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Drak2 is not required for tumor surveillance and suppression

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SHORT COMMUNICATION

Abstract

Drak2 is a promising therapeutic target to treat organ-specific autoimmune diseases such as type 1 diabetes and multiple sclerosis without causing generalized immune suppression. Inhibition of Drak2 may also prevent graft rejection following organ transplantation. However, Drak2 may function as a critical tumor suppressor, which would challenge the prospect of targeting Drak2 for therapeutic treatment. Thus, we examined the susceptibility of *Drak2*$^{-/-}$ mice in several tumor models. We show that Drak2 is not required to prevent tumor formation in a variety of settings. Therefore, Drak2 does not function as an essential tumor suppressor in *in vivo* tumor models. These data further validate Drak2 as a viable therapeutic target to treat autoimmune disease and graft rejection. Importantly, these data also indicate that while Drak2 may induce apoptosis when overexpressed in cell lines, it is not an essential tumor suppressor.

Keywords: autoimmunity, regulation of immune responses, tumor surveillance

Introduction

Drak2 is a serine/threonine kinase expressed highest in B cells and T cells (1–3). In the absence of *Drak2*, mice are resistant to autoimmune disease in models of type 1 diabetes and multiple sclerosis (1, 4). In addition, *Drak2*$^{-/-}$ mice display increased survival of tissue grafts in organ transplant models (5). Nevertheless, the absence of *Drak2* does not alter the ability to eliminate several infectious pathogens including mouse hepatitis virus (6), West Nile virus (7) and lymphocytic choriomeningitis virus (4). Thus, Drak2 is an ideal protein to target to treat autoimmune disease and prevent graft rejection, without compromising immunity to infectious pathogens. However, Drak2 may function as a tumor suppressor, which would render it a less suitable target for long-term pharmacological inhibition.

Conflicting reports indicate that Drak2 can function as a tumor suppressor or as an oncogene. In many cancer cell lines, overexpression of *Drak2* caused cell death, suggesting Drak2 functions as a tumor suppressor (3, 8–11). Similarly, increased susceptibility to apoptosis was also seen *in vivo* with transgenic expression of *Drak2* via the actin promoter in pancreatic β islet cells and activated T cells cultured with IL-2 (12, 13). In addition, the MYB (v-myb avian myeloblastosis viral oncogene homolog) oncogene was shown to bind to the *Drak2* promoter and suppress apoptosis in acute myeloid leukemia cells by decreasing Drak2 expression (14). Inhibition of MYB expression increased cell death, which was dependent on *Drak2*, supporting a role for Drak2 as a tumor suppressor. Likewise, in a colorectal cell line, a cyclooxygenase-2 inhibitor increased cell death, which was also dependent on increased *Drak2* expression, further implicating that Drak2 functions in tumor suppression (15).

Conversely, it was demonstrated that Drak2 is expressed at high levels in cutaneous T-cell lymphomas as well as basal-like and human epidermal growth factor receptor 2-enriched breast tumors, implicating a possible role for Drak2 in oncogenesis (16, 17). In support of this, depletion of *Drak2* in a breast cancer cell line suppressed the tumorigenic ability of the cells

and inhibited tumor growth in a xenograft model (17). These authors suggest that increased *Drak2* expression may promote tumors by inhibiting the tumor suppressor activity of transforming growth factor (TGF)-β via enhanced negative regulation of downstream signaling molecules. In addition, *Drak2*$^{-/-}$ mice displayed reduced rejection of an allogeneic transplanted tumor, which was because of decreased T-cell survival (5). Thus, even if Drak2 does not function as an oncogene, *Drak2*$^{-/-}$ mice may be more susceptible to tumors because of reduced tumor surveillance resulting from decreased T-cell survival.

Additional data indicate that Drak2 functions as neither a tumor suppressor nor an oncogene. *Drak2*$^{-/-}$ mice aged 1 year were not more susceptible to spontaneous tumors (1). Moreover, *Drak2* overexpression did not induce death in all cancer cell lines (2, 8). Furthermore, transgenic expression of *Drak2* with a T-cell-specific promoter did not render T cells more sensitive to apoptosis (2, 18). Therefore, it is unclear whether pharmacological inhibition of Drak2 would render patients more susceptible to tumors. To investigate this, we tested *Drak2*$^{-/-}$ mice in several different tumor models and found that in the absence of *Drak2*, the mice were not more susceptible to a variety of tumors.

