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The *Trp53* delta proline (*Trp53 P*) mouse exhibits increased genome instability and susceptibility to radiation-induced, but not spontaneous, tumor development

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Abstract

The tumor suppressor *TP53* can initiate a plethora of anti-proliferative effects to maintain genomic integrity under conditions of genotoxic stress. The N-terminal proline-rich domain (PRD) of TP53 is important in the regulation of TP53 activity and stability. A common polymorphism at codon 72 in this region has been associated with altered cancer risk in humans. The *Trp53 P* mouse, which carries a germline homozygous deletion of a region of the PRD, does not develop spontaneous tumors in a mixed 129/Sv and C57BL/6 genetic background, but is highly susceptible to a broad range of tumor types following total body exposure to 4 Gy gamma (γ) radiation. This contrasts with the tumor spectrum in *Trp53* null ($-/-$) mice, which mainly develop thymic lymphomas and osteosarcomas. Analysis of genomic instability in tissues and cells from *Trp53 P* mice demonstrated elevated basal levels of aneuploidy, but this is not sufficient to drive spontaneous tumorigenesis, which requires an additional DNA damage stimulus. Levels of genomic instability

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

did not increase significantly in *Trp53*^P mice following irradiation exposure, suggesting that other radiation effects including tissue inflammation, altered metabolism or autophagy, may play an important role. The *Trp53*^P mouse is a novel model to dissect the mechanisms of tumor development induced by radiation exposure.

Keywords

Trp53; cancer; radiation; genomic instability

INTRODUCTION

Ionizing radiation is a carcinogen that has been implicated in the causation of numerous malignancies including breast cancer, gliomas, lymphomas and sarcomas [1]. Radiation damages DNA directly, by inducing double-strand breaks, and indirectly, by generating reactive oxygen species that damage sugar and base residues leading to a variety of chromosomal aberrations [2]. However, the exact role of radiation in the induction of carcinogenesis still remains controversial [3], as radiation induces, in addition to DNA damage, a complex multicellular stress response that elicits effects at the whole tissue level [4]. The incidence of radiation-induced cancers is expected to rise with increased use of medical radiation such as CT scanning, and in long-term survivors of cancer in whom radiation therapy plays an important role.

The tumor suppressor TP53 can initiate a multiplicity of anti-proliferative effects to maintain genomic integrity, including cell cycle arrest, DNA repair, senescence, differentiation and apoptosis [5, 6]. The PRD region of TP53, spanning amino acid residues 58–98 in human TP53, is implicated in both the stabilization of TP53 and transcriptional activation of TP53-dependent genes, thereby acting as a molecular switch promoting induction of either apoptosis or cell cycle arrest [7–9]. The PRD of TP53 contains binding sites for SH3 (Src homology 3) domains, and interacts with a wide range of proteins including Mdm2, p300, mSin3a and the ASPP family [10–12]. A common human polymorphism occurs within the PRD at codon 72 (Pro72Arg), which modifies the induction of apoptosis [13]. Deletion of amino acids 75–91 of the PRD domain in the *Trp53*^P mouse [14] enhances sensitivity of Trp53 to Mdm2-dependent degradation and reduces Trp53-dependent transactivation of target gene expression, resulting in compromised cell cycle and apoptotic responses [14]. Interestingly, deletion of amino acids 58–88 of the PRD in a similar model, the m^{pro} mouse, resulted in a complete deficiency of apoptosis [15].

The *Trp53* heterozygous mouse (*Trp53*^{+/-}) is highly sensitive to ionizing radiation and develops lymphomas and soft tissue sarcomas after exposure to a single dose of 4 Gy [16–21]. However, this model has limitations including a relatively high rate of spontaneous tumor development, and a narrow spectrum of tumors, mainly limited to lymphomas and sarcomas [22]. Here we show that the homozygous *Trp53*^P allele on a mixed 129/Sv and C57BL/6 background develops no spontaneous tumors, but is exquisitely sensitive to 4Gy γ radiation-induced tumorigenesis, developing a wide spectrum of solid tumors including hemangiosarcomas and carcinomas as well as aggressive lymphomas and other high-grade

or poorly differentiated soft tissue sarcomas. We propose that the very low spontaneous tumor rate in the *Trp53*^P mouse combined with its high sensitivity to radiation-induced tumorigenesis provides new opportunities to explore the mechanisms that contribute to radiation carcinogenesis.

