Cell Death and Differentiation (2005) 12, 94–97

www.nature.com/cdd

Letter to the Editor

Loss of TRAIL-R does not affect thymic or intestinal tumor development in p53 and adenomatous polyposis coli mutant mice

Cell Death and Differentiation (2005) **12,** 94–97. doi:10.1038/sj.cdd.4401523
Published online 29 October 2004

Dear Editor,

TRAIL (TNF-Related Apoptosis-Inducing Ligand) and the TRAIL receptors were originally isolated based to their homology to tumor necrosis factor (TNF) and TNF receptor (TNFR) family members.[1–3] In humans, five TRAIL receptors have been characterized. Two of these receptors, DR4 and DR5, contain an intracellular protein motif known as a death domain, which can transmit an apoptotic signal. Two additional receptors, DcR1 and DcR2, lack a functional death domain and appear to function as decoy receptors to inhibit signaling by TRAIL.[4] The fifth receptor, osteoprotegerin (OPG), has been shown to bind TRAIL *in vitro* but has low affinity for the ligand at physiological temperatures;[5] therefore, it is unclear as to whether it is a true receptor for TRAIL. In mice, one full length signaling receptor (TRAIL-R) and two decoy receptors lacking death domains have been identified.[6,7] Like Fas and TNFR, TRAIL-R induces apoptosis in a broad range of transformed cell lines in a FADD- and caspase-8-dependent manner.[8–11] In contrast to Fas and TNFR, most nontransformed cells are resistant to TRAIL-R-mediated death;[1,12,13] as a result, there is considerable interest in the potential role of TRAIL as a cancer therapeutic.

Several studies have examined the *in vivo* role of TRAIL/TRAIL-R signaling in tumor immunity. TRAIL expression has been observed on the surface of activated $CD8^+$ T cells, NK, and NKT cells, which correlated with their ability to kill tumor cells via a TRAIL-dependent mechanism.[14–16] TRAIL blocking antibodies can also inhibit NK cell cytotoxicity against mouse fibrosarcoma L929 target cells.[17] *In vivo* TRAIL administration led to regression of exogenously introduced TRAIL-sensitive tumors in mice.[13] In addition, blocking antibodies to TRAIL can increase tumor growth and metastasis in methylcholanthrene (MCA)-treated mice as well as mice inoculated with TRAIL-sensitive tumors.[18,19] Finally, TRAIL-deficient mice are more sensitive to MCA-induced tumorigenesis, less able to reject implanted TRAIL-sensitive tumors, and less responsive to the immunotherapeutic agent alpha-galactosylceramide (α-GalCer).[20,21] These data indicate that TRAIL can induce rejection or apoptosis of tumor cells *in vivo*, but its role in immune surveillance of spontaneously developing tumors has not been fully investigated. We previously described the generation of TRAIL-R-deficient mice, which do not spontaneously develop tumors (Diehl G, *et al*. submitted). To determine what role, if any, the TRAIL/TRAIL-R pathway plays in the control of spontaneously developing tumors, we crossed the TRAIL-R null allele into the p53-deficient and Min (for Mouse Intestinal Neoplasia) tumor models.

The p53 tumor suppressor gene is mutated in a large percentage of human malignancies, including tumors of the colon, breast, lung, and brain.[22] Individuals who inherit a mutant allele of p53 are susceptible to a wide range of tumor types. Mice deficient for p53 are viable and develop normally, but 50% develop tumors by 20 weeks of age and all develop tumors by 6 months of age.[22–24] The vast majority of tumors in p53-deficient animals are lymphomas, making these animals a good lymphoma model. Most of the lymphomas are of thymic origin and are composed primarily of immature CD4/CD8 double-positive cells.[25] $p53^{+/-}$ mice also develop tumors, although at a much later age, and these tumors are primarily sarcomas. In most cases, tumorigenesis in these animals is accompanied by loss of the wild-type p53 allele.[22]