Methods

Mice

Drak2$^{-/-}$ mice were made in the laboratory of Stephen Hedrick at University of California, San Diego (1). These mice were backcrossed to the C57BL/6 background for 19 generations. C57BL/6 mice obtained from Jackson Laboratories were bred in house and used as controls. Mice were held under specific pathogen-free conditions at St. Jude Children's Research Hospital. Animal studies met the approval of the Animal Ethics Committee.

Sarcoma cell lines and transplantation

Regressor and progressor methylcholanthrene (MCA) sarcoma cell lines were derived from C57BL/6 or *Rag*$^{-/-}$ male mice by injecting MCA as previously described (19, 20). The phenotype of these lines was previously characterized as regressor (4654), intermediate regressor/progressor (6727 and 7727) and progressor (9614) based on their growth when transplanted into syngeneic mice at a dose of 1 million per mouse (20). Prior to injection, the cells were washed in PBS three times and resuspended at 1×10^7 ml^{-1}. One million cells were injected subcutaneously along the right flank of male C57BL/6 or *Drak2*$^{-/-}$ mice that were anesthetized with isofluorane. The following week, mice were shaved and monitored for tumor growth. Volumes of tumors were calculated according to the formula (length × width × width × π/6) as previously described (21). Tumors ≥65 mm^3 were included in graphs.

B16-F10 melanoma transplantation

B16-F10 cell line was obtained from American Type Culture Collection and maintained in Roswell Park Memorial Institute with 10% FCS. Cells were washed three times in PBS, and 1×10^6 cells were injected subcutaneously along the right flank of C57BL/6 or *Drak2*$^{-/-}$ mice. The following week, mice were shaved and monitored for tumor growth. Tumor volumes were calculated as described above. For the lung metastasis, 1.25×10^5 B16-F10 cells were injected intravenously. Three weeks later, the lungs were harvested and treated with 30% hydrogen peroxide to visualize the tumors. Tumors that were >1 mm were counted.

Tumorigenesis study of p53$^{-/-}$ *mice*

p53$^{-/-}$ mice (B6.129S2-*Trp53*^{tm1Tyj}/J) were obtained from Jackson Laboratory and bred to *Drak2*$^{-/-}$ mice. *p53*$^{-/-}$ and *p53*$^{-/-}$·*Drak2*$^{-/-}$ mice were monitored weekly for tumor growth. Mice with visible tumors, moribund mice and mice with severely compromised health conditions were euthanized and analyzed for tumors.

MCA-induced tumors

Mice were anesthetized with isofluorane and injected subcutaneously with 400 μg of 3-MCA, in peanut oil (Sigma) and monitored for tumor growth for at least 30 weeks. Tumor volume was determined as described above.

Azoxymethane/dextran sodium sulfate

Mice were injected intraperitoneally with 10 mg kg^{-1} azoxymethane (AOM, Sigma) on day 0. Five days later mice were fed 3% (w/v) dextran sodium sulfate (DSS, molecular mass 36–40 kDa; MP Biologicals) in the drinking water for 5 days. After 2 weeks of normal drinking water, mice were given a second cycle of DSS for 5 days, and 2 weeks later a third cycle for 5 days. Colons were harvested from mice 80 days after AOM injection and the number of tumors in the whole colon was counted. Tissues were fixed in 10% formalin and embedded in paraffin for histological analysis. Sections of colon were stained with hematoxylin and eosin and scored by an experienced pathologist for inflammation, ulceration, hyperplasia and inflamed area as previously described (22). The pathologist was blinded to the experimental groups for the scoring.

