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Mice lacking Dok-1, Dok-2, and Dok-3 succumb to aggressive histiocytic sarcoma

Ryuichi Mashima[1], Kazuho Honda[2], Yi Yang[3], Yohei Morita[4], Akane Inoue[1], Sumimasa Arimura[1], Hiroshi Nishina[5], Hideo Ema[4], Hiromitsu Nakauchi[4], Brian Seed[6], Hideaki Oda[2] and Yuji Yamanashi[1]

Histiocytic sarcoma (HS), a rare hematological malignancy, is an aggressive neoplasm that responds poorly to therapy. The molecular etiology and pathology of this disease remain unclear, hampering the development of an effective therapy, and there remains a need for more, and more realistic, animal models. HS cells typically show a histiocytic (ie, tissue macrophage-like) morphology and express histiocyte/macrophage markers in the absence of lymphocyte markers. In this study, we report that *Dok-1*$^{-/-}$*Dok-2*$^{-/-}$*Dok-3*$^{-/-}$ mice develop HS, but do not exhibit elevated incidence of other types of tumors. These mutant mice showed earlier mortality than wild-type (WT) or the other mutant mice, and this mortality was associated with HS. In total, 17 of 21 tumor-bearing *Dok-1*$^{-/-}$*Dok-2*$^{-/-}$*Dok-3*$^{-/-}$ mice necropsied at 25–66 weeks of age showed multiple organ spread, with osteolytic lesions and orthotopic invasion from the bone marrow to skeletal muscle. Tumors from the mice were transplantable. In addition, all *Dok-1*$^{-/-}$*Dok-2*$^{-/-}$*Dok-3*$^{-/-}$ mice, but only a small proportion of *Dok-3*$^{-/-}$ mice and no *Dok-1*$^{-/-}$*Dok-2*$^{-/-}$ mice, exhibited abnormal accumulation of macrophages in the lung on necropsy at 8–12 weeks of age. Macrophages derived from *Dok-1*$^{-/-}$*Dok-2*$^{-/-}$*Dok-3*$^{-/-}$ mice displayed an exaggerated proliferative response to macrophage colony-stimulating factor (M-CSF) or granulocyte-macrophage colony-stimulating factor (GM-CSF) compared with WT and mutant controls. Together, these findings indicate that Dok-1, Dok-2, and Dok-3 cooperatively suppress aggressive HS, and commend *Dok-1*$^{-/-}$*Dok-2*$^{-/-}$*Dok-3*$^{-/-}$ mice as a useful model for the study of this neoplasia.

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Histiocytic sarcoma (HS) is a malignant proliferation of cells showing morphological and immunophenotypic features of mature histiocytes, which represent tissue-resident macrophages.[1,2] Until recently, HS, which was also known as malignant histiocytosis, was often confused with anaplastic large B-cell lymphoma or with other malignant lymphomas. However, it has been established that true HS is a distinct and rare disease that is only about 0.1% as frequent as malignant lymphomas, which can be identified by the presence of B- or T-cell markers and/or CD30.[2] By definition, HS is negative for lymphocyte markers, but positive for histiocyte/macrophage markers such as CD163 in humans.[1,2] The tumor comprises a diffuse noncohesive proliferation of large cells, round to oval in shape, with abundant, eosinophilic cytoplasm and nuclear atypia. HS is an aggressive neoplasm with most patients dying of progressive disease. Patients may present with a solitary mass of HS, which is predictive of a relatively favorable outcome, but some patients show a systemic pattern of tumor spread.[1,2] As the molecular etiology of this disease is unknown, the development of rational therapeutics has been difficult. Although the generation of animal models is an essential step for the study of etiology, the paucity of mouse models that represent aggressive HS has remained an outstanding problem.

We and others previously identified Dok-1 as a common substrate of protein-tyrosine kinases (PTKs) including Bcr-Abl, a cause of chronic myelogenous leukemia,[3,4] and we further demonstrated that Dok-1 is a negative regulator of

[1]Division of Genetics, Department of Cancer Biology, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan; [2]Department of Pathology, Tokyo Women's Medical University, Tokyo, Japan; [3]Novartis Institute for Biomedical Research, Cambridge, MA, USA; [4]Laboratory of Stem Cell Therapy, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan; [5]Department of Developmental and Regenerative Biology, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan and [6]Department of Pediatrics, Center for Computational and Integrative Biology, Massachusetts General Hospital, Boston, MA, USA
Correspondence: Dr Y Yamanashi, PhD, Division of Genetics, The Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan.
E-mail: yyamanas@ims.u-tokyo.ac.jp

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PTK-mediated proliferation and transformation of cells.[5,6] Indeed, mice lacking both Dok-1 and its closest homolog Dok-2 developed a myeloproliferative disorder with a non-aggressive phenotype.[7,8] It is believed that Dok-1 and Dok-2 have virtually identical roles in myeloid lineages.[7–9]

The Dok family consists of seven members, Dok-1 to Dok-7, which share structural similarities characterized by the NH_2-terminal pleckstrin homology and phosphotyrosine-binding domains, followed by the src homology 2 target motifs in the COOH-terminal moiety, suggesting an adaptor function.[3,4,10–14] Among these members, only Dok-1, Dok-2, and Dok-3 are preferentially expressed in hematopoietic cells, or myeloid cells in particular, and comprise a closely related subgroup with regard to primary structure.[7,9,15] Similar to Dok-1 and Dok-2, Dok-3 is also a negative regulator of PTK-mediated signaling, despite being a relatively distant member of this subgroup.[11,16–19] However, mice lacking Dok-3 alone or Dok-1 and its closest homolog Dok-2 in combination do not develop aggressive tumors of hematopoietic cells.[7,8,18] In this study, we demonstrate that mice lacking Dok-1, Dok-2, and Dok-3 provide a model system for aggressive HS. Triple null mutant mice showed an early lethal phenotype that is associated with HS, and the tumor, which was transplantable, showed multiple organ invasion.

