Genetics

Genetic background influences loss of heterozygosity patterns in radiation-induced mouse thymic lymphoma

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Previous studies have revealed that p53 heterozygous (p53+/-) mice are extremely susceptible to radiation-induced tumorigenesis. To investigate whether genetic background influences radiation induced tumor susceptibility, we crossed p53+/- 129/Sv mice with genetically diverse strains to generate p53+/- F1 hybrids. The results showed that genetic background had a profound impact on tumor latency after exposure to gamma radiation, while the tumor spectrum did not change. We further characterized the thymic lymphomas that arose in the p53+/- mice by genome-wide loss of heterozygosity (LOH) analyses and found that genetic background strongly influenced the frequency of LOH and the loss of which parental allele on different chromosomes. Further research is needed to identify which genetic variations control the LOH patterns in radiation-induced thymic lymphomas and to evaluate its relevance to human cancers. *Journal of Nature and Science, 1(5):e96, 2015*

genetic susceptibility | thymic lymphoma | loss of heterozygosity | ionizing radiation

Introduction

Genetic background plays a determining role in susceptibility to cancer from environmental insults including radiation in both humans and mice [1-5]. Many genes have been discovered to confer a strong predisposition to tumor development when inherited in the mutant form through the germ-line [5, 6]. Thus, the identification of individuals who are particularly susceptible to radiation-induced cancer is of high interest in terms of occupational and medical radiation protection. Efforts underway to detect genetic variants in human populations are likely to be fraught with difficulties. The genetic heterogeneity and variable etiology of carcinogenesis in humans will necessitate the collection of large numbers of DNA samples from cancer patients and control populations, with no guarantee that the methods presently available would allow the detection of the most important loci. Furthermore, the inability to quantify the different levels of environmental carcinogens to which different individuals are exposed adds a further dimension of uncertainty to the resolution of these questions. Because of these obstacles, the literature on genetic susceptibility to radiation induced tumors is small. Moreover, the current approaches used in many of the existing human studies aimed at identifying susceptibility genes result in many potential candidate genes, whereas unbiased and rigorous genetic studies aimed at identifying genomic loci that contribute to tumor susceptibility are rare. Parallel studies in mice offer many advantages for the study of the genetic basis of complex traits, including radiation-induced cancers. These include precise exposures, well-designed populations for a specific question, standardized husbandry to control environmental components of risk, and comprehensive analysis of phenotypes.

It is well documented that the p53 tumor suppressor gene controls cellular responses to DNA damage, including those induced by radiation exposure, and forms a critical link to downstream effectors of growth arrest and cell death. Tumors have evolved a number of mechanisms to circumvent the effects of the p53 signaling pathway, including abrogation of the upstream signals leading to activation of p53 (e.g. mutations in *ATM*, deletions of *p19 Arf*), mutations or loss of the p53 gene itself, or loss of downstream effector molecules (e.g. mutations in *Bax*). We previously used mouse models to show that the p53 gene protects mice in vivo from the tumorigenic effects of radiation exposure [7]. A single dose of 4Gy radiation decreased the average lifespan of p53 heterozygous knockout mice by about 35 weeks, without affecting the lifespan of control wildtype animals [7]. In this study, we investigated how genetic background affects this sensitization to radiation resulting from p53 deficiency and somatic changes in these arisen tumors.

Materials and Methods

Mice and tumor induction

F1 hybrid p53+/- mice were generated by crossing female p53 null 129/Sv with male p53 wild type *Mus spretus* or C57BL/6J. The p53 knockout mice were obtained from Donehower et al [8], *Mus spretus* mice from S. Brown (MRC Harwell, England). The 5-week old 129/Sv and F1 hybrids p53+/- mice of both sexes were exposed to a single dose of 4Gy whole body irradiation from a Cobalt 60 source at a dose rate of 0.7Gy/min and observed daily until moribund, then euthanized and autopsied. Mice were bred and treated under UK Home Office regulations.

