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SHORT REPORT

MBD2 deficiency does not accelerate p53 mediated lymphomagenesis

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Recent studies using hypomorphic DNA methyltransferase 1 (DNMT1) alleles have suggested that strategies aiming to reduce DNA methylation may increase genomic instability and lymphomagenesis. Given our recent finding that loss of methyl-binding domain protein 2 (Mbd2) suppresses intestinal tumorigenesis, we have tested whether loss of Mbd2 increases lymphomagenesis by intercrossing Mbd2 deficient mice with p53 deficient and p53 heterozygous mice. Unlike DNMT1, loss of Mbd2 does not accelerate lymphomagenesis, arguing that MBD2 may represent a better potential therapeutic target than DNMT1.

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Human tumours have been shown to have widespread changes in the patterns of DNA methylation, showing regional hypermethylation at CpG islands accompanied by global hypomethylation (Lengauer, 2003). It is known that inhibition of DNA methylation through reduction of DNA methyltransferase 1 (DNMT1) (Laird *et al*., 1995; Eads *et al*., 2002) or perturbation of a protein that interprets the DNA methylation signal through knockout of *Mbd2*, suppresses intestinal tumorigenesis in $Apc^{Min/+}$ mice (Sansom *et al*., 2003). However, recent murine studies have raised concerns about the effects of proposed antitumour strategies that reduce DNMT1 as global genomic hypomethylation has been shown to induce lymphomas with increased genomic instability (Eden *et al*., 2003; Gaudet *et al*., 2003). The relevance of these finding to treatment of human cancers is uncertain, as the degree of hypomethylation produced by the hypomorphic allele concerned ($DNMT1^{chip}$) is far greater than following treatment with DNA methylation inhibitors such as 5-aza-2′-deoxycytidine or other previously reported *DNMT1* hypomorphic alleles ($DNMT^R$ and $DNMT^S$) (Trinh *et al*., 2002; Eden *et al*., 2003; Gaudet *et al*., 2003; Yang *et al*., 2003). However, all of the DNMT1 hypomorphic alleles tested thus far have shown increased lymphomagenesis either alone ($DNMT^{chip/}$) or when crossed to mismatch repair deficient $Mlh1^{-/-}$ mice ($DNMT^N$ and $DNMT^R$) (Trinh *et al*., 2002). Given these adverse effects of DNMT1 inhibition and the strong suppression of intestinal tumorigenesis by Mbd2, we have tested whether inhibition of Mbd2 (which is thought to interpret rather than maintain DNA methylation) also accelerates lymphomagenesis.

Loss of Mbd2 does not accelerate p53-mediated lymphomagenesis

Unlike $DNMT1^{-/-}$ and $DNMT^{N/N}$ (which are lethal), and $DNMT^{chip/-}$ mice (which are runted), $Mbd2^{-/-}$ mice are apparently healthy (Li *et al*., 1992; Hendrich *et al*., 2001; Trinh *et al*., 2002; Gaudet *et al*., 2003). To address whether the loss of *Mbd2* accelerated lymphomagenesis, we intercrossed *Mbd2* deficient mice to *p53* deficient mice (Sansom and Clarke, 2000). *p53* deficiency is the most studied murine model of lymphomagenesis, and unlike MMR deficiency does not induce intestinal tumorigenesis (Trinh *et al*., 2002). Therefore, the potential confounding problem caused by suppression of tumorigenesis in one organ and acceleration in another was avoided.

Cohorts of at least 18 $Mbd2^{+/+}p53^{-/-}$ and $Mbd2^{-/-}p53^{-/-}$ mice were aged until they showed signs of disease. Given that we have previously shown a dose-dependent reduction in intestinal tumorigenesis afforded by *Mbd2* deficiency (Sansom *et al*., 2003), cohorts of mice heterozygous for *Mbd2* null allele were also examined ($Mbd2^{+/-}p53^{-/-}$).

Figure 1a shows a Kaplan–Meier plot of survival for $Mbd2^{+/+}p53^{-/-}$, $Mbd2^{+/-}p53^{-/-}$ and $Mbd2^{-/-}p53^{-/-}$ mice. No significant differences were observed in survival between the three different cohorts of at least 18 mice (all statistical analyses, $P \geqslant 0.7$, Log rank). We next analysed the tumour spectra and frequency of each tumour type to assess whether there was increased lymphomagenesis in doubly mutant $Mbd2^{-/-}p53^{-/-}$ mice (Figure 1b). As expected, $p53^{-/-}$ mice died predominantly of lymphoma (Sansom and Clarke, 2000). However, there was no significant difference between the different genotypes with 83% (15/18) of $Mbd2^{-/-}p53^{-/-}$ mice developing lymphoma compared to 85% (23/27) of $Mbd2^{+/+}p53^{-/-}$ mice ($P \geqslant 0.8$, χ^2 test). Histological analysis of the tumours revealed no clear genotype dependent differences. Thus, in both single