Results and discussion

Drak2 is not required for tumor surveillance of transplanted tumors

We first tested whether Drak2 plays an important role in immunosurveillance of transplanted tumors. 3-MCA-induced sarcoma cell lines were generated from primary MCA-induced tumor masses and passaged *in vitro* (19, 20). These cell lines were previously characterized as regressors, progressors or intermediate regressor/progressors based on their tumorigenicity in syngeneic wild-type mice (20). Importantly, it was found that regressor tumor lines only developed when MCA-induced sarcoma lines were generated in *Rag2*$^{-/-}$ mice and not when generated in wild-type mice (20). This indicates that the adaptive immune system is required for clearance of the regressor tumors, which is important as we found that the role of Drak2 in T cells is particularly important for the resistance to autoimmunity (4). We tested four different sarcoma cell lines with a range of tumorigenicity to enable the

observance of partial or subtle differences in tumor clearance in the absence of *Drak2*. The incidence of tumor formation after transplant of either regressor, progressor, or intermediate regressor/progressor sarcomas was similar between wild-type and *Drak2*$^{-/-}$ mice (Fig. 1). Moreover, the size of the tumors that progressed was comparable between wild-type and *Drak2*$^{-/-}$ mice, indicating that Drak2 is not important for tumor immunosurveillance of sarcoma cell lines. This was true for all tumors tested, even those with an intermediate progressor/regressor phenotype. If Drak2 was important for tumor surveillance, we would have expected the *Drak2*$^{-/-}$ mice to be more susceptible to tumors that were rejected in