MATERIALS AND METHODS

Animal studies

Trp53^P mice were maintained on a mixed 129/SvJ and C57BL/6J background and have been described previously [14]. A total of 36 *Trp53*^P mice (18 female and 18 male) between 5 to 6 weeks of age were irradiated with 4 Gy from a ¹³⁷Cs source at a dose of roughly 220 rad/min. Twenty-two sham-irradiated *Trp53*^P mice served as controls. Mice were sacrificed and autopsied when they developed palpable tumors or became moribund. Tissues were snap frozen and a small section fixed in 10% phosphate-buffered formalin for histology purposes. All tumor samples were independently reviewed by two certified pathologists. Animal husbandry was carried out per national and institutional guidelines. All mouse experiments were approved by the University of California San Francisco Laboratory Animal Resource Centre.

Immunostaining

Immunofluorescence studies were performed as described previously [23] on either MEFs mounted on cover-slips or formalin-fixed paraffin embedded thymus sections. The following antibodies and concentrations were used: pericentrin - Covance (PRB-432C) 1:250, alpha-tubulin – Sigma-Aldrich (T6199) 1: 500 or gamma-tubulin Sigma-Aldrich (T5192) 1:200, followed by Alexa Fluor-488 or 555 conjugated secondary antibodies (1:500) (Molecular Probes). Staining was visualized with Olympus BX60 fluorescence and Leica Sp5II confocal microscopes.

Immunohistochemical staining was performed on formalin-fixed paraffin embedded tumor sections as previously described [24]. The following antibodies and concentrations were used: CD31- Santa Cruz biotechnology (SC1506) – 1:600, CD3 - DAKO (A0452) - 1:600 and B220- Pharmingen (550286) -1:800.

Flow cytometry

Early passage MEFs of WT, *Trp53*^{-/-} and *Trp53*^P genotypes were irradiated with 4 Gy γ radiation or sham irradiated and analyzed at 24 and 48 hours post-radiation. Cell cycle analysis of DNA content by propidium iodide staining was performed as previously described [14].

Statistical analysis

Cox proportional hazard and Students t-test (unpaired) were performed using GraphPad Prism version 5.00 for Windows. Linear modeling was performed to evaluate the relationship between genotype, timepoint/radiation, and aneuploidy. The fraction of aneuploid cells was predicted by radiation exposure timepoint and *Trp53* genotype. Significance was evaluated by ANOVA. All modeling was performed in R version 3.1.0.

RESULTS

Trp53^{-/-} P mice develop a broad spectrum of tumors post-irradiation

Despite the compromised cell cycle arrest and apoptotic function of the *Trp53*^{-/-} P mice, they are relatively resistant to spontaneous tumorigenesis [14]. Because *Trp53*^{+/+} (WT) mice are highly resistant to radiation-induced oncogenesis after a single dose of total body radiation [16], we investigated the impact of radiation exposure on tumor development in homozygous *Trp53*^{-/-} P mice by exposing 5–6 week old animals to 4Gy γ total body radiation. During the 500 day monitoring period, none of the age-matched control sham-irradiated *Trp53*^{-/-} P mice (n=20) developed tumors (Figure 1a), consistent with previous observations of a very low rate of spontaneous tumor development [14]. In contrast, 83%, (30 of 36) of the irradiated *Trp53*^{-/-} P mice developed tumors, and of these, 6 developed multiple tumor types (Table 1). For clarity, each different tumor type is treated as separate, resulting in a total of 36 tumors from 30 mice. The remaining 6 (17%) died of non-tumorigenic causes, such as encephalitis, hydrocephalus of unclear etiology and pulmonary hemorrhage. There was a large range in time of tumor onset, ranging from 111 to 476 days post-irradiation (Figure 1a). The median total survival of the irradiated *Trp53*^{-/-} P mice (329 days) was far greater than the median survival of irradiated *m^{-/-}* pro mice (220 days) [15], and also slightly higher than that of irradiated *Trp53*^{+/-} mice (280 days)[16].