MATERIALS AND METHODS

Mice

Dok-1$^{-/-}$*Dok-2*$^{-/-}$ and *Dok-3*$^{-/-}$ mice were generated as previously described.[7,20] *Dok-1*$^{-/-}$*Dok-2*$^{-/-}$*Dok-3*$^{-/-}$ (TKO) mice were obtained by crossing *Dok-1*$^{-/-}$*Dok-2*$^{-/-}$ and *Dok-3*$^{-/-}$ mice (Supplementary Figure S1). Mice were genotyped by a standard PCR using DNA isolated from the tail tips. Primers to amplify the wild-type (WT) and targeted *dok-1*, *dok-2*, and *dok-3* loci have been previously described.[7,20] All mice were maintained in a mixed genetic background of strains 129/SvJ and C57BL/6 under pathogen-free conditions in the animal care facilities at Tokyo Medical and Dental University and The University of Tokyo. The experimental protocols have been approved by the animal ethics committees of the two institutions.

Histological Analysis

For hematoxylin and eosin (H&E) staining, tissue samples, aside from bones, were fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned, and stained. Bones were fixed similarly and decalcified in 14% EDTA solution for 3 days at room temperature with gentle stirring before staining with H&E. For immunohistochemistry using antibodies to Mac-2, PCNA, and Ki-67, paraffin sections were processed with 10 mM citrate buffer (pH 6.0) in a microwave (95°C, 15 min) and subjected to standard immunohistochemical staining using the streptavidin–biotin–peroxidase complex method. For immunohistochemistry using antibodies to F4/80, CD68, CD3, and B220, cryostat sections were fixed in 95% acetone at 4°C for 20 min and were also subjected to standard immunohistochemical staining using the streptavidin–biotin–peroxidase complex method. The slides were counterstained with hematoxylin. Endogenous peroxidase activity was inactivated by incubation with 3% hydrogen peroxide in methanol (at room temperature for 15 min). The following antibodies were used: anti-Mac-2 rat monoclonal antibody (M3/38, Cedarlane, Burlington, ON, Canada; dilution 1:400); anti-F4/80 rat monoclonal antibody (BM8, BD Biosciences, San Diego, CA, USA; dilution 1:100); anti-CD68 rat monoclonal antibody (FA-11, Serotec, Kidlington, UK; dilution 1:100); anti-B220 rat monoclonal antibody (RA3-6B2, BD Biosciences; dilution 1:100); anti-CD3 hamster monoclonal antibody (145–2C11, BD Biosciences; dilution 1:100); anti-PCNA mouse monoclonal antibody (PC10, Dako, Glostrup, Denmark; dilution 1:100); and anti-Ki-67 rat monoclonal antibody (TEC-3, Dako; dilution 1:50). Only nonautolyzed tissues from moribund or recently deceased mice were subjected to histological analysis.

Microscopy

Sections were viewed using an AX80 microscope (Olympus, Tokyo, Japan) with either a $\times 20$ or $\times 40$ PlanApo objective. Images were captured using a DP70 digital camera and DP Controller software (Olympus).

Transplantation

Nucleated bone marrow cells or splenocytes (2×10^6 cells) prepared from the donor were intravenously injected into lethally irradiated WT recipient mice. Irradiation was performed using an IBL-437C instrument (^{137}Cs, CIS Bio-International, Gif-sur-Yvette, France) at 9 Gy, and no survival was observed beyond 2 weeks after irradiation in the absence of transplantation. In Figure 3, TKO and WT mice at 78–88 weeks of age were used as donors and WT mice at 8–16 weeks of age were used as recipients. In Figure 4c, TKO and WT mice at 6–10 weeks of age were used as donors and WT mice at 8–16 weeks of age were used as recipients: these recipient mice were killed 8–10 weeks after transplantation and histological studies were performed.

Bone Marrow-Derived Macrophages

Bone marrow cells were cultured in DME medium containing 100 ng/ml of recombinant murine macrophage colony-stimulating factor (M-CSF, PeproTech, Rocky Hill, NJ, USA) and 15% FCS. After 7 days of culture, adherent cells were maintained in the absence of M-CSF for 16 h and were used as bone marrow-derived macrophages.

Cell Viability Assay

Bone marrow-derived macrophages (1×10^4) were plated in 96-well plates in quadruplicate, cultured in RPMI-1640 medium containing 15% FCS in the presence or absence of M-CSF or recombinant murine granulocyte-macrophage colony-stimulating factor (GM-CSF, PeproTech) for 5 days.