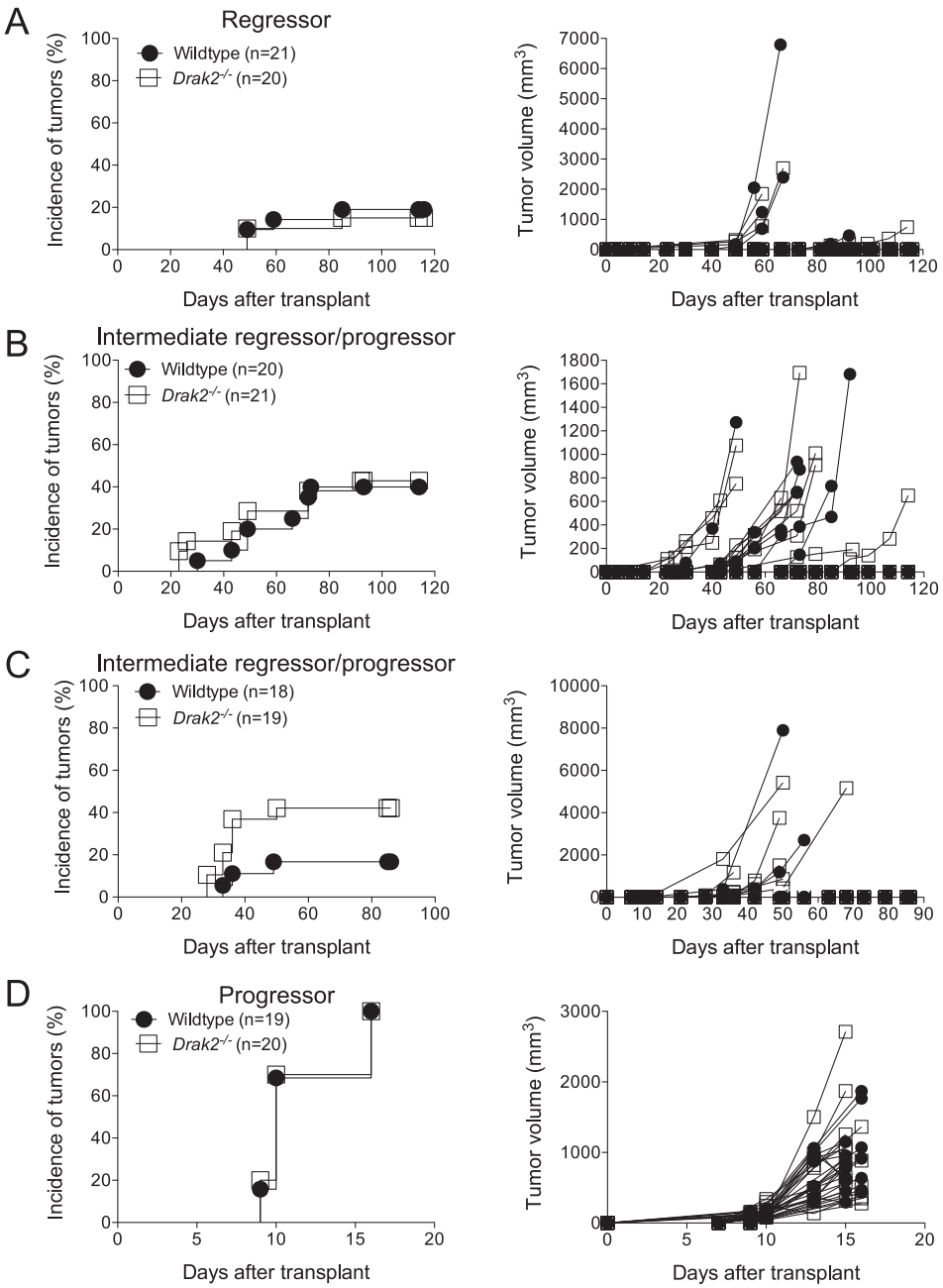

Fig. 1. Drak2 is not required for tumor surveillance of transplanted tumors. C57BL/6 wild-type or *Drak2*$^{-/-}$ mice were inoculated subcutaneously with 10^6 syngeneic sarcoma tumor cells previously characterized as a (A) regressor (4654), (B) intermediate regressor/progressor (6727), (C) intermediate regressor/progressor (7727) and (D) progressor (9614). Mice were monitored for tumor growth. Volumes of tumors were calculated according to the formula (length × width × width × π/6) as previously described (21). The incidence of mice with a tumor ≥65 mm^3 is plotted along with growth curves of individual tumors. Data are a combination of two independent experiments each with at least 10 mice per group. For all tumors, the incidence of tumors was not significantly different between wild-type and *Drak2*$^{-/-}$ mice according to the Log-rank (Mantel–Cox) test.

the majority of wild-type mice. Thus, Drak2 is not required for immunosurveillance of transplanted syngeneic tumors.

Drak2 does not affect incidence of an aggressive melanoma or metastasis to the lung

We next examined whether *Drak2*$^{-/-}$ mice would be more susceptible to transplanted tumors caused by the aggressive B16 melanoma cell line. B16-F10 cells were injected subcutaneously into wild-type or *Drak2*$^{-/-}$ mice, which were then monitored for tumor formation. Again, the incidence and size of the tumors was comparable between wild-type and *Drak2*$^{-/-}$ mice, also suggesting that Drak2 is not required for tumor surveillance of this tumor cell line (Fig. 2A and B).

To examine whether Drak2 was important for tumor surveillance in the lung, we injected B16-F10 melanoma cells intravenously and measured the number of lung metastases after 3 weeks. Again, the wild-type and *Drak2*$^{-/-}$ mice had similar numbers of tumors in the lung (Fig. 2C). Together, these data show that *Drak2*$^{-/-}$ mice are not more susceptible to tumor formation in transplantable tumor models.

Drak2$^{-/-}$ *mice are not more susceptible to MCA-induced tumor development*

To test whether Drak2 was important in preventing spontaneous carcinogen-induced tumors, wild-type or *Drak2*$^{-/-}$ mice were injected subcutaneously with the chemical carcinogen, MCA and monitored for tumor development for 30 weeks. T cells also impact the formation of tumors in this model as *Rag*$^{-/-}$ mice have a significantly increased incidence of tumors compared with wild-type mice (19, 20). Two hundred days following MCA administration, ~80% of the wild-type and *Drak2*$^{-/-}$ mice developed visible carcinomas (Fig. 3A and B). The incidence of tumors, time of tumor onset and tumor size was comparable in wild-type and *Drak2*$^{-/-}$ mice, indicating that the *Drak2*$^{-/-}$ mice are not more susceptible to spontaneous tumors induced by a chemical carcinogen. Thus, the absence of *Drak2* in both the T cells and the epithelial cells does not alter the incidence of tumorigenesis.

Drak2 is not important for protection against spontaneous tumor formation in the absence of p53

To further examine the requirement for Drak2 in the protection against spontaneous tumors caused by the removal of a tumor suppressor, we bred *Drak2*$^{-/-}$ mice to *p53*$^{-/-}$ mice and monitored the mice for tumors. If Drak2 functioned as an essential tumor suppressor, we would expect *Drak2*$^{-/-}$ mice to be more susceptible to tumor formation in the absence of the p53 tumor suppressor. However, both *p53*$^{-/-}$ and *p53*$^{-/-}$·*Drak2*$^{-/-}$ mice developed tumors with a similar incidence, time of onset and severity, again indicating that the absence of *Drak2* does not render mice more susceptible to tumors even in the absence of a critical tumor suppressor (Fig. 3C). In the absence of *p53*, several different types of cells can become tumorigenic, and in all cases, a germ line deficiency of *Drak2* did not increase the incidence or severity of these tumors. These data indicate that Drak2 does not function as a critical tumor suppressor in a variety of cell types.