The most frequently observed tumor type in the *m^{-/-}* pro and *Trp53*^{+/-} mice is lymphoma. In contrast, *Trp53*^{-/-} P mice developed a broad spectrum of tumor types in a wide range of tissues. These included the lymph nodes and lymphatic system (33%, 12 of 36) and mesenchymal tissues (45%, 16 of 36), but also in epithelial tissues (22%, 8 of 36) (Figure 1b). Of the epithelial tumors that developed in *Trp53*^{-/-} P mice, 50% (4 of 8) were malignant carcinomas, including squamous cell carcinoma of the head and neck, adenocarcinoma of the mammary gland and bronchio-alveolar carcinoma of the lung (Figure 1c & 2a). An additional two mice developed squamous cell tumors of the skin (keratoacanthomas), and the remaining two mice developed papillary bronchio-alveolar adenoma of the lung. The similarity of median survival time between *Trp53*^{-/-} P and *Trp53*^{+/-} suggests that the broader tumor spectrum observed in the irradiated *Trp53*^{-/-} P is not likely due to a longer life span in *Trp53*^{-/-} P mice. We sequenced all exons of *Trp53* in 20 radiation-induced tumors (thymic lymphomas, mammary and skin tumors) from *Trp53*^{-/-} P homozygous mice to determine if any additional mutations in *Trp53* are required to drive tumor development. No mutations were found (data not shown), suggesting that susceptibility to radiation-induced tumors is caused by the compromised allele (*Trp53*^{-/-} P) and does not require additional *Trp53* mutations.

Angiosarcomas (including lymphangiosarcomas and hemangiosarcomas) of the breast account for >50% of all sarcomas in humans following treatment of primary breast carcinomas with radiation therapy and have an extremely poor prognosis (<35% at 5 years) [25]. Included among the radiation-induced sarcomas in the *Trp53*^{-/-} P mouse were mammary hemangiosarcomas, confirmed by immunostaining for CD31, an endothelial marker of blood vessels, for which expression was uniformly positive (Figure 2b). Additional angiosarcomas were detected in other tissues including the spleen, intestinal stroma and spinal cord (Figure

2a & 1c). Despite the range of malignant tumors and organs involved in tumorigenesis, there is no significant difference in latency of carcinoma or sarcoma onset, regardless of the involved organ ($P=0.81$) (Figure 2c).

Consistent with other mouse models of abrogated *Trp53* function, lymphomas were frequently observed post-irradiation (33%, 12 of 36). Thymic lymphomas were the predominant type seen in 42% (5 of 12) of the mice, while the remaining 7 mice displayed highly aggressive lymphomas in the lung, liver, bladder, and pancreas (Figure 1c & 2a). The *m pro* model, which lacks Trp53-dependent apoptosis, is resistant to thymic lymphomas, and develops relatively late onset B-cell lymphomas [15, 22]. Analysis of latency of lymphoma onset in *Trp53*^{-/-} mice identified two distinct populations ($P>0.0001$), classified as early onset (<300 days) or late onset (>300 days) survival post-radiation (Figure 2c, Table 1). We performed immunostaining using B-cell marker CD45R (B220) and the T-cell marker CD3 to determine the lineage of lymphomas (Figure 2d). Early onset lymphomas were predominantly of T-cell origin (71%, 5 of 7) whilst the majority of late onset lymphomas (60%, 3 of 5) were negative for both CD3 and B220 expression.