A previous study has suggested a role for TRAIL in the control of p53 tumorigenesis.[19] However, in these experiments, TRAIL neutralizing antibodies were injected every 5 days into $p53^{+/-}$ animals for 1.5 years. To examine the role of TRAIL/TRAIL-R in p53-mediated tumorigenesis directly, we generated mice deficient for both TRAIL-R and p53 to determine if TRAIL-R deficiency would enhance tumor formation caused by loss of p53. Comparison of $TRAIL\text{-}R^{-/-};p53^{-/-}$ animals with $TRAIL\text{-}R^{+/-};p53^{-/-}$ or $TRAIL\text{-}R^{+/+};p53^{-/-}$ animals at both 16 and 20 weeks of age revealed no differences in the frequency of tumor-bearing mice (Figure 1a). No tumors were observed in $p53^{+/-}$ or $p53^{+/+}$ animals at this age (data not shown). Interestingly, while some wild-type animals developed nonthymic tumors at 16 weeks of age, tumors in TRAIL-R-deficient animals were exclusively thymomas at this age (Figure 1b). However, nonthymic tumors did develop in slightly older (20 weeks old) $TRAIL\text{-}R^{-/-};p53^{-/-}$ mice.

To determine if TRAIL-R deficiency affected the growth of tumors, the weight of splenic and thymic tumors was recorded. At 16 weeks, there was no difference in the average thymoma weight between TRAIL-R wild-type and knockout animals in the p53-deficient background (Figure 1c). No

