Figures and figure supplements

Deletion of the MAD2L1 spindle assembly checkpoint gene is tolerated in mouse models of acute T-cell lymphoma and hepatocellular carcinoma

Floris Foijer et al
Figure 1. Tissue specific loss of Mad2l1 leads to T-cell acute lymphoblastic lymphoma in T cells in a permissive Trp53<sup>−/−</sup> background. (A) Schematic overview of the Mad2l1 conditional allele before and after Cre-mediated recombination. The red triangles refer to the loxP sites that surround exon 2 to exon 5; roman numerals refer to exons. (B, C) PCR for Mad2l1 genotypes and recombination of the Mad2l1<sup>f</sup> allele in (B) thymocytes and (C) liver tissue (L) or tail tissue (T). (D) Kaplan Meier plots showing survival of the indicated genotypes for Lck-Cre::Mad2l1<sup>f/f</sup>::Trp53<sup>f/f</sup> compared to control mice. Statistical tests for compared Mad2l1<sup>f/f</sup> and Mad2l1<sup>+/+</sup> having same Trp53 genotype, **p<0.01 (Mantel-Cox test). Control curves (Lck-Cre::Trp53<sup>f/f</sup> and Lck-Cre) were same animals as used in Foijer et al. (2014). (E) Images showing enlarged thymus and spleen in a Lck-Cre::Mad2l1<sup>f/f</sup>::Trp53<sup>f/f</sup> mouse.
Figure 1 continued

compared to a healthy control. (F) Average thymus and spleen weights for tumor-bearing Lck-Cre::Mad2l1<sup>f/f</sup>:
Trp53<sup>f/f</sup> mice compared to unaffected control mice. (G) Representative H&E staining of control thymus (upper
panel) and Lck-Cre::Mad2l1<sup>f/f</sup>::Trp53<sup>f/f</sup> acute T acute lymphoblastic lymphoma sample with staining indicating an
undifferentiated cell state (lower panel). Scale bar 10 microns. (H) Forward and side scatter (FSC, SCC) plots for
normal (appearing) thymuses and a T-ALL showing the emergence of a larger blasting population, before thymus
size increased (upper panels). FSC and SCC plots for thymus, blood and spleen of a tumor-bearing mouse,
showing blasting cells in thymus and spleen, but not blood (lower panels).
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Figure 1—figure supplement 1. Complete Targeting Vector and Generation of Mad2l1<sup>f/f</sup> Mice. (A) Targeting vector and locus with restriction site changes highlighted. Recombination of floxed allele results in excision of exons 2–5. Arrows indicate PCR primer sites. (B) Restriction digest and southern blot showing correct recombination at the murine Mad2l1<sup>f/f</sup> locus.

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Figure 1—figure supplement 2. Representative array CGH profiles for 3 Lck-Cre::Mad2l1^{f/f}::Trp53^{f/f} tumors showing clonal loss at the TCR loci on chromosomes 6 and 14 indicating tumor clonality.
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Figure 2. Loss of Mad2l1 in hepatocytes results in multifocal hepatocellular carcinoma. (A) Kaplan Meier plots showing survival of the indicated genotypes for Alb-Cre::Mad2l1f/f::Trp53f/f compared to control mice. Statistical tests compared Mad2l1f/f::Trp53f/f and Mad2l1f/f::Trp53f/+ to Mad2l1f/+::Trp53f/+ mice, **p<0.01, ***p<0.001 (Mantel-Cox test). (B) Images showing multifocal disease in a Alb-Cre::Mad2l1f/f::Trp53f/f mouse compared to a healthy control. (C) Histological sections from Alb-Cre::Mad2l1f/f::Trp53+/+ mice. Left panels show regenerative nodules (RN) marked by black arrows in low magnification field and cells in one nodule at high magnification. Centre panels show an HCA marked by the asterisk. Right panels show an HCC marked by an asterisk (top) or the entire field (bottom). Scale bars in top fields 1 mm, bottom fields 0.1 mm. (D) Incidence of HCA and HCC in the livers of Control (Mad2l1+/+::Trp53+/+ or Alb-Cre), Alb-Cre::Mad2l1f/f::Trp53+/+, and Alb-Cre::Mad2l1f/f::Trp53f/f. (E) Kaplan Meier plot showing survival of Alb-Cre::Mad2l1f/f::Trp53R246Sf/f mice compared to control. **p<0.001 (Mantel-Cox test). (F) Representative MRI images of an Alb-Cre::Mad2l1f/f::Trp53f/f with a tumor (white arrow) over time in weeks. EOVIST is used as a contrast agent and tumors exclude the reagent and are dark. (G) Volumetric measurements of tumors over time. Each symbol represents a different mouse. (H) Doubling time as determined by semi-log regression of data in (G).

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Figure 2—figure supplement 1. Characterizing aneuploid T-ALLs and HCCs. Workflow from MRI imaging to histological examination of liver malignancies.
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Figure 3. Excision of Mad2l1 DNA and loss of SAC function in normal and tumor cells. (A) Recombination efficiency of Mad2l1 and Trp53 alleles in T-ALL samples as measured by genomic PCR. Numbers refer to tumor ID. (B) Recombination efficiency at Mad2l1 and Trp53 loci in T-ALL or samples as measured by array CGH. Each rectangle represents a single aCGH probe value, three probes values are shown per conditional gene: one probe recognizing the Mad2l1 or Trp53 deleted fragment (middle) and two probes flanking the 5' and 3' sides of the deleted region. (C) Quantitative PCR showing complete deletion of Mad2l1 (probe A) and Trp53 (probes A and B) in T-ALL samples. Error bars show SEM for six (Mad2l1, Trp53) and three (Trp53) tumors (biological replicates). (D) Western blots showing loss of Mad2l1 expression in T-ALL samples. (E) Recombination efficiency at Mad2l1 and Trp53 loci in HCC samples as measured by array CGH. (F) Genomic PCR of tumor tissue for WT, FLOX, and recombined alleles of Mad2l1 and Trp53. Black vertical line shows where an empty lane was removed.