These data show that while *Trp53*^{-/-} mice very rarely develop spontaneous tumors [14], they are highly sensitive to a wide spectrum of radiation-induced malignancies ($P<0.0001$, Figure 1a) including those with lymphoid, mesenchymal, or epithelial characteristics.

Trp53^{-/-} MEFs have elevated basal levels of polyploidy

Normal cells from *Trp53*^{-/-} mice show high levels of aneuploidy, which is associated with elevated genomic instability and increased spontaneous tumor development [26, 27]. To assess basal levels of aneuploidy and the impact of radiation exposure on the DNA content of *Trp53*^{-/-} cells, we exposed mouse embryonic fibroblasts (MEFs) of WT (*Trp53*^{+/+}), *Trp53*^{-/-} and *Trp53*^{-/-} genotypes to 4 Gy γ radiation and performed flow cytometry for DNA content at 24 and 48 hours post-radiation. For both timepoints, taking into consideration radiation, genotype was strongly associated with the percentage of aneuploidy cells ($P=7.016\text{e-}09$, 24 hour and $P=6.173\text{e-}11$, 48 hour). We suspected this significance may be driven by WT samples, which had a far lower percentage of cells in aneuploidy (Figure 3a). Indeed, upon exclusion of WT samples from this analysis genotype was no longer significant at the 24 hour timepoint, and at the 48 hour timepoint significance was reduced, but not lost ($P = 3.954\text{e-}07$), implying at 48 hours post-IR differences in aneuploidy fractions exist between all genotypes (Figure 3a). This suggests that, despite *Trp53*^{-/-} mice being resistant to spontaneous tumorigenesis, un-irradiated *Trp53*^{-/-} cells have an elevated level of chromosomal instability (CIN) in comparison to WT. At no point was radiation a significant driving factor of aneuploidy, therefore irradiation with 4 Gy γ (the dose used for tumor induction) did not increase the aneuploidy populations compared to those of the sham group at the same time point regardless of genotype (Figure 3a). Additional studies with 10 Gy γ radiation consistently increased levels of aneuploidy across all genotypes (Supplementary Figure 1).

Trp53^{-/-} cells exhibit centrosome hyperamplification, leading to multiple spindle poles and mis-segregation of chromosomes, ultimately resulting in aneuploidy [26]. The *Trp53*^{-/-} MEFs frequently have three or more centrosomes per cell, and display abnormal multipolar

spindle formation, similar to *Trp53*^{-/-} cells (Figure 3b). Quantification of co-immunostaining with pericentrin and gamma-tubulin further confirmed that the number of centrosomes in both un-irradiated and irradiated *Trp53*^P MEFs frequently exceeded two per cell (Figure 3c). Taking into account radiation, genotype was significantly associated with the size of the aneuploid population ($P=1.813\text{e-}12$), which appeared to be mainly driven by WT samples. As previously, upon exclusion of WT samples from our analysis the significance fell dramatically ($P=0.04618$) although a modest difference still exists between *Trp53*^P and *Trp53*^{+/-} genotypes (Figure 3c). Taken together, these data suggest that, similar to *Trp53*^{-/-} cells *Trp53*^P cells have an elevated level of aneuploidy in comparison to WT cells in both basal and irradiated conditions.

***Trp53*^P mice have high levels of genomic instability in vivo**

We next asked whether centrosome abnormalities could be detected *in vivo* in the thymus, a tissue highly susceptible to radiation-induced malignancies. We quantified centrosome numbers in the thymus by co-immunostaining for pericentrin and gamma-tubulin at various times after exposing *Trp53*^P, *Trp53*^{WT}, and *Trp53*^{-/-} mice to 4 Gy γ total body radiation (Figure 4a). After taking into account timepoint post-radiation we found genotype was significantly associated with the fraction of aneuploid cells ($P=1.279\text{e-}06$). This difference appeared to be driven by WT mice, which showed reduced levels of aneuploidy relative to both *Trp53*^P and *Trp53*^{-/-} mice (Figure 4b). Congruent with this hypothesis, after removing WT mice from the analysis genotype was no longer significant ($P=0.4$). This demonstrates that both *Trp53*^P and *Trp53*^{-/-} genotypes display similarly elevated aneuploidy levels relative to WT mice. Of note, timepoint post-IR was never a significant predictor of fraction of cells in aneuploidy. Taken together, these results demonstrate that the level of genetic instability as measured by aneuploidy is consistently high in *Trp53*^P *in vitro* and *in vivo*, at both baseline and post-radiation exposure conditions.