Cells were then treated with 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT, Sigma, St Louis, MO, USA) for 4 h at 37°C, cellular formazan product was dissolved with acidic isopropanol, and the absorbance at 570 nm was measured spectrophotometrically to evaluate viable cells using an Emax microplate reader (Molecular Devices, Sunnyvale, CA, USA).

RESULTS

TKO Mice Succumb to HS

To examine whether Dok-1, Dok-2, and Dok-3 cooperatively suppress malignant tumor formation *in vivo*, we generated TKO mice. These mice were born at the expected Mendelian frequency without any abnormality in appearance evident by visual inspection. However, nearly half (15 of 33) of the TKO mice died between 14–51 weeks after birth, whereas all WT and *Dok-1*$^{-/-}$*Dok-2*$^{-/-}$ mice as well as all *Dok-3*$^{-/-}$ mice but one remained alive (Figure 1a). To gain insight into the early lethal phenotype of TKO mice, we first performed conventional histological studies. H&E staining of tissue sections revealed that TKO mice, but neither the other mutants nor the WT controls, showed a markedly high incidence (24 of 41) of large cell tumors at <65 weeks of age, characterized by the accumulation of abnormal cells with histiocytic morphology in the bone marrow, spleen, and/or liver (Figure 1b). The aberrant cells are distinguished morphologically from other hematopoietic cells by their round shape, prominent nuclear atypia, and eosinophilic cytoplasm, features that

