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SHORT COMMUNICATION

Jdp2 downregulates Trp53 transcription to promote leukaemogenesis in the context of Trp53 heterozygosity

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We performed a genetic screen in mice to identify candidate genes that are associated with leukaemogenesis in the context of Trp53 heterozygosity. To do this we generated Trp53 heterozygous mice carrying the T2/Onc transposon and SB11 transposase alleles to allow transposon-mediated insertional mutagenesis to occur. From the resulting leukaemias/lymphomas that developed in these mice, we identified nine loci that are potentially associated with tumour formation in the context of Trp53 heterozygosity, including AB041803 and the Jun dimerization protein 2 (Jdp2). We show that Jdp2 transcriptionally regulates the Trp53 promoter, via an atypical AP-1 site, and that Jdp2 expression negatively regulates Trp53 expression levels. This study is the first to identify a genetic mechanism for tumour formation in the context of Trp53 heterozygosity.

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INTRODUCTION

Genetic alterations of TP53 are frequent events in tumourigenesis and promote genomic instability, impair apoptosis, and contribute to aberrant self-renewal.1-4 The spectrum of mutations that occur in TP53 in human cancers is diverse. Missense mutations that deregulate the DNA-binding domain are common, and prevent or impair the transcriptional regulatory activity of TP53.1,2 Cytogenetic alterations that delete or disrupt TP53 impair the transcriptional regulatory activity of TP53 and promote genomic instability, impair apoptosis, and contribute to aberrant self-renewal.1-4

Results and discussion

Mouse lines carrying the mutant Trp53 allele, Trp533/4, which are null for Trp53,15,16 the SB (Sleeping Beauty) transposon array, T2/Onc,16 and the SB transposase allele, Rosa26SB11 (see Dupuy et al.17) were intercrossed to generate mice that were homozygous, heterozygous or wild type for the Trp53 allele (hereafter referred to as Trp53+/−, Trp533/−/− or Trp533/−/− mice, respectively) with or without SB transposition occurring (that is, on a T2/Onc+/−, Rosa26+/−/− or T2/Onc+/−/−, Rosa26+/−/− background, respectively). These mice were aged until they became moribund, and, as expected, SB transposition significantly accelerated tumour latency in mice of all genotypes (Figure 1a). The predominant tumour type of all genotypes was a widely disseminated CD3+ T-cell lymphoma (Figures 1b and c). A number of solid tumours, mainly undifferentiated sarcomas, were also observed, but only in Trp53+/− or Trp533/−/− mice (Figures 1b and c).

Genomic DNA from 36 Trp53+/−, 116 Trp533/−/− and 9 Trp533/−/− SB-induced leukaemic/lymphomic tissues (typically spleen, thymus or lymph node) was extracted and subjected to a previously described linker-mediated PCR approach16 to amplify barcoded genomic fragments containing transposon–genome junction sequences. These products were then pooled and sequenced on the 454 platform, from which we generated 487 586 uniquely mapped sequence reads (approximately 3000 per tumour). After merging overlapping reads originating from the same sample and removing any on chromosome 1 (because SB
transposons frequently reintegrate into regions adjacent to the donor locus—a phenomenon known as 'local hopping'16), we obtained 7538 (Trp53$^{+/+}$), 21 975 (Trp53$^{+/−}$) and 1829 (Trp53$^{−/−}$) unique, non-redundant insertion sites (for the respective tumour genotypes indicated in brackets). Using a previously described Gaussian Kernel Convolution statistical method for determining common insertion sites (CISs),19,20 we identified 42, 63 and 9 CISs in Trp53$^{+/+}$, Trp53$^{+/−}$ and Trp53$^{−/−}$ tumours, respectively, ($P < 0.05$ on a chromosome-adjusted scale; Figure 2 and Supplementary Table 1). Many of these genes have been previously implicated in the pathogenesis of T-cell lymphomagenesis/leukaemogenesis, including NOTCH1, PTEN and IKZF1 (reviewed in Demarest et al.21). There were 12 CIS genes in common between the Trp53$^{+/+}$ and Trp53$^{+/−}$ tumours, specifically Mecom.
development of T-ALL and PTEN inactivation can compensate for some Notch-mediated processes in T-ALL.\textsuperscript{21} In addition, retroviral insertional mutagenesis recently identified ikzf1, Krox\textsuperscript{4120} and notch1 as a novel genetic pathway in T-lineage leukaemogenesis.\textsuperscript{28}