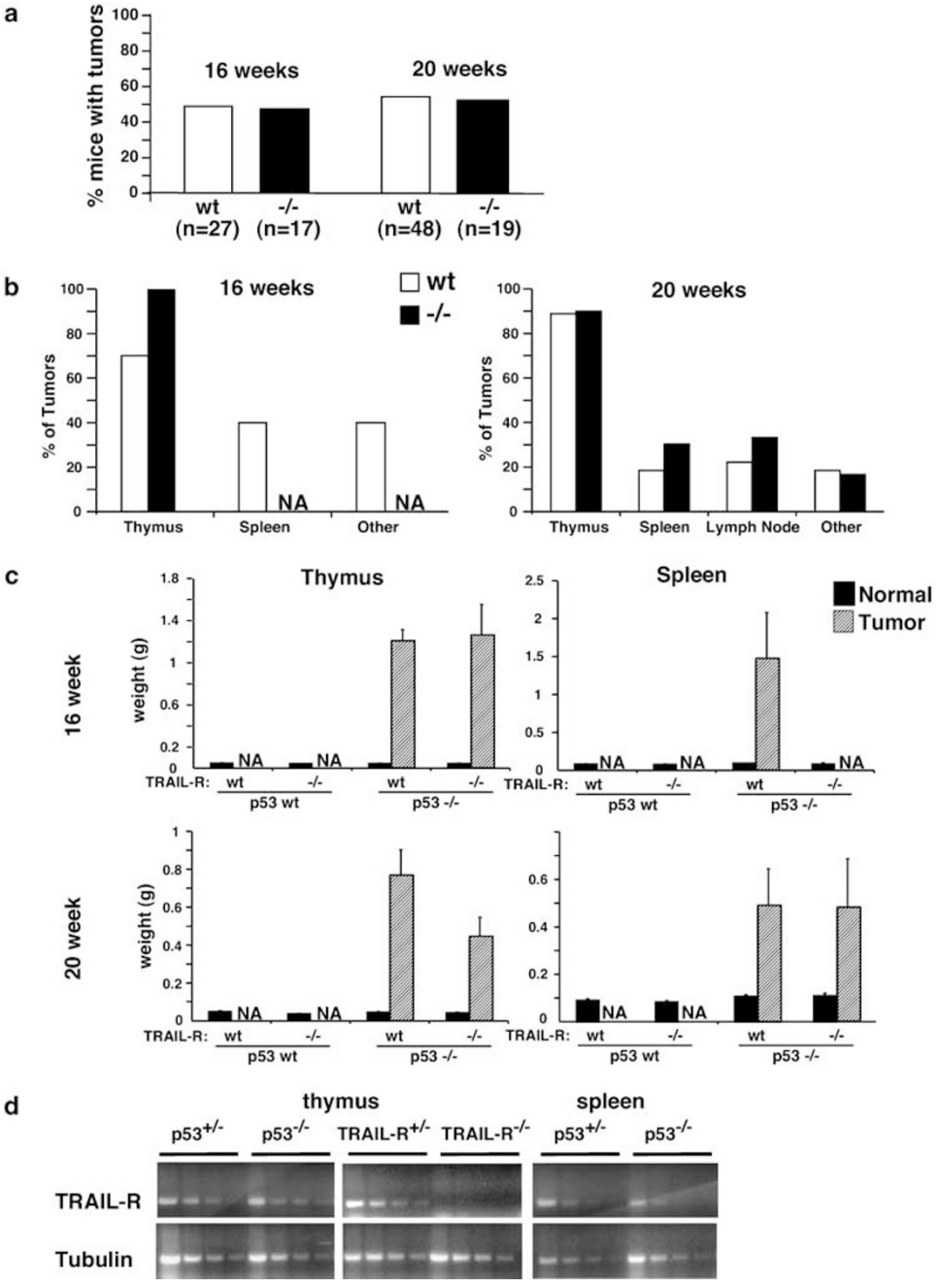

Figure 1 (**a**) TRAIL-R deficiency does not increase the frequency of tumor development in *p53*$^{-/-}$ mice. TRAIL-R-deficient mice were generated as described elsewhere (Diehl G, *et al.* submitted) and crossed with *p53*$^{-/-}$ mice (from The Jackson Laboratory, genotyped as described on The Jackson Laboratory website) to obtain *TRAIL-R*$^{-/-}$;*p53*$^{-/-}$ mice in the C57BL/6 background. Animals were killed at 16 or 20 weeks of age and dissected to assess tumor development. Spleens and thymi were scored as tumorigenic if the organ weight was more than threefold over p53 wild-type littermate controls. (**b**) Distribution of tumors in mice dissected at 16 or 20 weeks of age. Other tumors refers to nonthymic, splenic, or lymph node-associated tumors. (**c**) Average spleen and thymus organ weights from 16- and 20-week-old *TRAIL-R*$^{+/-}$;*p53*$^{-/-}$ and *TRAIL-R*$^{-/-}$;*p53*$^{-/-}$ animals. Weights for both normal organs and organs scored as tumorigenic are shown. NA: not applicable; wt: wild-type littermates. (**d**) Loss of p53 does not alter TRAIL-R expression. Semiquantitative RT–PCR was performed on RNA isolated from *p53*$^{+/-}$, *p53*$^{-/-}$ thymus and spleen and *TRAIL*$^{+/-}$ and *TRAIL*$^{-/-}$ thymus. The TRAIL-R oligonucleotides used are ttgggcatcttggcataagc and tgtggttagagtcatttgtcgtgc. The tubulin oligonucleotides used are tgttcgtagacctggaacccac and ctcaaccacagcagtggaaacc

comparison of splenic tumors could be made, as no splenic tumors were found in *TRAIL-R*$^{-/-}$ animals at this age. At 20 weeks, the average thymic and splenic tumor weights were similar between the TRAIL-R wild-type and knockout mice in the p53-deficient background (Figure 1c). Thus, loss of TRAIL-R has no effect on the generation or growth of *p53*$^{-/-}$ thymic tumors but may slightly delay the formation of nonthymic tumors.

Other groups have shown that TRAIL-R expression can be upregulated by p53.[6,26] To address the possibility that p53 deficiency might lead to loss of TRAIL-R expression, we examined TRAIL-R transcripts in *p53*$^{+/-}$ and *p53*$^{-/-}$ thymus and spleen. As shown in Figure 1d, TRAIL-R expression was not altered by p53 deficiency. No product was observed in TRAIL-R$^{-/-}$ thymus as expected. This demonstrates that steady-state expression of TRAIL-R is not dependent on p53 and should still be functional in *p53*$^{-/-}$ tumors.