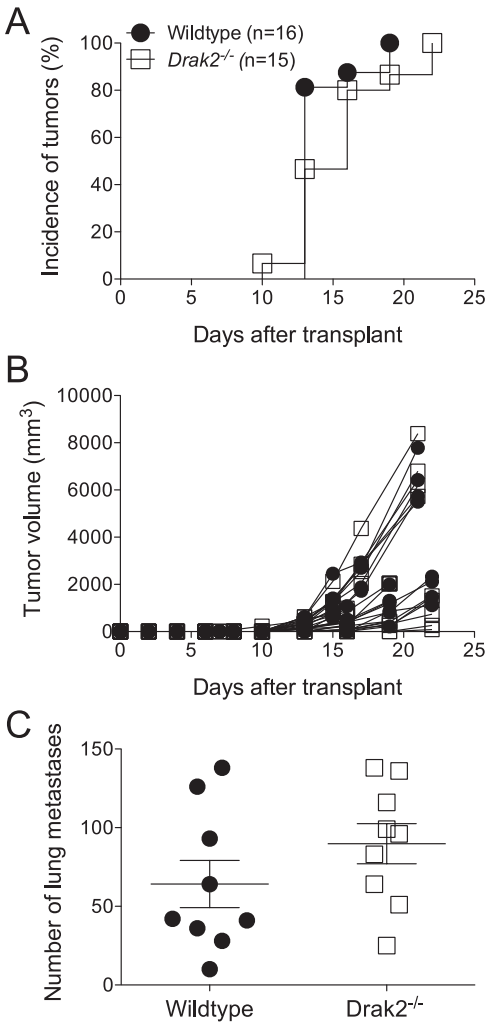

Fig. 2. Drak2 does not affect incidence of an aggressive melanoma or metastasis to the lung. (A) C57BL/6 wild-type or *Drak2*$^{-/-}$ mice were inoculated subcutaneously with 10^6 B16-F10 melanoma cells and monitored for tumor development. Tumor volumes were determined as described in Fig. 1 and the incidence of mice with tumors ≥65 mm^3 is plotted along with (B) growth curves of individual tumors. (C) 1.25×10^5 B16-F10 cells were injected intravenously. Three weeks later, lungs were harvested and the number of tumors per lung that were >1 mm was recorded. The incidence of subcutaneous tumors was not significantly different between wild-type and *Drak2*$^{-/-}$ mice according to the Log-rank (Mantel–Cox) test, and the number of lung metastases was not significantly different according to the Mann–Whitney test.

Drak2 is not important for protection from inflammation-induced tumors in the colon

Finally, we tested whether *Drak2*$^{-/-}$ mice were more susceptible to colon tumors caused by a carcinogen in the presence of inflammation. Mice were injected intraperitoneally with the carcinogen, AOM and subsequently fed DSS in their drinking water for 3 cycles of 5 days per cycle (22). This treatment models colon cancer as the DSS disrupts the epithelial barrier in the colon causing chronic inflammation, which is associated with colon cancer in humans. After 12 weeks, the colons were harvested from the mice and the number of

