospital and Medical School, Dundee DD1 9SY, United Kingdom.

* Corresponding author:

Department of Genetics, University of Leicester, University Road, Leicester LE1 7RH, UK

Telephone: +44-116-252-5654 Fax: +44-116-252-3378

E-mail: yed2@le.ac.uk

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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.

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 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 x 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 *AluI*. 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 1xTBE 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 (Figure 1A, 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 3-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-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-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 gain or loss of repeat units was essentially the same in control and exposed groups (Table 1). For each genotype, the data for control and exposed males were therefore combined for further analyses. The frequency of gains and losses did not significantly differ between the three genotypes (*Msh2*^{+/+} males, 11 gains vs. 16 losses; *Msh2*^{+/-} males, 14 gains vs. 10 losses; *Msh2*^{-/-} males 32 gains vs. 17 losses; $\chi^2=4.20$, $df=2$, $P=0.1222$).

We next determined the spectra of ESTR mutations. This analysis was restricted by the resolution of agarose gel electrophoresis within the size-range of progenitor alleles (6-21 kb). Given that the bands showing a shift of at least 1 mm relative to the progenitor allele were scored as mutants, the smallest mutational change corresponded to the gain or loss of 6 repeats. Within each genotype the mutation spectra for the exposed and non-irradiated males did not significantly differ (Kruskal-Wallis test, $P>0.15$; data not shown). The combined distributions of length changes at ESTR loci were indistinguishable between the three genotypes (Fig. 1D). We therefore conclude that neither the loss of MSH2 function, nor exposure to ionising radiation affect the spectrum of ESTR mutations. A similar result was previously obtained by scoring ESTR mutations in offspring non-exposed and irradiated *scid* and *PARP-1*^{-/-} males [9].

The effects of MSH2 deficiency on the spectrum of spontaneous mutations in the germline and somatic tissues of KO mice have been addressed in a number of publications [5-8, 15-19]. The analysis of mutation spectrum at transgenic bacterial reporter genes has shown an increased proportion of +/-1 bp frameshift mutations in the non-cancerous tissues of *Msh2*^{-/-} mice [6, 8]. However given that ESTR mutation is almost exclusively attributed to events altering the number of repeats, the size of which for *Ms6-hm* and *Hm2* loci is 5 and 4 bp [16, 17], such small changes cannot influence mutation spectrum at these loci. Nevertheless, some other data provide strong evidence for significant alterations in the spectrum of mutations detected in transgenic *Msh2*^{-/-} animals, carrying human trinucleotide-repeat microsatellite loci [18-22]. The main result of these studies is that MSH2 deficiency either stabilises transgenes [18, 19] or practically abolishes their expansion [20-22]. At first glance, our data showing that the loss MSH2 substantially increases spontaneous ESTR mutation rate and, at the same time, does not affect the spectrum of ESTR mutations, are at odds with the results of these publications. However it should be noted that in contrast to the transgenic data, it was reported that endogenous microsatellite loci are unstable in somatic tissues of *Msh2*^{-/-} mice [4]. Similar data were obtained for transgenic reporter genes, all of which are highly unstable in *Msh2* KO animals [6-8]. It therefore appears that the effects of MSH2 deficiency vary dramatically across different transgenic loci. Further studies should address this important issue.

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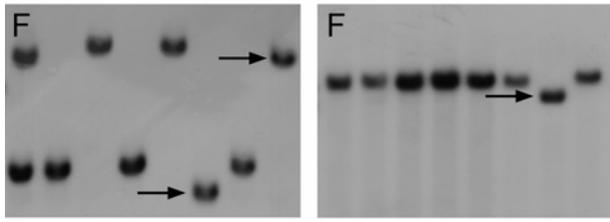
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Figure legend