High levels of DR4 and DR5 expression on neoplastic cells were observed on human colonic epithelial cells and adenoviral expression of TRAIL on human colorectal cancer cells reduced their spread when injected into mice.[27,28] These studies suggest that TRAIL-R might play a role in regulating intestinal tumorigenesis. Min mice carry a loss of function allele of the adenomatous polyposis coli (*Apc*) gene. While homozygosity for this allele results in embryonic lethality, heterozygous (*Apc*^{Min}/+) animals develop normally but are prone to the development of intestinal polyps, which is accelerated by a high-fat diet.[29–31] In the C57BL/6 background, Min animals develop an average of 29±10 tumors along the length of their intestine.[32] This correlates highly with loss of heterozygosity at the *Apc* gene locus.[33] The Min mice represent a clinically relevant rodent model for FAP (familial adenomatous polyposis),[30] a genetic condition that predisposes individuals to colorectal cancer and is also caused by a loss of function mutation in the *Apc* gene.[34]

To examine the role of TRAIL and TRAIL-R in intestinal tumor formation, we generated *TRAIL-R*$^{-/-}$;*Apc*$^{Min/+}$ mice. After 100 days on a high-fat diet, formation of intestinal tumors in these mice were compared to their *TRAIL-R*$^{+/+}$;*Apc*$^{Min/+}$ littermate controls. As shown in Figure 2, no difference in intestinal polyp numbers was found between *TRAIL-R*$^{-/-}$ and wild-type mice in the Min background (Figure 2). In addition, the average size, morphology, and distribution of the polyps within the intestine were identical between these mice (data not shown). These data show that TRAIL-R deficiency does not affect the onset or growth of intestinal polyps caused by the Min mutation.

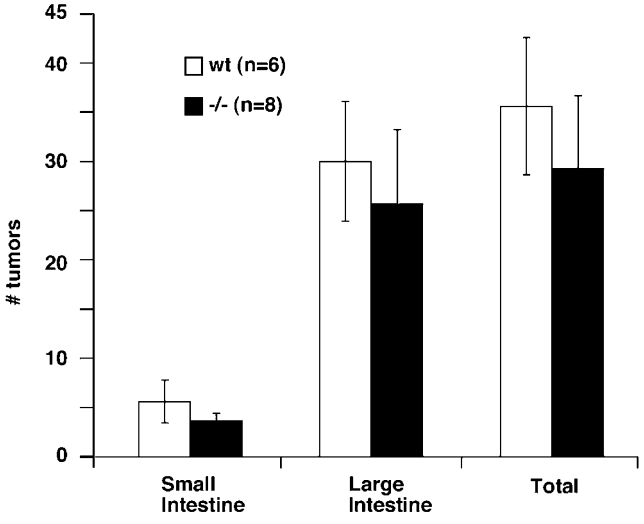

Figure 2 TRAIL-R deficiency does not increase the number of intestinal tumors in Min mice. The TRAIL-R-deficient animals were backcrossed at least five generations into the C57BL/6 background before being crossed with *Apc*^{Min}/+ mice (from The Jackson Laboratory, genotyped as described on The Jackson Laboratory website) to generate *TRAIL-R*$^{-/-}$;*Apc*^{Min}/+ mice. Mice were weaned at 3 weeks of age onto a high-fat diet (Harlan, Teklad 7904) and killed 100 days later. Intestines were washed, fixed in 10% neutral-buffered formalin, transferred to phosphate-buffered saline, and scored for intestinal polyps. wt: wild-type littermates