Loss of heterozygosity (LOH) analysis in tumors by PCR using microsatellite markers

Preparation of DNA from thymic lymphomas and normal tissues were same as our previous studies [9]. LOH was determined by PCR of microsatellite markers, with normal and tumor DNA from the same mice. PCR reactions were set up in a total volume of 20μl, containing 2μl of 10x PCR buffer with 15mM $MgCl_2$ (Bioline), 1.6μl 0f 2.5 mM dNTPs (Pharmacia Biosystem Ltd), 1μl (5μM) of each primer, 0.2μl Taq polymerase (Bioline), 2μl (40ng/μl) tumor or normal tissue DNA, and 12.2μl dH_2O. Amplifications were initially denatured at 94°C for 3 min, followed by 35 cycles at 94°C for 30 s, 55°C or 52°C for 30 s and 72°C for 30 s, and then a final incubation at 72°C for 5 min. The PCR products were then mixed with loading buffer and electrophoresed in 4% (3% NuSieve/1% agarose) agarose gels with 0.5 mg/ml ethidium-bromide, then photographed and saved in image files for analysis of band density.

Statistical analysis

The Kaplan-Meier method was used to compare the survival time post-radiation between different genetic backgrounds. Differences in frequencies of LOH between different genetic backgrounds were analyzed by the Fisher exact test ($p<0.05$ was used as a threshold for significance). All analyses were carried out using the SPSS statistical package.

Results and Discussion

Genetic background strongly influences radiation-induced tumor latency in p53+/- mice

Loss of a single copy of *p53* confers sensitization to radiation-induced lymphoma. A single dose of 4Gy radiation decreased the average lifespan of *p53* heterozygous knockout (*p53*+/-) mice by about 35 weeks, without affecting the lifespan of wildtype control animals [7].

Conflict of interest: No conflicts declared.

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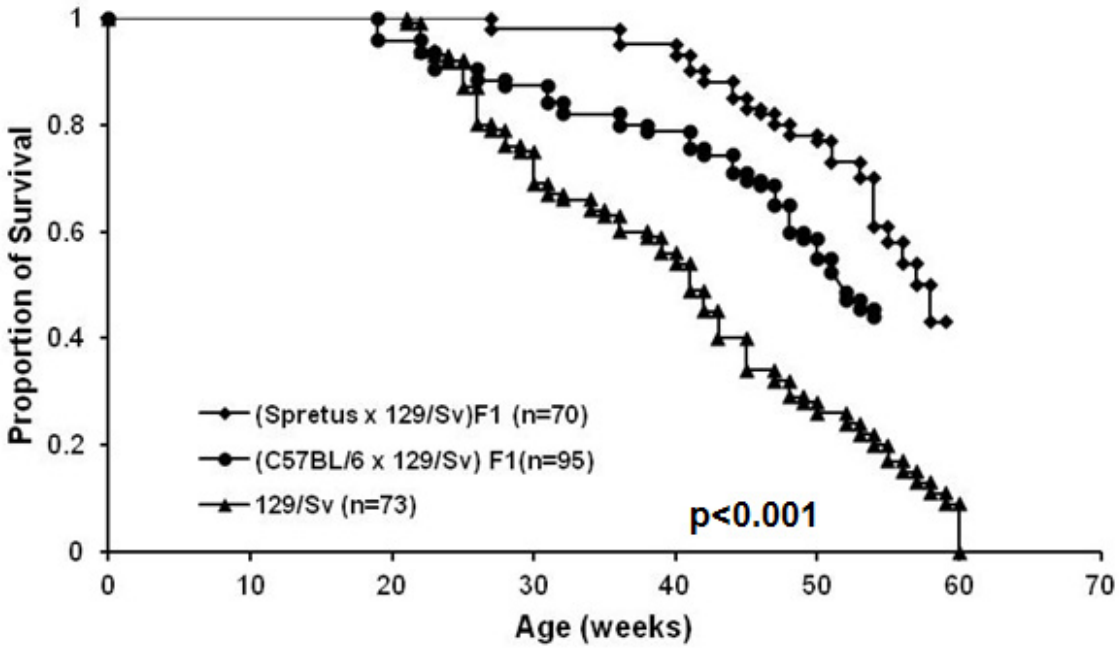

Figure 1. Effect of genetic background on radiation-induced tumorigenesis. The figure shows three different Kaplan-Meier survival curves for mice that all have a single functional p53 gene (p53+/-), but differ in genetic background. There is a highly statistically significant difference between F1 hybrids and 129/Sv ($p<0.001$). The p-value was obtained from long rank test.