Quantitative PCR was performed on all tumours from Trp53\textsuperscript{+/—} mice to identify those that had retained a wild-type copy of Trp53 and those that carried two copies of the targeted Trp53\textsuperscript{797} allele (presumably having lost the wild-type allele by mitotic recombination; Figure 3a). From the 111 Trp53\textsuperscript{+/—} tumours analysed, we identified 40 that had retained a wild-type Trp53 allele (defined as having a normalised wild-type allele content of $0.28$ and a Trp53\textsuperscript{797} allele content of $0.72$) and 27 tumours that carried two targeted Trp53\textsuperscript{797} alleles and no wild-type allele signal (defined as having a normalised wild-type allele content of $<0.1$ and a Trp53\textsuperscript{797} allele content of $>0.8$). To determine if there were any somatic mutations in the intact wild-type copy of Trp53, genomic DNA from all 111 Trp53\textsuperscript{+/—} tumours (as well as some tail samples to facilitate the identification of somatic mutations) underwent Trp53 sequencing on the Illumina platform (Illumina, San Diego, CA, USA) to scan for point mutations (using the primers shown in Supplementary Table 2). Paired-end sequencing of PCR amplified fragments was followed by base-calling with SAMTOOLS mpile-up,\textsuperscript{29} which identified three possible mutations, specifically MMU11:69400422 (T-C), MMU11:69403089 (G-A) and MMU11:69403110 (G-A) in single tumours. All other tumours appeared to have retained the wild-type Trp53 allele. A further two required changes at MMU11:69401065 and MMU11:69401996 were discovered in 22 and 44 of the samples, respectively, and are therefore likely to be germline variants (as these mice were on a mixed C57BL/6J-129Sv background and the sequencing data was compared with the C57BL/6J reference genome). These data suggest point mutations of the wild-type Trp53 are infrequent in our model.

Taking the insertion sites found in tumours from Trp53\textsuperscript{+/—} mice, we performed CIS analysis in two ways. First, the tumours were divided into two groups: those that had either retained a wild-type copy of Trp53 or those that had lost the wild-type copy to identify the CISs that were unique and common to each group (Figure 3b). We found a set of nine CIS loci enriched in Trp53\textsuperscript{+/—} mice that developed tumours despite retaining a wild-type copy of the gene, including AB041803, Akt2, Eras, Ikzf1, Jdp2, Myb, Rapgef6 and two intergenic regions. Second, we pooled the insertion sites from both groups together and then distinguished genotypic-specific CISs using a P-value generated by Fisher’s Exact test analysis.\textsuperscript{30} Using this more ‘stringent’ method of CIS calling, we identified two CISs that were ‘enriched’ in Trp53\textsuperscript{+/—} tumours that had retained a wild-type copy of Trp53, specifically AB041803 and Jdp2. Little is known about AB041803 and as yet no role in tumourigenesis is evident. In addition, it was also found to be a CIS in leukaemia/lymphoma of wild-type mice (Supplementary Table 1).\textsuperscript{22} Thus we focused on Jdp2.

Transcription factor JDP2 (also known as JUNDM2) is an AP-1 repressor protein\textsuperscript{31} that has a paradoxical role in tumour formation. Overexpression of Jdp2 has been shown to promote hepatocellular carcinoma in mice\textsuperscript{32} and retroviral insertions predicted to activate the gene have been reported in mouse lymphoma models.\textsuperscript{33,34} In contrast, downregulation of JDP2 has been associated with a poor prognosis in pancreatic cancer.\textsuperscript{35} Loss of Jdp2 has also been associated with resistance to replicative senescence.\textsuperscript{36,37} and Jdp2 expression has been shown to suppress cell-cycle progression by downregulation of cycler-A2.\textsuperscript{38} However, hypomethylation of the Jdp2 promoter or upregulation of Jdp2 expression in common myeloid progenitors and in granulocyte-macrophage progenitors has led to suggestions that it functions as a regulator of myelopoiensis.\textsuperscript{39} Here, we find that transposon insertions in the Jdp2 promoter occur exclusively in tumours from Trp53\textsuperscript{+/—} mice that retain a wild-type allele of Trp53.

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Figure 2. Analysis of common insertion sites. A Circos plot showing the common insertion sites (CISs) called in tumours from Trp53\textsuperscript{+/—} (black), Trp53\textsuperscript{+/+} (blue) and Trp53\textsuperscript{−/−} (red) mice. Lines crossing the circle indicate statistically significant co-occurring mutations with the thickness of the line indicating the level of significance. All tumour DNA was extracted using GenePure kits (Qiagen, Sussex, UK) and transposon insertion site sequences were generated on the 454 platform (Roche, West Sussex, UK), as described previously.\textsuperscript{28} Processing of 454 reads, identification of insertion sites, and the Gaussian Kernel Convolution statistical methods used to identify CISs have been described previously.\textsuperscript{19,20} The P-value for each CIS was calculated using an adjusted-by-genome cutoff of $P < 0.05$. A complete list of the CISs is given in Supplementary Table 1.