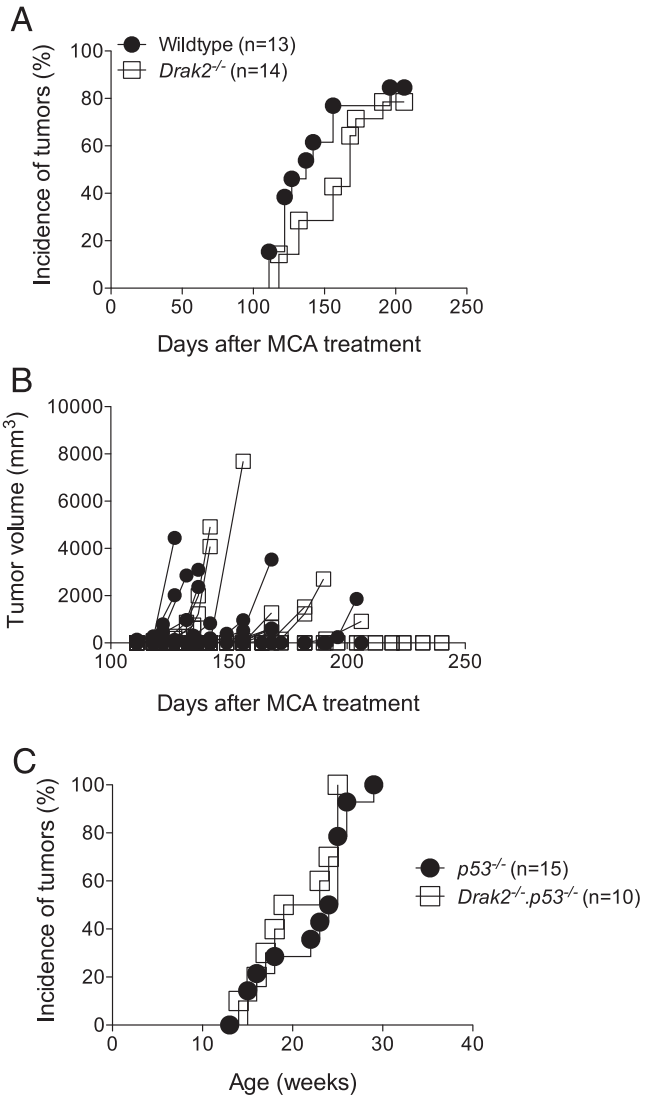

Fig. 3. Drak2 is not required for protection from spontaneous MCA-induced carcinomas or spontaneous tumor formation in the absence of p53. (A and B) Wild-type and *Drak2*$^{-/-}$ mice were injected subcutaneously with 400 μg of 3-MCA, in peanut oil and monitored for tumor growth for at least 30 weeks. Tumor volume was determined as described in Fig. 1. (A) The incidence of mice with tumors ≥65 mm^3 is plotted over time. (B) Growth curves of individual tumors are shown. The data are a combination of two independent experiments. The incidence of tumors was not significantly different between wild-type and *Drak2*$^{-/-}$ mice according to the Log-rank (Mantel–Cox) test. (C) *p53*$^{-/-}$ mice were bred to *Drak2*$^{-/-}$ mice. *p53*$^{-/-}$ and *p53*$^{-/-}$·*Drak2*$^{-/-}$ mice were monitored weekly for tumor growth. Mice with visible tumors, moribund mice and mice with severely compromised health conditions were euthanized and analyzed for tumors. The incidence of mice with tumors or mice that spontaneously died is plotted over time. There was no significant difference in the incidence of tumors between *p53*$^{-/-}$ and *p53*$^{-/-}$·*Drak2*$^{-/-}$ mice according to the Log-rank (Mantel–Cox) test.

tumors was counted. In addition, sections of the colons were analyzed by histology and given a score based on inflammation, ulceration, hyperplasia and inflamed area as previously described (22). As in the other models that we tested, the wild-type and *Drak2*$^{-/-}$ mice had comparable numbers of tumors and pathology scores (Fig. 4).