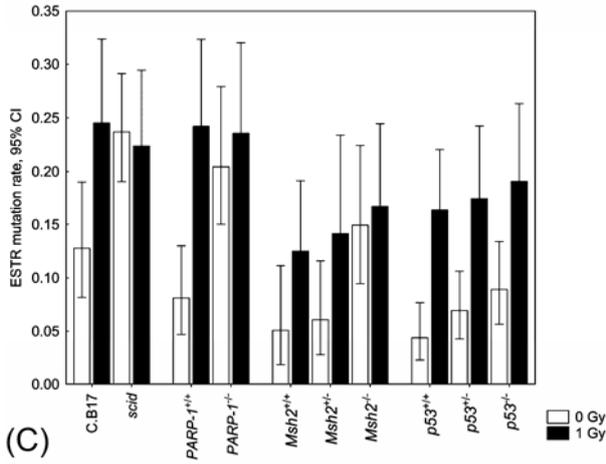
Fig. 1. ESTR mutation data for DNA-repair deficient male mice. Representative images of ESTR mutations: (A) *Ms6-hm*, (B) *Hm-2*. DNA samples from the father (F) and offspring are shown; mutants are indicated with arrowheads. (C) Spontaneous and radiation-induced ESTR mutation rates in the germline of wild type and DNA repair deficient male mice. Data for *scid* mice and their isogenic wild-type C.B17 controls, *PARP-1*, and *p53* mice are taken from refs. 9, 10. (D) Spectrum of germline ESTR mutations in male mice with different *Msh2* genotypes (Kruskal-Wallis test, $P=0.3174$). The progenitor allele was assumed to be the paternal allele closest in size to the mutant allele.

Figure 1

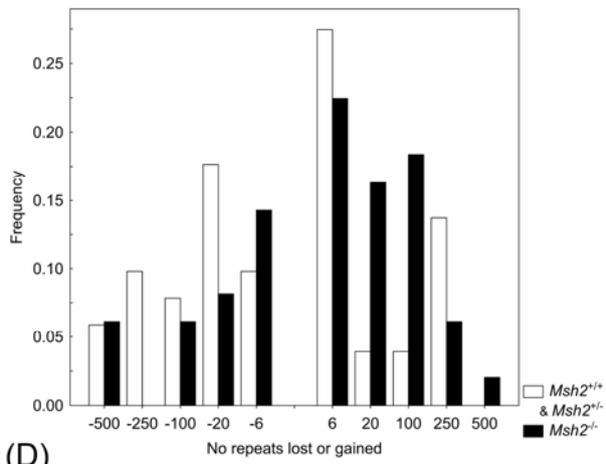


(A)

(B)



(C)



(D)

Table 1
Summary of mutation data

Genotype, dose	No males	No offspring	No mutations ^a	No alleles	Rate	Ratio	P	Type of mutants	
								Gains	Losses
<i>Msh2</i> ^{+/+}									
Control	6	59	6 (4+2)	118	0.0508	-	-	2	4
1 Gy	6	87	21 (12+9)	168	0.1250	2.46 ^b	0.0514 ^b	9	12
								0.16	P=0.8494
<i>Msh2</i> ^{+/-}									
Control	6	77	9 (6+3)	148	0.0608	1.20 ^c	0.9417 ^c	6	3
1 Gy	4	53	15 (11+4)	106	0.1415	2.32 ^b	0.0525 ^b	8	7
								0.39	P=0.6779
<i>Msh2</i> ^{-/-}									
Control	6	87	23 (16+7)	154	0.1494	2.94 ^c	0.0132 ^c	15	8
1 Gy	6	83	26 (18+8)	156	0.1667	1.12 ^b	0.7931 ^b	17	9
								0.00	P=0.9998

^a Number of mutations detected at *Ms6-hm* and *Hm-2* loci is given in parenthesis.

^b Ratio to mutation rate in the non-exposed males of the same genotype and probability of difference (Fisher's exact test, two-tailed).

^c Ratio to mutation rate in the non-exposed *Msh*^{+/+} males and probability of difference (Fisher's exact test, two-tailed).

^d Chi-square test for homogeneity of the type of mutants between control and exposed males.