DISCUSSION

The widely studied *Trp53*^{-/-} mouse has a very high spontaneous tumor incidence which has been associated, at least in part, with high levels of genomic instability in adult tissues [26, 27]. Here we demonstrate that, while basal levels of aneuploidy are similar (both *in vitro* and *in vivo*) in *Trp53*^P and *Trp53*^{-/-} mice, *Trp53*^P mice on a mixed 129/Sv and C57BL/6 background develop no spontaneous tumors in this study. The *Trp53*^P mouse is, however, sensitive to radiation-induced tumorigenicity and develops a broad spectrum of tumor types in multiple tissues. Exposure to 4 Gy γ radiation does not significantly increase levels of genomic instability in *Trp53*^P mice, suggesting that perturbation of other radiation-induced responses, such as inflammation, metabolism or autophagy may be responsible for driving tumorigenesis in this model.

Notably, the latency to tumor onset was increased and tumor spectrum broader in radiation-exposed *Trp53*^P mice, in comparison to the similar irradiated *m pro* mouse model [15]. The *m pro* mouse develops mainly late onset B cell lymphomas, and is resistant to thymic lymphomas [15]. In contrast thymic lymphomas are the most frequently observed lymphoma type in the *Trp53*^P mouse, along with a range of carcinomas and sarcomas which are not

observed in the m^{pro} mouse. There are several potential reasons for these differences: apoptotic function is diminished in Trp53^P mice, but is not eliminated as in the m^{pro} mice, which may contribute to the lack of spontaneous tumors and increased latency to tumor onset in Trp53^P mice. The differential tumor spectrum may rest in inherent differences in mouse strain-specific susceptibility to different types of cancers. The m^{pro} mice are on the C57BL/6 background whereas Trp53^P mice are on a mixed 129/SvJ and C57BL/6 strain, increases in the 129/Sv genetic background can lead to a rise in the incidence of carcinomas with reduction of lymphomas [28]. Furthermore, the exact PRD regions deleted between the two models differs (m^{pro} 58–88, Trp53^P 75–91). This difference may alter structural conformation and potentially binding sites for proteins such as iASPP or ATM and subsequent post-translational modifications, all of which may differentially modulate p53 transactivational activity and impact upon tumorigenesis [10, 12, 29].