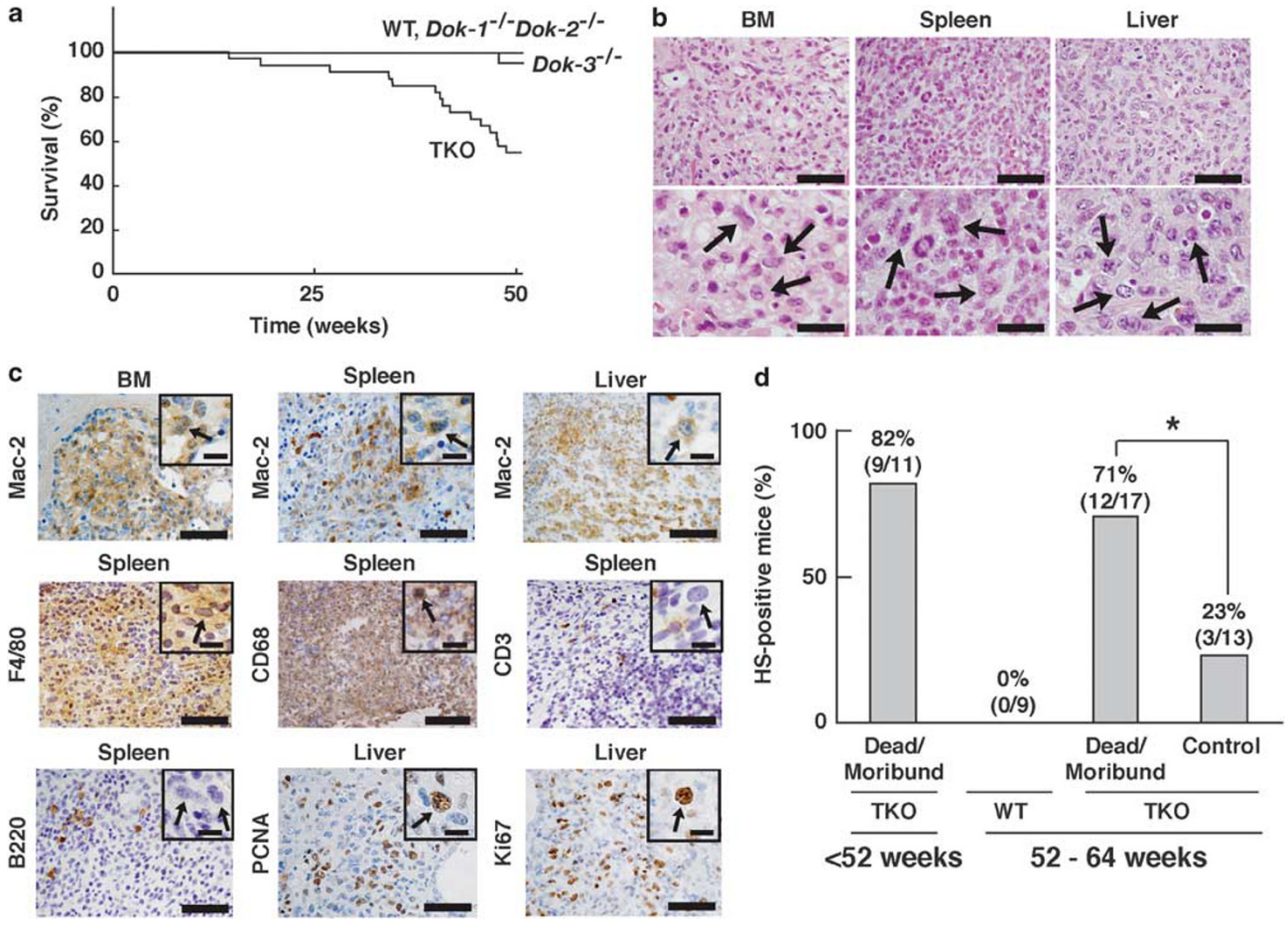

Figure 1 TKO mice develop HS with early mortality. (**a**) Survival of TKO ($n=33$), *Dok-1*$^{-/-}$*Dok-2*$^{-/-}$ ($n=20$), *Dok-3*$^{-/-}$ ($n=22$), and wild-type (WT, $n=26$) mice are presented. (**b** and **c**) Identification of tumor cells developed in TKO mice as HS by H&E (**b**) and immunohistochemical (**c**) staining. In panel (**b**), histology of tissue sections prepared from the bone marrow (BM), spleen, and liver of TKO mice is presented. Tumor cells are diffusely distributed (top), and are large and rounded and have atypical nuclei (bottom). Arrows indicate representative tumor cells. Scale bars show 100 (top) and 50 μm (bottom). In panel (**c**), tumor cells in the BM, spleen, and liver of TKO mice are stained with antibodies to Mac-2, F4/80, and CD68, but not by antibodies to CD3 and B220. Many of the tumor cells are stained with antibodies to PCNA and Ki67. Arrows indicate representative tumor cells. Scale bars show 100 and 20 μm (inset). (**d**) Association of the early lethal phenotype of TKO mice with HS. The percentage of mice that are HS positive in the BM, spleen, and/or liver was determined for each genotype and condition (dead/moribund or not) and is presented along with the exact fraction in parenthesis. Dead/moribund TKO mice at 52–64 weeks of age showed a significantly higher rate of HS positivity than TKO mice that were neither dead nor moribund (control). No tumor was observed in the age-matched WT mice. TKO mice that died or became moribund before 52 weeks of age also showed a high rate of HS positivity. Fischer's exact test was used to calculate the statistical significance. $^*P<0.05$.

are characteristic of HS tumor cells (Figure 1b). Moreover, tumors showed monotonous morphology with tissue destruction and mass lesions, suggesting a malignant transformation (Figure 1b and Supplementary Figure S2). Immunohistochemical staining revealed the abnormal cells to be positive for the histiocyte/macrophage markers, Mac-2, F4/80, and CD68, but negative for the lymphocyte markers, CD3 and B220, consistent with the interpretation that these tumor cells represent HS (Figure 1c).[21,22] In addition, these cells stained positively with antibodies to MHC class II molecules, a marker of antigen-presenting cells including macrophages (Supplementary Figure S3). Further immunohistochemical examination showed HS cells to stain positively with antibodies to PCNA and Ki67, confirming that these cells are proliferative (Figure 1c).

As most patients with HS die of progressive disease, we examined whether HS is associated with the early lethal phenotype of TKO mice. As shown in Figure 1d, 9 of 11 moribund or dead mice at <52 weeks of age that could be necropsied bore HS in the bone marrow, spleen, and/or liver. Furthermore, no similar cells were found in WT mice even at 52–64 weeks of age. By contrast, 12 of 17 dead or moribund mice that could be necropsied at matched age developed HS, whereas only 3 of 13 nondead/moribund mice were positive for the tumor, indicating a significant link of HS with the lethal phenotype of TKO mice (Figure 1d).

HS Developed in TKO Mice is Highly Invasive and Transplantable

To evaluate the tumorigenic potential of HS developed in TKO mice, we wished to know whether the tumors spread into multiple organs. We examined six organs, the bone marrow, spleen, lung, skeletal muscle (femoral muscle), liver, and kidney. In all, 17 of the 21 tumor-bearing TKO mice at 25–66 weeks of age whose organs could be analyzed at necropsy showed multiple organ spread of the tumor (Figure 2a). As nearly all (20 of 21) TKO mice had HS in the bone marrow, this tissue might be the primary site of tumorigenesis in these mice (Figure 2b). Moreover, the majority (11 of 20) of bone marrow lesions were associated with osteolytic and direct invasions to the skeletal muscle (Figure 2b and c), indicating a strong invasiveness of the tumor. Next, to further examine the tumorigenic potential of HS developed in TKO mice, we examined whether the tumor was transplantable. Cells prepared from bone marrow or spleen of TKO mice or WT controls at 78–88 weeks of age were transplanted into lethally irradiated WT recipients intravenously. The mice transplanted with TKO cells from HS-positive tissues died within 10 weeks after transplantation, whereas control recipients that had been transplanted with cells from WT animals or from HS-negative sources of TKO animals survived, suggesting that the tumor was transplantable (Figure 3a). Indeed, histological study con-