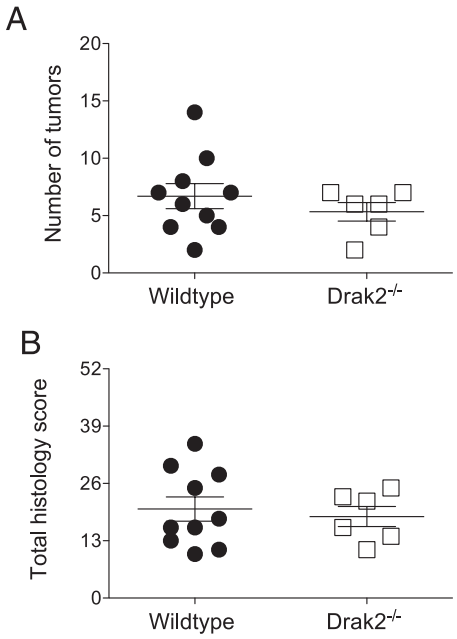

Fig. 4. Drak2 is not important for protection from inflammation-induced tumors in the colon. Wild-type and *Drak2*$^{-/-}$ mice were injected intraperitoneally with AOM on day 0. Five days later mice were fed 3% DSS in the drinking water for 5 days. After 2 weeks of normal drinking water, mice were given a second cycle of DSS for 5 days, and 2 weeks later a third cycle for 5 days. (A) Colons were harvested from mice 80 days after AOM injection and the number of tumors in the whole colon was counted. (B) Tissues were fixed in 10% formalin and embedded in paraffin for histological analysis. Sections of colon were stained with hematoxylin and eosin and scored by an experienced pathologist for inflammation, ulceration, hyperplasia and inflamed area. A cumulative score based on these four parameters was assigned as described previously (22). The data are representative of three independent experiments where there was no significant difference in the number of tumors or histology score between wild-type and *Drak2*$^{-/-}$ mice according to the Mann–Whitney test.

Together, our data indicate that *Drak2*$^{-/-}$ mice are not more susceptible to tumors in a variety of tumor models. This includes transplant tumor models, spontaneous tumors caused by carcinogen or deletion of a tumor suppressor and a tumor model involving inflammation. In all of these models, the absence of *Drak2* did not affect the onset, severity or incidence of tumorigenesis. Thus, Drak2 remains a viable target for potential pharmacologic inhibition. While mice only have Drak2, humans express both Drak1 and Drak2, which are 55% homologous at the amino acid level. Therefore, it is possible that in humans, inhibition of both Drak1 and Drak2 may be necessary to prevent autoimmunity. However, the function of Drak1 has not been tested in human T cells, and further experiments will be required to determine if Drak1 and Drak2 have overlapping functions. It is also conceivable that a small molecule inhibitor of Drak2 may also inhibit Drak1. Thus, the homology of Drak1 and Drak2 does not necessarily reduce the potential of Drak2 as a target for treatment of autoimmunity.

Our data further suggest that Drak2 typically does not function as a tumor suppressor or as an oncogene. It is possible that Drak2 contributes to very specific types of tumors, such

as those that are suppressed by TGF-β; however, we found that *Drak2*$^{-/-}$ T cells were not more sensitive to TGF-β signaling as would be expected if Drak2 negatively regulates TGF-β signaling (T. L. Harris and M. A. McGargill, manuscript in preparation). Moreover, the fact that Drak2 overexpression induces apoptosis in certain cell lines may not be indicative of its physiological role, but rather may be an artifact of overexpression. Although our data do not rule out the possibility that Drak2 contributes to certain types of tumors in specific tissues, we have shown, using various tumor models, that there is not an overt increased susceptibility to tumors in the absence of *Drak2*.

These data raise the question of why Drak2 is required for survival of T cells specific for self-antigens, but not T cells responding to tumors. While the answer to this question is not clear at this point, it is possible that in a tumor microenvironment, other cells such as NK cells and NKT cells compensate for *Drak2*$^{-/-}$ tumor-specific T cells. Alternatively, *Drak2*$^{-/-}$ T cells may only be more susceptible to death in the context of the inflammatory environment that accompanies autoimmunity. It is probable that the tumor microenvironment contains significantly more immunosuppressive components such as regulatory T cells and tumor-associated macrophages, which may affect survival of *Drak2*$^{-/-}$ T cells differently than an autoimmune setting. The reason that Drak2 is only required for the survival of T cells in specific situations is the focus of ongoing research.