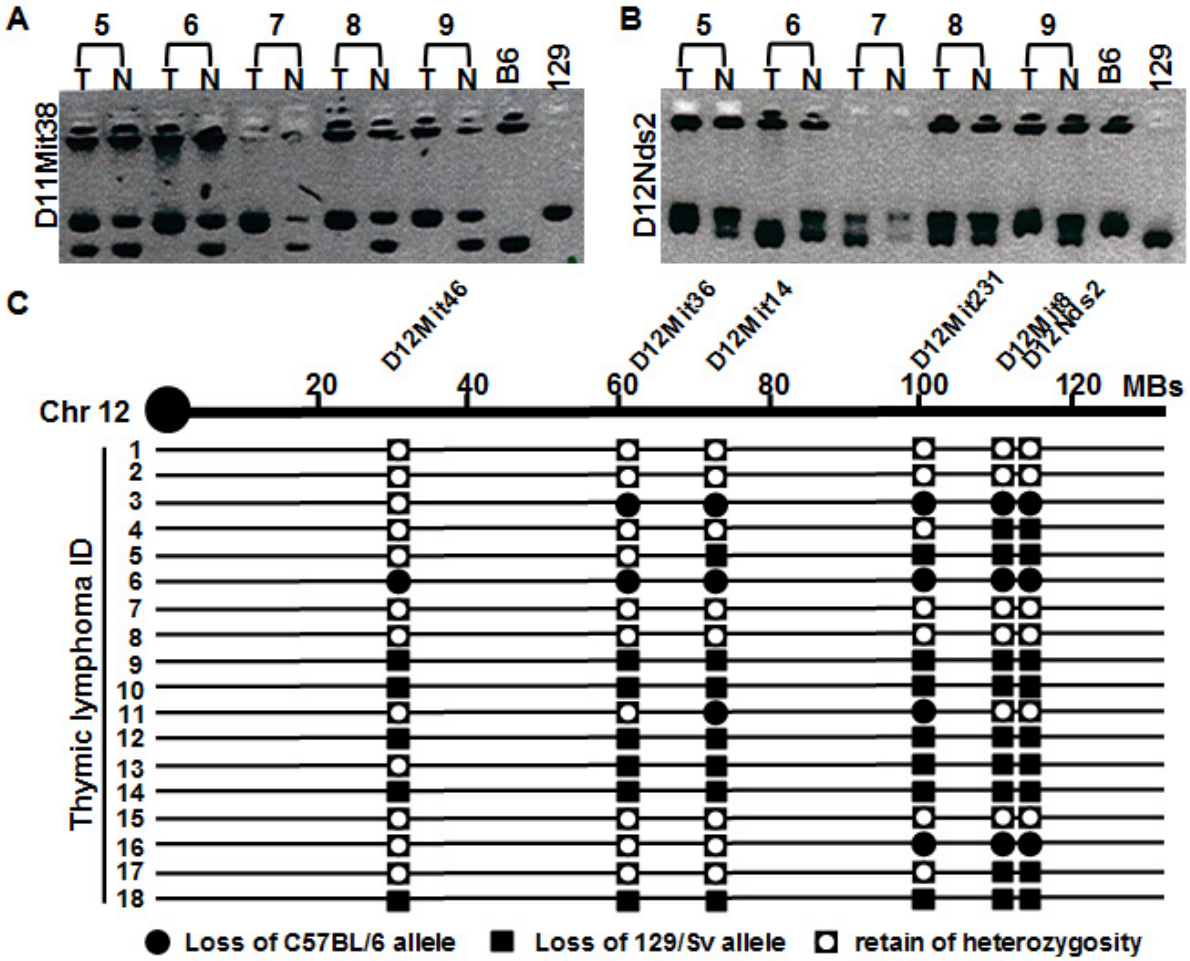

Figure 2. LOH analysis of radiation-induced thymic lymphomas. (A-B) Representative gel images for detecting LOH of microsatellite marker D11Mit38 (A) and D12Nds2 (B). Various types of genetic changes were detected: both parental alleles retained (e.g. Tumor 5 at D11Mit38, and Tumor 8 at D12Nds2), loss of a single allele (e.g. Tumor 6 to 9 at D11Mit38, Tumor 5, 6 and 9 at D12Nds2) and imbalance of one of the alleles (e.g. Tumor 7 at D12Nds2). B6 stands for C57BL/6, 129 for 129/Sv, T for thymic lymphoma tissue, N for normal tail tissue. (C) Representative LOH pattern on chromosome 12. Various patterns were detected: retention of parental chromosomes (e.g. Tumor 1 and 2), loss of one of parental chromosomes (e.g. Tumor 6 and 9) and partial loss of one of the parental chromosomes (e.g. Tumor 3 and 4).