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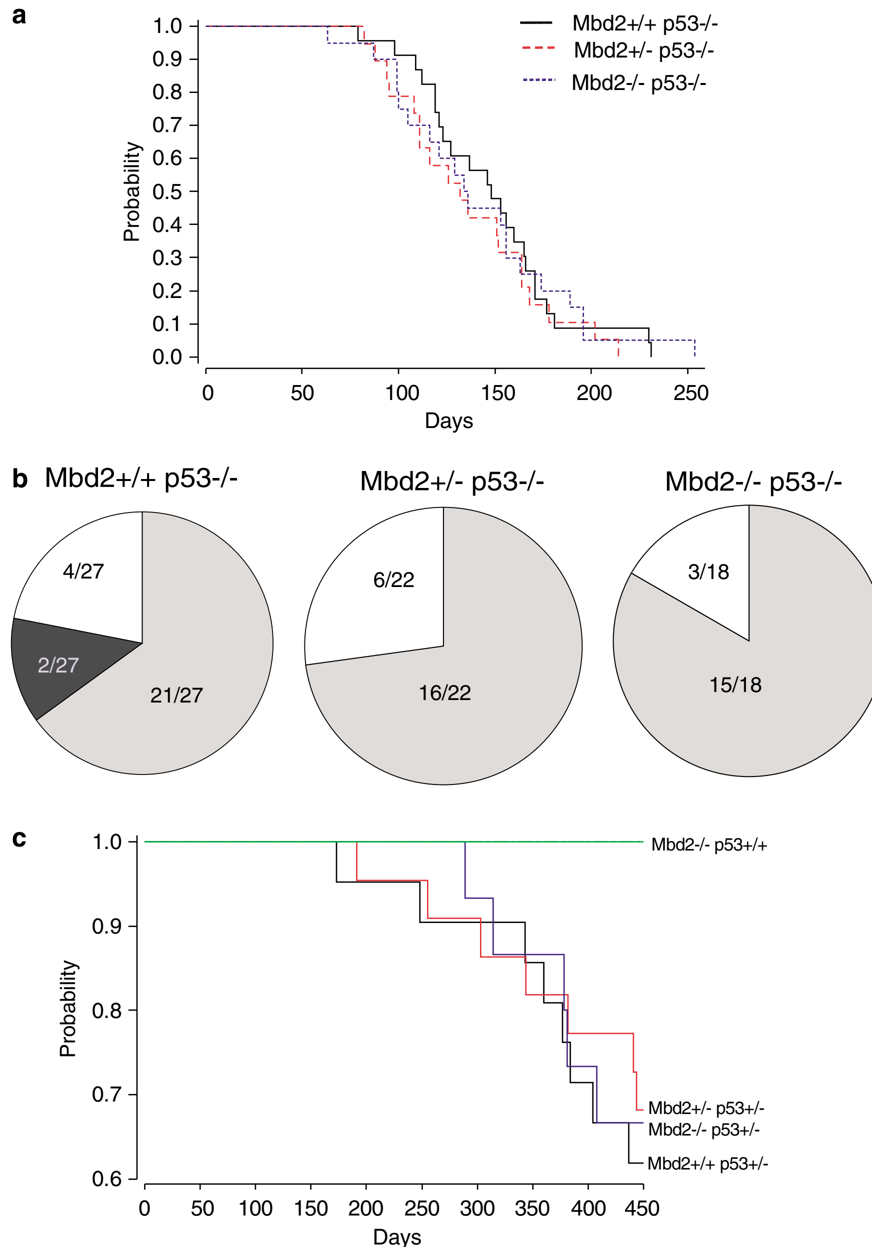