Recently the mechanisms by which Trp53 maintains tumor suppression have been re-assessed and the importance of Trp53-dependent cell cycle arrest and apoptotic activity in tumor suppression questioned [6, 30–33]. Notably, the acute Trp53-dependent DNA damage response is dispensable for tumor suppression [34, 35]. Indeed, the key role of Trp53 in maintaining genomic integrity in the absence of cell cycle arrest or apoptotic activity, has been demonstrated using the “*Trp53*^{25,26}” mouse model, in which Trp53 transactivation function is severely compromised. Although *Trp53*^{25,26} cannot transcriptionally induce *p21*, *Puma* or *Noxa* in these animals, they are resistant to spontaneous tumors, *Kras* driven non-small cell lung cancers and *Eμ-myc* driven lymphomas [30, 31]. *Trp53*^{25,26} can still induce cellular senescence, which initially may suggest that this phenotype is required for Trp53-dependent tumor suppression. However, mutation of three lysine residues within the DNA binding domain that are acetylated in response to DNA damage, (the *Trp53*^{3KR} mutant), abrogates Trp53-dependent senescence, cell cycle arrest and apoptosis *in vivo* [33]. Unexpectedly, these mice are also resistant to spontaneous tumorigenesis, demonstrating the diverse pathways by which Trp53 can implement tumor suppression [33]. Trp53-dependent apoptosis and cell cycle arrest *in vivo* are compromised in a model deficient for *p21*, *Puma* and *Noxa* expression, yet Trp53-dependent tumor suppression remains intact in this model [32]. These findings underscore the complexity of Trp53-dependent tumor suppression. Our data address the role of Trp53 in controlling an additional phenotype – genomic instability – and demonstrate that loss of this control in the *Trp53*^P mouse is not sufficient for induction of spontaneous tumors. Despite high basal levels of genomic instability *Trp53*^P mice remain tumor free in this genetic background, indicating that this partially deficient allele maintains tumor suppressor activity by alternative mechanisms. These data suggest that loss of any single function of *Trp53* by germline mutation in these engineered models is not sufficient for tumor development, and that spontaneous tumorigenesis is only unmasked upon combined loss of multiple biological pathways conferring protection. The mechanistic explanation as to why, in the presence of elevated genomic instability, *Trp53*^P can suppress spontaneous, but not radiation-induced tumorigenesis, remains to be elucidated.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

Trp53 P	Trp53 delta proline
PRD	Proline-rich domain
γ	Gamma
CT	Computerized tomography
MEFs	Mouse embryonic fibroblasts
CIN	Chromosomal Instability

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Figure 1. Tumor-free survival and spectrum

a) Kaplan-Meier curve shows overall survival in days of *Trp53* P homozygous mice on a mixed 129/SvJ and C57BL/6J background exposed to 4Gy γ radiation (n=36, red line), versus sham-irradiated control mice, (n=20, blue line) ($P < 0.0001$, log-rank test). **b)** Pie chart shows types of tumors developed post-irradiation based on differentiation; lymphoid (12 of 36), mesenchymal (16 of 36) and epithelial (8 of 36). **c)** Further details of tumor category, type and involved organ with the frequency of occurrence indicated. BA = bronchio-alveolar.

Figure 2. Radiation induces a broad tumor spectrum in a wide range of tissues

a) Hematoxylin and Eosin (H&E) of a range of tumor types developed in irradiated *Trp53* P mice, magnification is 20 $\times$, scale bar represents 200 μ m. **i)** ID:124- lymphoma infiltrating liver **ii)** ID:127- lymphoma infiltrating lung. **iii)** ID:151- hemangiosarcoma of the intestinal stroma. **iv)** ID:169- hemangiosarcoma invading the spinal cord, surrounding bone and of soft tissues. **v)** ID:166- papillary bronchio-alveolar adenoma of the lung. **vi)** ID:162- adenocarcinoma of the mammary gland. **b)** Representative H&E of two angiosarcomas showing vascular clefts lined by neoplastic endothelial cells, and, IHC for CD31 of both

angiosarcomas showing strongly positive membrane staining for CD31. ID numbers corresponding to Table 1 are indicated, magnification is 20×. Scale bar represents 100 μm. **c)** Scatter graph depicting days survival post-irradiation of various tumor types; bars indicate p values as determined by unpaired Student's t-tests, *= <0.05 **= <0.008 ***= <0.0001 , error bars represent 95% confidence interval. **d)** Representative CD3 and B220 IHC staining of two lymphomas. ID:128 is a thymic lymphoma, which stains positive for CD3 and is interpreted as a T-cell lymphoma. ID:124 is a lymphoma in the liver and has a mixed cell infiltrate. The majority of cells with large dysplastic nuclei are positive for B220 staining indicating origin as a B-cell lymphoma. ID numbers correspond to those in Table 1, magnification is 20×. Scale bar represents 200μm.