The difference in our results compared to previous studies may be due to several reasons. With one exception, all the previous studies examined the growth of either exogenously introduced tumors known to be sensitive to TRAIL killing or chemically induced tumors. Many of these injected cell lines and chemically induced tumors are known to be highly immunogenic compared to tumors of spontaneous origin. TRAIL expression has been previously shown on activated T and NK cells; activation and/or recruitment of these cells to the sites of immunogenic tumor cells may lead to bystander killing of TRAIL-sensitive cells. In contrast, spontaneously developing tumors may not normally induce a vigorous immune response; as a result, their development and growth could remain unaltered by TRAIL/TRAIL-R interactions. The one report examining the role of TRAIL/TRAIL-R in spontaneous tumor development observed increased tumor development when TRAIL neutralizing antibodies were administered into *p53*$^{+/-}$ mice.[19] However, *p53*$^{+/-}$ animals develop different types of tumors than those that arise in *p53*$^{-/-}$ mice;[24] therefore, TRAIL may be important in the control of nonlymphoid tumors. It is also possible that continuous injection of anti-TRAIL antibodies for 18 months might lead to unknown effects unrelated to the TRAIL/TRAIL-R pathway. Our observation that 16-week-old *TRAIL-R*$^{-/-}$;*p53*$^{-/-}$ animals do not develop nonthymic tumors might suggest that thymic and nonthymic tumors exhibit differential growth regulation by TRAIL. However, TRAIL-R would enhance tumor development in this situation because loss of TRAIL-R led to an unexpected absence of nonthymic tumor development. No difference in the frequency of nonthymic tumors in 20-week-old animals was observed, suggesting that the TRAIL/TRAIL-R pathway would at the most have a modest effect on tumor development. Further studies will be needed to dissect the exact physiological role of TRAIL and TRAIL-R *in vivo*.

Acknowledgements

We thank Kristina Hsieh for help in mouse husbandry and genotyping and James P Allison for helpful discussions about triangular mice. This work is supported by the National Institute of Health Grant CA92000 to AW. HHY is a National Science Foundation Predoctoral Research Fellow.

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1. Wiley SR *et al.* (1995) Immunity 3: 673–682
2. Pan G *et al.* (1997) Science 276: 111–113

3. Pan G *et al.* (1997) Science 277: 815–818
4. LeBlanc HN *et al.* (2003) Cell Death Differ. 10: 66–75
5. Truneh A *et al.* (2000) J. Biol. Chem. 275: 23319–23325
6. Wu GS *et al.* (1999) Cancer Res. 59: 2770–2775
7. Schneider P *et al.* (2002) J. Biol. Chem. 278: 5444–5454
8. Schneider P *et al.* (1997) FEBS Lett. 416: 329–334
9. Kuang AA *et al.* (2000) J. Biol. Chem. 275: 25065–25068
10. Kischkel FC *et al.* (2000) Immunity 12: 611–620
11. Sprick MR *et al.* (2000) Immunity 12: 599–609
12. Sheridan JP *et al.* (1997) Science 277: 818–821
13. Walczak H *et al.* (1999) Nat. Med. 5: 157–163
14. Mariani SM *et al.* (1998) Eur. J. Immunol. 28: 1492–1498
15. Smyth MJ *et al.* (2001) J. Exp. Med. 193: 661–670
16. Kayagaki N *et al.* (1999) J. Immunol. 162: 2639–2647
17. Kayagaki N *et al.* (1999) J. Immunol. 163: 1906–1913
18. Takeda K *et al.* (2001) Nat. Med. 7: 94–100
19. Takeda K *et al.* (2002) J. Exp. Med. 195: 161–169
20. Cretney E *et al.* (2002) J. Immunol. 168: 1356–1361
21. Sedger LM *et al.* (2002) Eur. J. Immunol. 32: 2246–2254
22. Jacks T *et al.* (1994) Curr. Biol. 4: 1–7
23. Donehower LA *et al.* (1992) Nature 356: 215–221
24. Harvey M *et al.* (1993) Nat. Genet. 5: 225–229
25. Donehower LA *et al.* (1995) Mol. Carcinogen. 14: 16–22
26. Wu GS *et al.* (1997) Nat. Genet. 17: 141–143
27. Koornstra JJ *et al.* (2003) J. Pathol. 200: 327–335
28. Mohr A *et al.* (2004) Gene Ther. 11: 534–543
29. Moser AR *et al.* (1990) Science 247: 322–324
30. Su LK *et al.* (1992) Science 256: 668–670
31. Yang K *et al.* (1998) Cancer Res. 58: 5713–5717
32. Moser AR *et al.* (1992) J. Cell Biol. 116: 1517–1526
33. Luongo C *et al.* (1994) Cancer Res. 54: 5947–5952
34. Groden J *et al.* (1991) Cell 66: 589–600