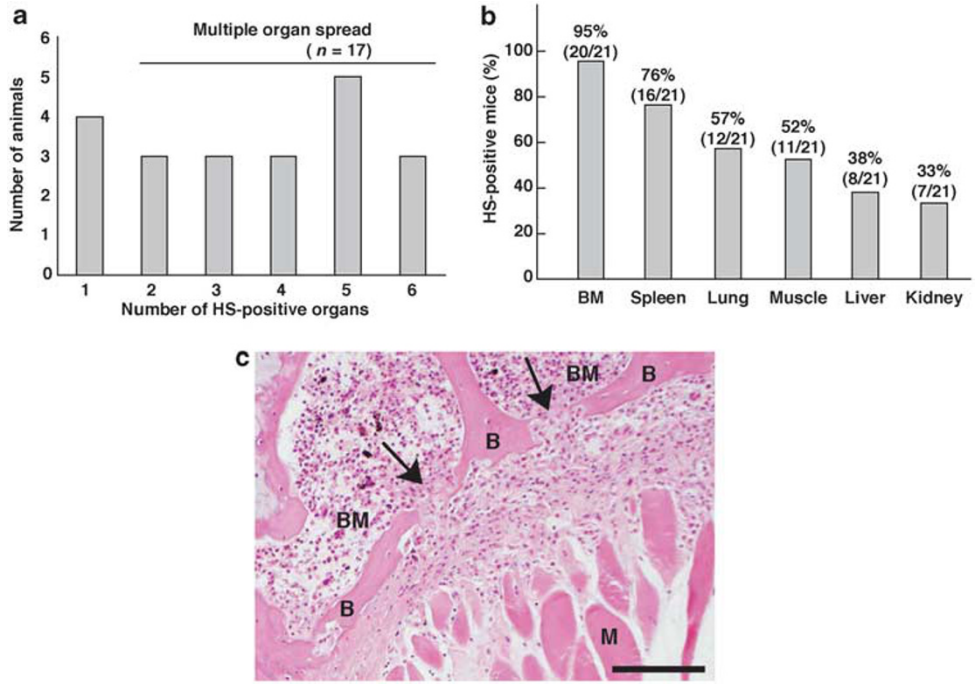

Figure 2 TKO mice show multiple organ spread of HS. (**a**) The number distribution of HS-affected organs in individual TKO mice. The bone marrow, spleen, lung, skeletal muscle, liver, and kidney from a total of 21 TKO mice were examined, and the number of HS-positive organs in each mouse was counted and plotted against the number of the corresponding mice. In all, 4 mice had only one HS-positive organ but the remaining 17 mice showed multiple organ spread of the tumor. (**b**) The organ distribution of HS positivity in TKO mice. From the data obtained in (**a**), the percentage of HS positivity by organ in affected mice is presented along with the exact fraction in parenthesis. (**c**) Histology of osteolytic invasions of HS into the skeletal muscle in TKO mice (H&E staining). B, bone; BM, bone marrow; M, muscle. Arrows indicate osteolytic lesions. Scale bar shows 100 μm.