Figure 1 (**a**) Deficiency of Mbd2 does not alter survival of p53 deficient mice. *Mbd2* and *p53* mutant animals were derived from a colony segregating for Ola/129 and C57BL6J genomes, which had been backcrossed three generations onto the C57BL6J background and so were predominantly (87.5%) C57/BL6J. Mice were genotyped by PCR as previously described (Toft *et al*., 2002; Sansom *et al*., 2003). Cohorts of at least 18 mice were monitored and harvested when they exhibited symptoms of disease. Kaplan–Meier plot of survival for $Mbd2^{+/+}p53^{-/-}$ ($n=27$), $Mbd2^{+/-}p53^{-/-}$ ($n=22$) and $Mbd2^{-/-}$ $p53^{-/-}$ mice ($n=18$). Black line, $Mbd2^{+/+}p53^{-/-}$ mice; red line $Mbd2^{+/-}p53^{-/-}$; $Mbd2^{-/-}$ $p53^{-/-}$ blue line. No differences in survival were observed between any of the genotypes ($P\geqslant0.7$, Log rank). (**b**) Mbd2 does not alter tumour distribution in p53 deficient mice. Pie charts of tumour distribution in (**a**) $Mbd2^{+/+}p53^{-/-}$ ($n=27$); (**b**) $Mbd2^{+/-}p53^{-/-}$ ($n=22$) and (**c**) $Mbd2^{-/-}$ $p53^{-/-}$ ($n=18$) mice. These pie charts indicate the tumour burden identified at death in each mouse. Grey slice, mice with lymphoma; white slice, mice with sarcoma; black slice, mice with concurrent lymphoma and sarcoma. No significant difference was observed in lymphomagenesis with 23/27 $Mbd2^{+/+}p53^{-/-}$ developing lymphoma (two of these also had concurrent sarcoma) compared to 15/18 $Mbd2^{-/-}$ $p53^{-/-}$ mice ($P\geqslant0.8$ χ^2). No significant difference was observed in sarcoma either with 6/27 $Mbd2^{+/+}p53^{-/-}$ developing sarcoma compared to 3/18 $Mbd2^{-/-}$ $p53^{-/-}$ mice. (**c**) Deficiency of Mbd2 does not alter survival of mice heterozygous for p53. Kaplan–Meier plot of survival for $Mbd2^{+/+}p53^{+/-}$, $Mbd2^{+/-}p53^{+/-}$ and $Mbd2^{-/-}p53^{-/+}$ mice. Black line, $Mbd2^{+/+}p53^{+/-}$ mice ($n=22$); red line $Mbd2^{+/-}p53^{+/-}$($n=19$); blue line $Mbd2^{-/-}p53^{+/-}$($n=20$); green line $Mbd2-/-$ $p53^{+/+}$ ($n=10$). No differences in survival were observed between any of the p53 +/− genotypes ($P\geqslant0.8$, χ^2). All experiments were performed according to UK Home Office regulations

and double null mice, thymic lymphomas predominated (63 and 67% respectively), with a reduced number of lymphomas having extra-thymic origin (22 and 17% respectively). In the $Mbd2^{+/+}p53^{-/-}$, the majority of sarcomas (67%) were poorly differentiated, although one was identified as a haemangiosarcoma and one an

osteosarcoma, both of which have previously been reported in $p53^{-/-}$ mice. All three of the sarcomas developing in the $Mbd2^{-/-}p53^{-/-}$ were poorly differentiated. These data therefore consolidates the survival data, showing that loss of *Mbd2* does not accelerate *p53* mediated lymphomagenesis.

Given the strong predisposition to lymphomagenesis and genomic instability associated with *p53* deficiency, any effect of *Mbd2* deficiency may be masked. We therefore examined the effect of *Mbd2* deficiency upon survival of *p53* heterozygous mice (Figure 1c). At 450 days there were no difference in survival between $p53^{+/-}Mbd2^{+/+}$ mice (42%, 9/21) and $p53^{+/-}Mbd2^{-/-}$ (6/15, 40%, $P > 0.8$ χ^2). In support of our previous studies we again saw no increase in lymphomagenesis in $Mbd2^{-/-}$ alone ($n = 5$) at least in mice up to 500 days (Hendrich *et al*., 2001; Sansom *et al*., 2003). These studies in conjunction with our previous studies showing no gross changes in methylation days (Hendrich *et al*., 2001; Sansom *et al*., 2003) highlight that complete depletion of *Mbd2* does not mimic the effects of the $DNMT1^{chip/-}$ hypomorph, namely increased tumorigenesis and genomic instability (Eden *et al*., 2003; Gaudet *et al*., 2003). Therefore, although Mbd2 deficiency fails to suppress lymphomagenesis, critically it does not accelerate neoplasia, even within the highly tumour prone *p53* null environment. This finding, coupled with the ability of *Mbd2* to suppress intestinal malignancy, enhances the potential of MBD2 as a target for clinical intervention. Indeed, recent studies have shown that depletion of Mbd2 using antisense inhibitors suppresses growth of human lung and colorectal cell lines *in vitro* and human cancer xenografts *in vivo* (Campbell *et al*., 2004). Therefore, by focussing on the proteins that interpret methylation rather that inhibiting the methylation *per se*, future antitumour strategies may avoid the possible side effects of global hypomethylation and genomic instability.

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