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References

1 McGargill, M. A., Wen, B. G., Walsh, C. M. and Hedrick, S. M. 2004. A deficiency in Drak2 results in a T cell hypersensitivity and an unexpected resistance to autoimmunity. *Immunity* 21:781.
2 Friedrich, M. L., Wen, B. G., Bain, G. *et al.* 2005. DRAK2, a lymphoid-enriched DAP kinase, regulates the TCR activation threshold during thymocyte selection. *Int. Immunol.* 17:1379.
3 Sanjo, H., Kawai, T., Akira, S. 1998. DRAKs, novel serine/threonine kinases related to death-associated protein kinase that trigger apoptosis. *J. Biol. Chem.* 273:29066.
4 McGargill, M. A., Choy, C., Wen, B. G. and Hedrick, S. M. 2008. Drak2 regulates the survival of activated T cells and is required for organ-specific autoimmune disease. *J. Immunol.* 181:7593.
5 Weist, B. M., Hernandez, J. B. and Walsh, C. M. 2012. Loss of DRAK2 signaling enhances allogeneic transplant survival by limiting effector and memory T cell responses. *Am. J. Transplant.* 12:2220.
6 Ramos, S. J., Hardison, J. L., Stiles, L. N., Lane, T. E. and Walsh, C. M. 2007. Anti-viral effector T cell responses and trafficking are not dependent upon DRAK2 signaling following viral infection of the central nervous system. *Autoimmunity* 40:54.
7 Wang, S., Welte, T., McGargill, M. *et al.* 2008. Drak2 contributes to West Nile virus entry into the brain and lethal encephalitis. *J. Immunol.* 181:2084.
8 Kuwahara, H., Nakamura, N. and Kanazawa, H. 2006. Nuclear localization of the serine/threonine kinase DRAK2 is involved in UV-induced apoptosis. *Biol. Pharm. Bull.* 29:225.
9 Kögel, D., Prehn, J. H. and Scheidtmann, K. H. 2001. The DAP kinase family of pro-apoptotic proteins: novel players in the apoptotic game. *BioEssays* 23:352.
10 Matsumoto, M., Miyake, Y., Nagita, M. *et al.* 2001. A serine/threonine kinase which causes apoptosis-like cell death interacts with a calcineurin B-like protein capable of binding Na(+)/H(+) exchanger. *J. Biochem.* 130:217.
11 Bialik, S. and Kimchi, A. 2006. The death-associated protein kinases: structure, function, and beyond. *Annu. Rev. Biochem.* 75:189.
12 Mao, J., Luo, H. and Wu, J. 2008. Drak2 overexpression results in increased beta-cell apoptosis after free fatty acid stimulation. *J. Cell. Biochem.* 105:1073.
13 Mao, J., Qiao, X., Luo, H. and Wu, J. 2006. Transgenic drak2 overexpression in mice leads to increased T cell apoptosis and compromised memory T cell development. *J. Biol. Chem.* 281:12587.
14 Ye, P., Zhao, L. and Gonda, T. J. 2013. The MYB oncogene can suppress apoptosis in acute myeloid leukemia cells by transcriptional repression of DRAK2 expression. *Leuk. Res.* 37:595.
15 Doherty, G. A., Byrne, S. M., Austin, S. C. *et al.* 2009. Regulation of the apoptosis-inducing kinase DRAK2 by cyclooxygenase-2 in colorectal cancer. *Br. J. Cancer.* 101:483.
16 Hartmann, T. B., Mattern, E., Wiedemann, N. *et al.* 2008. Identification of selectively expressed genes and antigens in CTCL. *Exp. Dermatol.* 17:324.
17 Yang, K. M., Kim, W., Bae, E. *et al.* 2012. DRAK2 participates in a negative feedback loop to control TGF-β/Smads signaling by binding to type I TGF-β receptor. *Cell Rep.* 2:1286.
18 Gatzka, M., Newton, R. H. and Walsh, C. M. 2009. Altered thymic selection and increased autoimmunity caused by ectopic expression of DRAK2 during T cell development. *J. Immunol.* 183:285.
19 Shankaran, V., Ikeda, H., Bruce, A. T. *et al.* 2001. IFNgamma and lymphocytes prevent primary tumour development and shape tumour immunogenicity. *Nature* 410:1107.
20 O'Sullivan, T., Saddawi-Konefka, R., Vermi, W. *et al.* 2012. Cancer immunoediting by the innate immune system in the absence of adaptive immunity. *J. Exp. Med.* 209:1869.
21 Tomayko, M. M. and Reynolds, C. P. 1989. Determination of subcutaneous tumor size in athymic (nude) mice. *Cancer Chemother. Pharmacol.* 24:148.
22 Zaki, M. H., Vogel, P., Malireddi, R. K.S., *et al.* 2011. The NOD-like receptor NLRP12 attenuates colon inflammation and tumorigenesis. *Cancer Cell* 20:649.