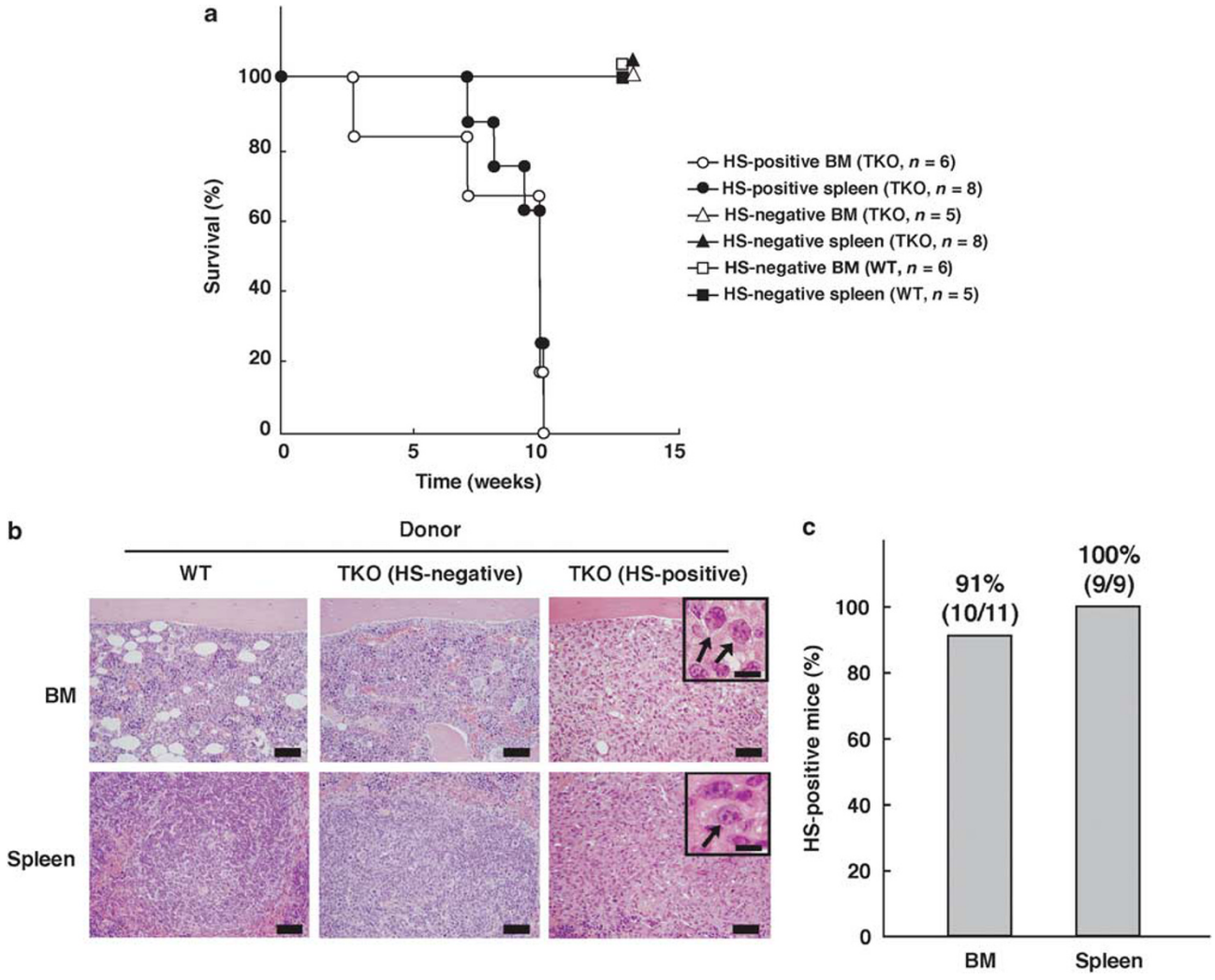

Figure 3 HS developed in TKO mice is transplantable. (**a**) Survival of transplanted mice. The survival rates of lethally irradiated (9 Gy) wild-type (WT) recipients that were transplanted with mononuclear cells from the bone marrow (BM) and spleen of the indicated donors are plotted against time after transplantation. The donor organs were scored as HS positive or negative, and only the recipients transplanted with WT cells or HS-negative TKO cells survived beyond 10 weeks after transplantation. (**b**) Histological analysis of transplanted mice. Histology of the BM and spleen sections prepared from the recipient mice that were transplanted with BM cells from the indicated donors is presented (H&E staining). Arrows indicate representative tumor cells. Scale bars show 100 and 20 μm (inset). (**c**) The percentage of HS-positive BMs and spleens in transplanted mice. The BMs and spleens from eleven and nine recipients, respectively, which had been transplanted with the HS-positive BM or spleen cells were examined. The percentage of HS positivity for BM and spleen was calculated and is presented along with the exact fraction in parenthesis.

firmed that almost all the recipient mice that had been transplanted with TKO cells from HS-positive animals and could be necropsied had HS in their bone marrow (10 of 11) and spleen (9 of 9) (Figure 3b and c). Furthermore, HS was not observed in control recipients that had been transplanted with WT cells ($n = 11$) or TKO cells from HS-negative sources ($n = 13$). We conclude that the malignant cell type that develops in TKO mice is highly invasive and transplantable.

TKO Mice Show Abnormal Accumulation of Macrophages in the Lung

During the course of histological analysis of TKO mice, we frequently found abnormal accumulation of Mac-2-positive macrophages in the lung (Figure 4a). These macrophages had a large cytoplasm without nuclear atypia and were not associated with tissue destruction, suggesting a non-tumorigenic nature. Almost all (13 of 14) TKO mice, but neither *Dok-1*$^{-/-}$*Dok-2*$^{-/-}$ (0 of 11) mice nor the WT controls (0 of 9), exhibited such abnormal proliferation of macrophages, which was uncorrelated with the presence or absence of HS at 50–59 weeks of age (Figure 4b). The incidence of macrophage accumulation in the lung was intermediate (3 of 8) in age-matched *Dok-3*$^{-/-}$ mice. Although development of lung adenocarcinoma, but not aggressive HS, has recently been reported in mice lacking Dok-1, Dok-2, and/or Dok-3 on a pure 129S1/SvImj genetic background even at the age of 11–15 months,[23] in this study mice mu-

tated in the same genes, but on a 129/SvJ and C57BL/6 mixed background, did not exhibit elevated incidence of lung adenocarcinoma. These different findings are likely due to the difference in the genetic backgrounds.

When bone marrow cells of TKO mice at 6–10 weeks of age were transplanted into lethally irradiated recipients, we invariably observed macrophage accumulation in the lung within 10 weeks after transplantation ($n = 4$), but not when bone marrow cells were transplanted from WT controls (Figure 4c). Similarly, the histological study of TKO mice at 8–12 weeks of age, before the onset of observable HS, confirmed abnormal proliferation of macrophages in the lung of all mutant mice (8 of 8) but not in WT controls (0 of 8) (Figure 4d). Again no age-matched *Dok-1*$^{-/-}$*Dok-2*$^{-/-}$ (0 of 5) mice, and only a subset of *Dok-3*$^{-/-}$ mice (1 of 5), showed such accumulation of macrophages. Given that Dok-1 and Dok-2 negatively regulate proliferation of bone marrow-derived macrophages *in vitro*,[7] these findings suggest that Dok-1, Dok-2, and Dok-3 cooperatively inhibit the proliferation of macrophages.