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Figure 4. Mad2l1 inactivation in mouse embryonic fibroblasts fully alleviates the spindle assembly checkpoint. (A) Recombination efficiency measured by genomic PCR (top) and Western blot (bottom) showing partial to complete Mad2l1 deletion in MEFs following retroviral doxycycline-inducible Cre. Actin serves as loading control. (B) Average phospho-Histone H3 staining of dox-inducible Cre-transduced MEFs following 6 hr of nocodazole treatment. Error bars show the SEM of at least two biological replicates. (C) Average mitotic index of thymocytes isolated from Paclitaxel or control-injected mice 4–6 hr post-treatment. Error bars show SEM of at least four biological replicates.

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Figure 5. Aneuploidy events are a recurrent genetic lesion in T-ALLs and HCCs. (A) Box and whiskers plot of whole chromosome gain-loss events in tumors, scored by averaging the log2 ratio of aCGH fluorescence from tumor over normal (aCGH ratio) for each chromosome with a ±0.3 cut-off. Line – median, Box – interquartile range, Whisker – range. (B) Box and whiskers plot of the aCGH ratio for each chromosome. Two-way ANOVA, Bonferroni post-test **p<0.01, ***p<0.001 (C) Scatter plot showing average chromosome aCGH ratio of HCC and thymus tumors. Gain of Chr15 (red), loss of Chr13 (green), and tissue specific gain or loss of Chr4, Chr12, and Chr18 (blue). (D, E) Scatter plots of average chromosome aCGH ratio plotted against average mRNA ratio for (D) HCC and (E) T-ALL. r is the Pearson Correlation with indicated P value. (F, G) Expression analysis of genes on chromosome 15 (F) and chromosome 18 (G) comparing HCC and T-ALL samples. (H) Scatter plot showing chromosome normalized aCGH ratio for every gene in HCC and T-ALL. The three listed genes are likely hybridization artifacts due to a mixed 129/C57BL6 background. (I, J) Number of focally amplified or deleted regions per tumor (I) and the number of genes amplified or deleted per tumor (J) scored with ±0.3 cut-off for the chromosome normalized aCGH ratio. *p<0.05, **p<0.01, Mann-Whitney Test comparing T-ALL and HCC.

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Figure 5—figure supplement 1. Copy number changes in T-ALL and HCC as assessed by aCGH. (A, B) Individual aCGH plots for T-ALLs (A) and HCCs (B) showing chromosome copy number alterations for individual tumors.

Figure 5—figure supplement 1 continued on next page.
Figure 5—figure supplement 2. T-ALL and HCC specific CNVs. (A) Graph of the chromosome normalized aCGH ratio for the genomic region around Pten for T-ALL samples. Colored lines have deletions in Pten. (B) Graph of the normalized aCGH ratio for Chr6 (left) focused on the genomic region around Met (right). Blue dots are individual aCGH probes. Red line is a moving average of 3 adjacent probes. (C, D) Met is over expressed by (C) mRNA as measured by quantitative PCR, mouse number indicates normal while mouse number + LT indicates tumor, and (D) protein as measured by Western Blot. NT is normal tissue, T is tumor tissue.

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Figure 6. Mad2l1 deficiency results in clonal abnormalities despite ongoing chromosomal instability in murine T-ALL. (A) Box and whiskers plot for each chromosome of unaffected liver (blue n = 12), HCA (green n = 10), and HCC (red n = 18) from Alb-Cre::Mad2l1\(^{fl/fl}\)::Alb-Cre mice with mixed Trp53 genotypes. Statistical significance assessed by Two-way ANOVA and Tukey multiple comparison test with comparison between each group shown in the table below, *p<0.05, **p<0.01, ***p<0.001. (B) Sum of the absolute value of the aCGH ratio for each chromosome. Statistical significance assessed by One-way ANOVA and Tukey multiple comparison test, *p<0.05, **p<0.01, ***p<0.001. (C–F) AneuFinder plots revealing perfect euploidy in control thymus (45 cells, C), and recurrent chromosomal abnormalities as well as intratumor karyotype heterogeneity in three Lck-Cre::Mad2l1\(^{fl/fl}\)::Trp53\(^{fl/fl}\) T-ALLs for which 46 (D), 44 (E) and 43 (F) primary tumor cells were analyzed by single cell sequencing, respectively. Colors refer to chromosome copy number state.
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Figure 6—figure supplement 1. Heterogeneity and aneuploidy scores for control thymus and individual T-ALLs analyzed by single cell sequencing.
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Figure 7. Overlaid TCGA copy number data at kilobase resolution for epithelial (Hepatocellular carcinoma and Breast Cancer, upper panel) and non-epithelial (Glioma and Glioblastoma multiforme, lower panel) represented as the number of tumors above a log2 threshold of 0.3 for gains and below −0.3 for losses. The Y-axis is scaled to the total number of tumors analyzed. Analyzed data was sequenced at the Broad institute (Boston, MA) on SNP6.0 chips.

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