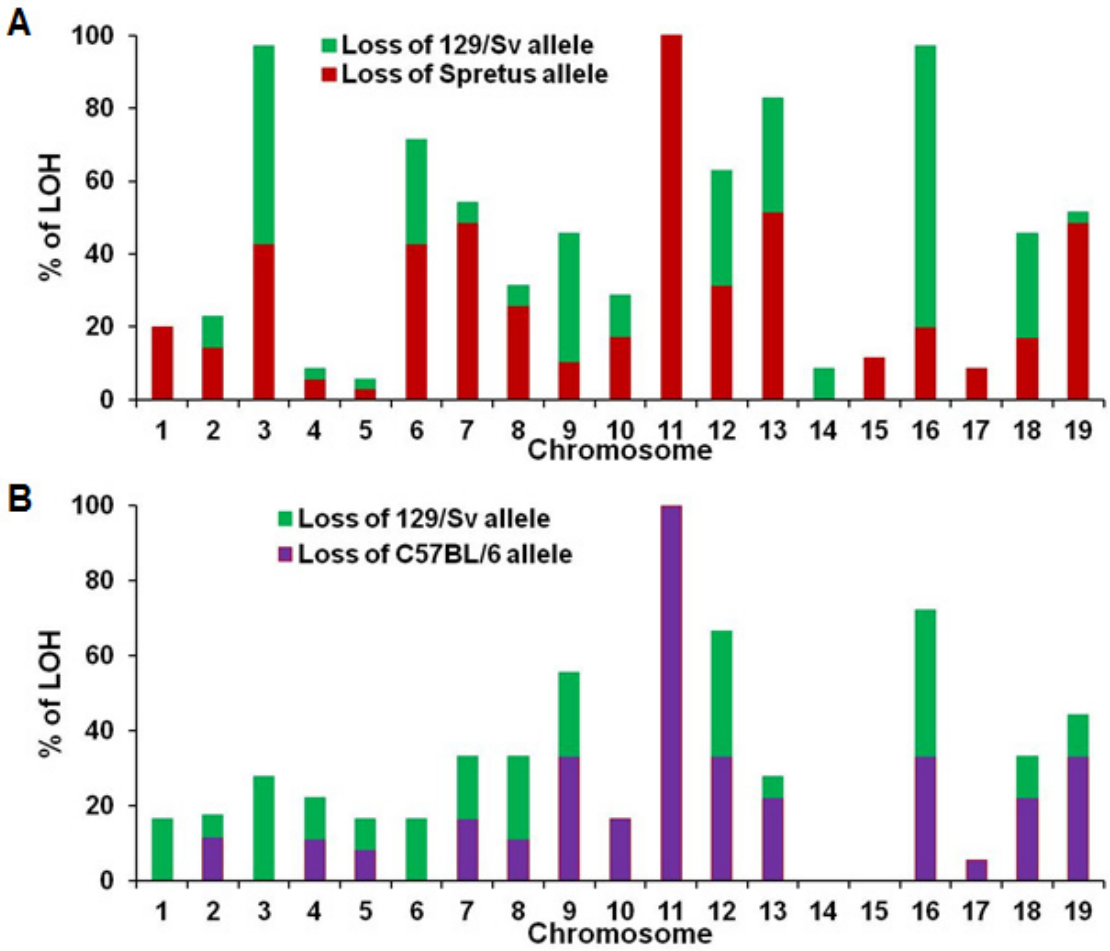

Figure 3. Frequency of allele-specific loss in radiation induced thymic lymphomas (A) from (Spretus X 129/Sv) F1 p53+/- mice and (B) (C57BL/6 X 129/Sv) F1 p53+/- mice.

To investigate whether such sensitization to radiation resulting from p53 deficiency is dependent on the host genetic background, we crossed female *p53*-/-129/Sv mice with male C57BL/6 and *Mus spretus* mice to generate *p53*+/- F1 hybrids. The F1 hybrids were exposed to a single dose of 4Gy whole body γ-ray radiation and were monitored for tumor development. As shown in Figure 1, *p53*+/- F1 hybrids between 129/Sv and C57BL/6 mice, or interspecific hybrids with *Mus spretus*, had a significantly longer life span than the irradiated 129/Sv mice ($p<0.001$). More than 80% of mice developed lymphomas, and the remaining mice developed fibrosarcomas, osteosarcomas, and few other types of tumor. There is no difference in tumor spectra between F1 hybrids and 129/Sv mice ($p>0.05$). These results suggest that genetic backgrounds strongly influence the sensitivity to radiation-induced lymphoma development in *p53*+/- mice.

Genome-wide screen of LOH in radiation-induced thymic lymphomas

We initially focused our search for loss of heterozygosity (LOH) in radiation induced thymic lymphomas using over 100 microsatellite markers distributed across the genome [9,10]. Representative examples of LOH in these tumors are shown in Figure 2A and B for microsatellite markers D11Mit38 and D12Nds2, respectively. We