(Mds1 and Evi1 complex locus), Myb, Notch1, Stat5b, Erg, Ikzf1, Raf1, Rasgrp1, Zmi1, Pten, AB041803 and Il2rb. Given that eight of these genes have also been identified as CISs in leukaemias/lymphomas from T2/Onc/Rosa26-SB11 mice on a wild-type background (Myb, Notch1, Erg, Ikzf1, Rasgrp1, Zmi1, Pten and AB041803),\textsuperscript{27} they likely represent genes involved in lymphomagenesis/leukemogenesis in general, and do not contribute to promotion of tumourigenesis in the context of Trp53 heterozygosity.

A CIS gene that was found in the Trp53\textsuperscript{+/−} and Trp53\textsuperscript{−/−} tumours, but not Trp53\textsuperscript{+/+} tumours, was Rapgef6. The Rap1 guanine nucleotide exchange factor RAPGEF6 (also known as PDZGEF2) has a critical role in the maturation of adherens junctions.\textsuperscript{25} Although no immediate role for RAPGEF6 in tumourigenesis is evident, it has been shown to form protein complexes that result in the activation of Rap1A and control of cell adhesion/migration.\textsuperscript{24,25} Interestingly, apart from Rapgef6, the CIS genes found in the Trp53\textsuperscript{−/−} tumours were not found in tumours of the other genotypes. These included genes Usp42 (ubiquitin specific peptidase 42) and Wdr33 (WD repeat-containing protein 33). Although little is known about Wdr33 gene, Usp42 gene has been recently identified as a fusion partner of RUNK1 in three cases of myeloid neoplasia, and the associated upregulated expression of USP42 suggests a role of this deubiquitinating enzyme in the pathogenesis of this leukaemia.\textsuperscript{26}

There were also four CISs that were found to co-occur in tumours (Figure 2), specifically Notch1 and Pten, Notch1 and Ikzf1, Pten and Ikzf1 and Pten and Akt2 in Trp53\textsuperscript{+/−} tumours. These genes have all been previously implicated in the pathogenesis of T-ALL.\textsuperscript{21,27} Our results are in keeping with the literature, as there is evidence for genetic co-operation of these genes in development of T-ALL. For example, loss of ikzf1, a direct repressor of Notch target genes, and suppression of p53-mediated apoptosis are essential for
insertions clustered in the promoter of \textit{Jdp2} (Figure 3c) and were mostly orientated so that the transposon was inserted in the same transcriptional orientation as the gene, suggesting that these insertions were functioning to drive overexpression (with a single insertion orientated on the reverse strand relative to the gene, which may represent an enhancer insertion\cite{40}). RT-PCR on RNA from these tumours showed splicing of the T2Onc transposon splice donor site directly onto \textit{Jdp2} exons 2 and/or 3 (Supplementary Figure 1). Indeed insertions in this exact location have been shown to activate \textit{Jdp2} expression,\cite{34} and consistent with this, qPCR on RNA from tumours containing insertions in \textit{Jdp2} showed a trend towards having higher expression levels of \textit{Jdp2} and lower expression levels of \textit{Trp53}, relative to \textit{Trp53}/+/+ tumours with no insertions in \textit{Jdp2} (randomly selected from mice on this study that had not lost the \textit{Trp53} allele by mitotic recombination; Figure 3d).

Co-transfection of \textit{JDP2} cDNA in an overexpression vector with a mouse \textit{Trp53} proximal promoter construct in murine NIH3T3 (Figure 3e) and human HEK293T cells (data not shown) resulted in significant repression of \textit{Trp53} promoter activity, confirming that overexpression of \textit{JDP2} functions directly on the \textit{Trp53} promoter to repress \textit{Trp53} expression. The ability of \textit{JDP2} to repress transcription of the \textit{p53} promoter is reported to occur via its binding to an atypical AP-1 site, termed the PF-1 site,\cite{41} When we mutated (deleted) this binding site in the proximal \textit{Trp53} promoter, this completely abrogated the suppressive effects of \textit{JDP2} (Figure 3e), confirming that over-expression of \textit{JDP2} mediates repression of \textit{Trp53} through the PF-1 site in the proximal promoter. Furthermore, overexpression of \textit{JDP2} in HEK293T cells was shown to repress endogenous \textit{TP53} expression (Figure 3f).
In conclusion, we show that overexpression of Jdp2 in tumours that are heterozygous for Trp53 precludes the need for loss of the wild-type allele of Trp53 during the process of leukaemogenesis. Jdp2 overexpression is the first genetic mechanism that has been identified as being responsible for tumour formation in the context of Trp53 heterozygosity.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the Oncogene website (http://www.nature.com/onc)