Dok-1, Dok-2, and Dok-3 are Negative Regulators of Proliferative Response of Macrophages to M-CSF or GM-CSF

To address whether Dok-1, Dok-2, and Dok-3 are negative regulators of macrophage proliferation, we examined growth responses of bone marrow-derived macrophages from WT, *Dok-1*$^{-/-}$*Dok-2*$^{-/-}$, *Dok-3*$^{-/-}$, and TKO mice at 10–12 weeks of age on stimulation with M-CSF and GM-CSF, both of which are critical for proliferation of macrophages.[24–26] TKO

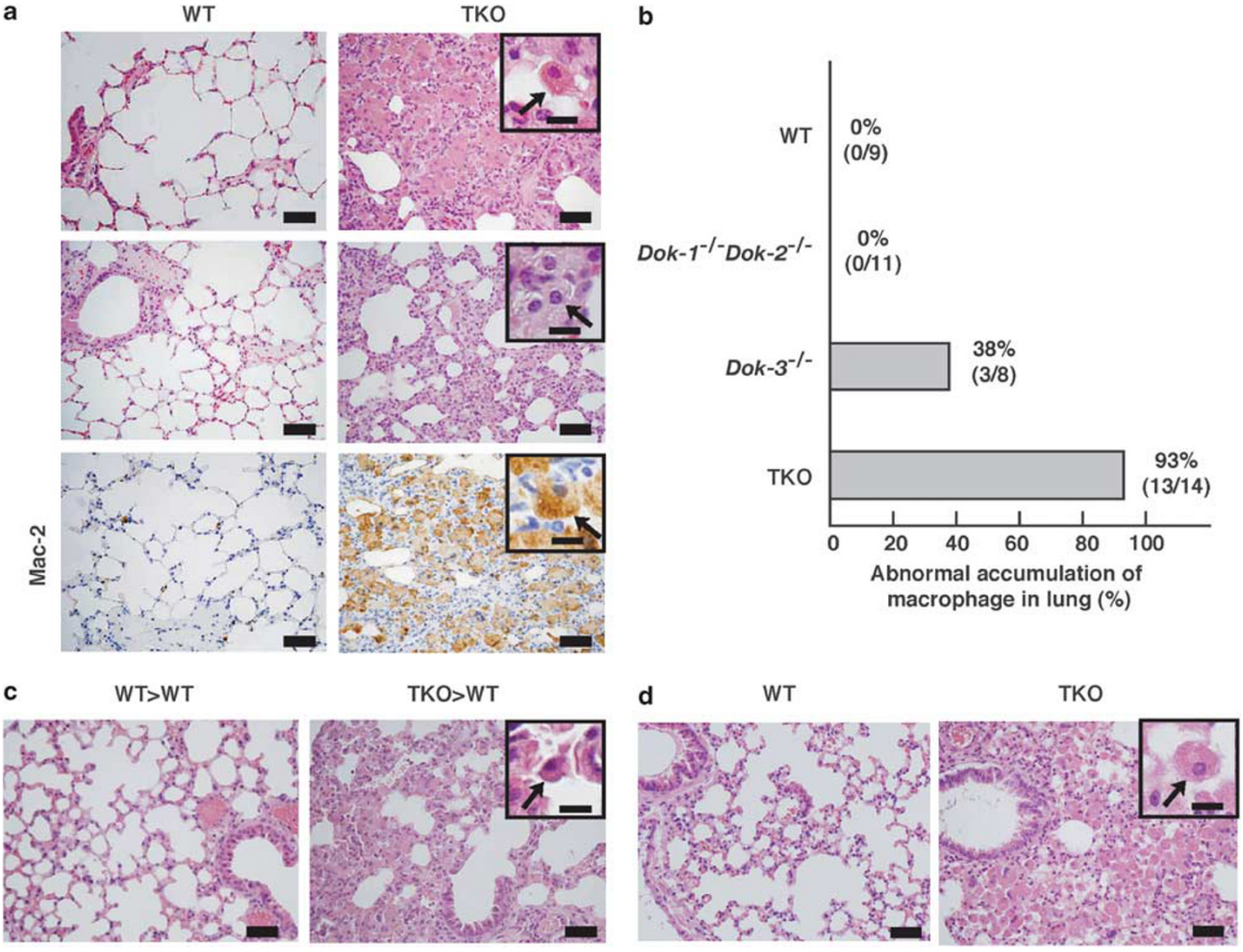

Figure 4 TKO mice show abnormal proliferation of macrophages in the lung. (**a**) Abnormal accumulation of macrophages in the lungs of TKO mice. Histology of lung sections prepared from wild-type (WT, left) and TKO (right) mice is presented (top, H&E staining; bottom, anti-Mac-2 staining). Arrows indicate representative macrophages present in the lung. Scale bars show 100 and 20 μm (inset). (**b**) The percentage of lungs with abnormal macrophage accumulations in various mice. The lungs from mice of the indicated genotypes were examined. The percentage of mice with abnormal lung macrophage accumulations was calculated and is presented along with the exact fraction in parenthesis. (**c**) Histology of lung sections prepared from recipient mice transplanted with bone marrow cells from WT (WT > WT, left) and TKO (TKO > WT, right) mice (H&E staining). An arrow indicates a representative macrophage present in the lung. Scale bars show 100 and 20 μm (inset). (**d**) Abnormal accumulation of macrophages in the lungs of younger (8–12 weeks of age) TKO mice. Histology of lung sections prepared from WT (left) and TKO (right) mice is presented (H&E staining). An arrow indicates a representative macrophage present in the lung. Scale bars show 100 and 20 μm (inset).