then generated tumor specific LOH patterns by mapping the LOH status for each marker along the axis of each chromosome. Figure 2C shows an example of an LOH pattern of chromosome 12 in eighteen independent thymic lymphomas. Next we calculated the frequencies of chromosomal LOH (one or more markers on the chromosome showed LOH) for each of the parental alleles separately (Fig. 3). In the tumors from *p53*+/- F1 hybrid mice derived by crossing 129/Sv *p53*-/- mice with *Mus spretus*, chromosomes 3, 6, 7, 11, 12, 13, 16, 19 showed LOH in over 50% of tumors, whereas in the tumors from *p53*+/- hybrid mice derived from 129/Sv *p53*-/- mice with C57BL/6, chromosomes 9, 11, 12, and 16 showed clear evidence of LOH in over 50% of the tumors examined, providing evidence that genetic background strongly affects LOH patterns (Fig. 3, Table 1). Notably, all of the radiation-induced thymic lymphomas lost the remaining wild type allele of *p53*, located on mouse chromosome 11.

Table 1. Genetic background dependent differences in LOH frequency for each chromosome

Chromosome	(Spretus X 129/Sv) F1 (% LOH)	(C57BL/6 X 129/Sv) F1 (% LOH)	p-Value*
1	20	16.7	1
2	22.9	16.7	0.75
3	97.1	27.8	1.89E-08
4	8.6	22.2	0.25
5	5.7	16.7	0.21
6	71.4	16.7	0.000049
7	54.3	33.3	0.18
8	31.4	33.3	1
9	45.7	55.6	0.6
10	28.6	16.7	0.36
11	100	100	1
12	62.9	66.7	0.79
13	82.9	27.8	0.000045
14	8.6	0	0.26
15	11.4	0	0.14
16	97.1	72.2	0.006
17	8.6	5.6	0.64
18	45.7	33.3	0.42
19	51.4	44.4	0.79

*The p-value was obtained from Fisher's exact test.