Figure 3. Abnormal centrosomes can participate in formation of multi-polar spindles in *Trp53*^{ΔP} MEFs and result in increased aneuploidy population

a) Flow cytometric analysis of cell cycle distribution by DNA content of MEFs of genotypes indicated. The average % of cells >4n (aneuploidy population) is 21.71 (WT), 34.43 (*Trp53*^{ΔP}) and 40.20 (*Trp53*^{-/-}). **b)** Representative image of immunostaining performed in MEFs of genotypes WT, *Trp53*^{ΔP}, and *Trp53*^{-/-} 24 hours 4Gy γ irradiation. Centrosomes are stained with pericentrin (green), mitotic spindles stained with alpha-tubulin (red). Nuclei were visualized with DAPI staining. All images are merged in the bottom panel. **c)**

Quantification of the fraction of cells with >2 centrosomes per cell as determined by gamma tubulin and pericentrin staining. MEFs of genotypes WT, *Trp53*^P, and *Trp53*^{-/-} were exposed to 4 Gy irradiation or sham irradiated and harvested at time points indicated. Studies were performed at minimum in triplicate. The mean number of cells counted per genotype/timepoint was 293.

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Figure 4. Centrosomal abnormalities in thymus

a) Representative confocal images of centrosome staining in thymus from mice 24 hrs after irradiation with 4 Gy γ radiation. Centrosomes were co-stained with pericentrin (green) and gamma-tubulin (red), DAPI was used to visualize nuclei; scale bar represents 10 μ m. **b)** Quantification of the fraction of cells with >2 centrosomes in thymus tissue. For each genotype and timepoint 2–3 mice were used and the mean number of cells counted was 609.

Table 1

Survival time and individual tumor description in chronological order of days post-radiation exposure.

ID	Gender	Days survival post-radiation	Tumor type	Involved Organ
137	M	111	Lymphoma *	Thymus
128	F	119	Lymphoma *	Thymus
171	M	146	Lymphoma *	Mesenteric lymph node
162#1	F	152	Adenocarcinoma	Mammary
162#2	F	152	Angiosarcoma	Leg
127	F	159	Lymphoma *	Lung
139	F	179	Lymphoma *	Neck, peri-salivary gland
135	F	202	Lymphoma *	Thymus
120	M	207	Sarcoma	Retropertitoneum
140	F	252	Hemangiosarcoma	Mammary
157	F	255	Sarcoma	Mammary
154	M	279	Angiosarcoma	Spleen
160	M	279	Lymphoma *	Thymus
126	F	284	Squamous cell carcinoma	Head and neck
129	M	284	Sarcoma	Small intestine
166	M	284	Papillary bronchio-alveolar adenoma	Lung
169	M	284	Hemangiosarcoma	Spinal cord
144	M	300	Keratoacanthoma	Skin
148#1	M	320	Bronchio-alveolar carcinoma	Lung
148#2	M	320	Hemangioma	Liver
152#1	F	320	Angiosarcoma	Leg
152#2	F	320	Hemangioma	Liver
163	F	362	Sarcoma	Spleen
146	F	369	Hemangiosarcoma	Mammary
151#1	F	400	Lymphoma	Bladder

ID	Gender	Days survival post-radiation	Tumor type	Involved Organ
151#2	F	400	Hemangiosarcoma	Intestinal stroma
123	M	403	Lymphoma	Mesenteric fat, thyroid, lung
124#1	M	417	Hemangiosarcoma	Mammary
124#2	M	417	Lymphoma	Liver, lymph node, Mammary
170	M	419	Keratoacanthoma	Skin
133	F	420	Adenocarcinoma	Mammary
164	M	425	Hemangioma	Spleen, liver
158	M	426	Lymphoma	Thymus
161	M	432	Papillary bronchio-alveolar adenoma	Lung
122#1	M	476	Lymphoma	Mesenteric lymph node
122#2	M	476	Sarcoma	Small intestine

A description of each individual tumor type and tissue with ID of the mouse for cross referencing. M=male, F=female. If multiple different tumors were observed in one mouse these are indicated as #1 and #2.

* represents lymphomas that develop in mice with survival <300 days, which are classified as early onset