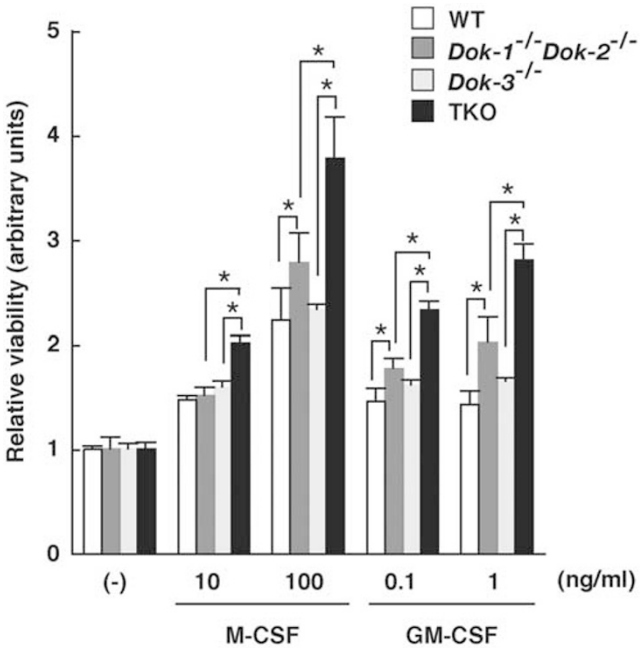

Figure 5 Augmented proliferative responses of bone marrow-derived macrophages prepared from TKO mice. Relative viabilities of bone marrow-derived macrophages of the indicated genotypes were evaluated by the MTT cell viability assay after 5 days of culture in the presence of M-CSF or GM-CSF at the indicated concentrations, in which the mean value in the absence of cytokine (−) was defined as 1 in arbitrary units for each genotype. WT, wild type. Data are expressed as mean ± s.d., and *P* values are calculated between WT and *Dok-1*$^{-/-}$*Dok-2*$^{-/-}$ or between TKO and *Dok-1*$^{-/-}$*Dok-2*$^{-/-}$ *or Dok-3*$^{-/-}$ macrophage proliferation. **P* < 0.05.

macrophages showed the highest proliferative response (Figure 5), consistent with the view that Dok-1, Dok-2, and Dok-3 are negative regulators of the proliferative response of macrophages to M-CSF or GM-CSF.

DISCUSSION

In this study, we have established that combined ablation of the adaptor proteins Dok-1, Dok-2, and Dok-3 has profound phenotypic consequences in mice. TKO mice, but not WT controls, develop and succumb to aggressive HS with multiple organ invasion. Although individual Dok proteins have the ability to inhibit PTK-mediated oncogenic signaling,[5–9,16] in this study mice lacking Dok-3 alone or Dok-1 and Dok-2 in combination did not develop aggressive tumors. The simplest interpretation for these data is that Dok proteins 1–3 can each suppress the aggressive transformation of HS. In addition, combined loss of Dok-1, Dok-2, and Dok-3 causes abnormal proliferation of macrophages in the lung, observable before the onset of morphologically recognizable HS. By contrast, combined ablation of Dok-1 and Dok-2 did not cause accumulation of macrophages in the lung. Although deficiency in Dok-3 caused abnormal proliferation of macrophages in the lung, the incidence was low. Therefore, the data suggest that these Dok proteins mutually compensate to inhibit the proliferation of macrophages. Indeed, our *in vitro* assay revealed that these proteins cooperatively downregulate proliferative response of macrophages on M-CSF or GM-CSF stimulation. Given that histiocytes are tissue-resident macrophages and HS is believed to arise from macrophage lineages, it is likely that the enhanced proliferative capacity of macrophages in mice lacking Dok-1, Dok-2, and Dok-3 contributes to the development of HS.

In general, Dok family proteins are believed to be activated as adaptors by tyrosine phosphorylation.[9] It was previously demonstrated that Lyn is required for the tyrosine phosphorylation of Dok-1 and Dok-3 in B cells (in which Dok-2 is typically undetectable) on B-cell receptor signaling,[6,27] suggesting that Lyn may activate these Dok proteins as negative regulators to suppress HS. Indeed, it has been reported that Lyn is expressed in macrophages, and that bone marrow-derived macrophages from Lyn-deficient mice showed enhanced growth responses to M-CSF and GM-CSF.[28] Furthermore, mice lacking Lyn develop macrophage tumors, which may be related to HS.[28] Unlike TKO mice, however, Lyn-deficient mice additionally develop severe renal disease.[29]

As mentioned above, HS in humans is an aggressive malignancy of unknown etiology and there remains a need for realistic animal models. Mouse models that have been reported for HS frequently show multiple lesions including lymphomas and severe renal failure. For example, the majority of mice lacking Pten, Ink4A, and Arf in combination develop both lymphoblastic B-cell lymphomas and HS with virtually the same incidence.[30] Similarly, mice lacking the cell-cycle regulator p21 develop a variety of tumors, including HS and B-cell lymphomas, and also suffered from severe renal failure,[31] unlike TKO mice. The syndrome elicited in mice lacking all the three proteins, Dok-1, Dok-2, and Dok-3, more specifically resembles the disease found in humans and hence may serve as a useful model for the study of HS. Although elucidation of the mechanisms by which the ablation of Dok proteins specifically causes HS and how the tumor gains its aggressive phenotype awaits further studies, such studies will help unveil the hidden etiology of this rare aggressive human malignancy.

Supplementary Information accompanies the paper on the Laboratory Investigation website (http://www.laboratoryinvestigation.org)

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DISCLOSURE/CONFLICT OF INTEREST

The authors declare no conflict of interest.

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