Genetic background effect on LOH in radiation-induced thymic lymphomas

Next we sought to investigate how genetic background influences LOH patterns in radiation-induced thymic lymphomas. In comparison to our previous studies in F1 hybrids between *Mus spretus* and 129/Sv [9,10], we found that chromosomes 3, 6, 13 and 16 showed a significant reduction in the LOH frequency in the tumors from (C57BL/6 x 129/Sv) F1 mice ($p<0.01$; Table 1 and Fig. 3), indicating that genetic background affects the frequency of certain genetic changes in these tumors. Interestingly, in addition to preferential loss of the wild-type C57BL/6 or *Mus spretus* p53 allele on chromosome 11, we also found in the tumors from *p53*+/- F1 hybrid mice derived by crossing 129/Sv *p53*-/- mice with *Mus spretus*, preferential loss of the 129/Sv allele on chromosome 16 and the *Mus spretus* allele on chromosome 7, 8 and 19 (Fig. 3A). On the other hand, in the tumors from *p53*+/- hybrid mice derived from 129/Sv *p53*-/- mice with C57BL/6 we observed preferential loss of the C57BL/6 allele on chromosome 13 and the 129/Sv allele on chromosome 3 (Fig. 3B). Our previous study showed that there is a polymorphism in the Fbxw7 gene located on chromosome 3 affecting protein function [11]. Interestingly, the 129/Sv and Spretus strains share the same allele of this gene, which is different from the allele in C57BL/6. Thus, we speculate that both the decrease in frequency of LOH and preferential loss of 129/Sv allele on chromosome 3 are due to *Fbxw7*. Our data indicates that allele-specific LOH is an alternative approach for cancer gene mapping.

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1. Balmain, A. (2002). Cancer as a complex genetic trait: tumor susceptibility in humans and mouse models. Cell *108*, 145-152.
2. Kleinerman, R.A. (2009). Radiation-sensitive genetically susceptible pediatric sub-populations. Pediatr Radiol *39 Suppl 1*, S27-31.
3. Miller, M.C., 3rd, Mohrenweiser, H.W., and Bell, D.A. (2001). Genetic variability in susceptibility and response to toxicants. Toxicol Lett *120*, 269-280.
4. Mao, J.H., and Balmain, A. (2003). Genomic approaches to identification of tumour-susceptibility genes using mouse models. Curr Opin Genet Dev *13*, 14-19.
5. Perez-Losada, J., Castellanos-Martin, A., and Mao, J.H. (2011). Cancer evolution and individual susceptibility. Integr Biol (Camb) *3*, 316-328.
6. Balmain, A., Gray, J., and Ponder, B. (2003). The genetics and genomics of cancer. Nat Genet *33 Suppl*, 238-244.
7. Kemp, C.J., Wheldon, T., and Balmain, A. (1994). p53-deficient mice are extremely susceptible to radiation-induced tumorigenesis. Nat Genet *8*, 66-69.
8. Donehower, L.A., Harvey, M., Slagle, B.L., McArthur, M.J., Montgomery, C.A., Jr., Butel, J.S., and Bradley, A. (1992). Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. Nature *356*, 215-221.
9. Mao, J.H., Perez-Losada, J., Wu, D., Delrosario, R., Tsunematsu, R., Nakayama, K.I., Brown, K., Bryson, S., and Balmain, A. (2004). Fbxw7/Cdc4 is a p53-dependent, haploinsufficient tumour suppressor gene. Nature *432*, 775-779.
10. Mao, J.H., Wu, D., Perez-Losada, J., Jiang, T., Li, Q., Neve, R.M., Gray, J.W., Cai, W.W., and Balmain, A. (2007). Crosstalk between Aurora-A and p53: frequent deletion or downregulation of Aurora-A in tumors from p53 null mice. Cancer Cell *11*, 161-173.
11. Perez-Losada, J., Wu, D., DelRosario, R., Balmain, A., and Mao, J.H. (2012). Allele-specific deletions in mouse tumors identify Fbxw7 as germline modifier of tumor susceptibility. PLoS One *7*, e31301.