Effects of expressing human T-cell leukemia virus type 1 (HTLV-I) oncoprotein Tax on DOK1, DOK2 and DOK3 gene expression in mice

Takeo OHSUGI1)*

1Department of Laboratory Animal Science, School of Veterinary Medicine, Rakuno-Gakuen University, S82 Bunkyodai-Midorimachi, Ebetsu, Hokkaido 069-8501, Japan

ABSTRACT. Transgenic mice expressing the tax gene from human T-cell leukemia virus type 1 (HTLV-I) genome developed T-cell leukemia or histiocytic sarcoma after at least 12 months. The transgenic mice showed low expression of the downstream of tyrosine kinase (DOK) family members, DOK1, DOK2 and DOK3, which were recently reported to be tumor suppressor genes. Mice showed low DOK2 expression at 5–6 months of age, before disease onset. The expression of DOK1 and DOK3 was not significantly reduced at any age tested. These results suggest that downregulation of DOK2 by the expression of the viral tax gene is the first step in the development of T-cell leukemia or histiocytic sarcoma.

KEY WORDS: adult T-cell leukemia/lymphoma (ATLL), downstream of tyrosine kinase (DOK), human T-cell leukemia virus type 1 (HTLV-1)

Human T-cell leukemia virus type 1 (HTLV-I) was the first human retrovirus to be isolated. Infection with HTLV-I can result in the aggressive malignancy adult T-cell leukemia/lymphoma (ATLL) or in inflammatory diseases, such as HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) and HTLV-I uveitis, after a prolonged period of latency, often lasting 20–50 years [15, 16]. HTLV-I encodes the oncoprotein Tax, which modulates the expression of several genes leading to T cell transformation and appears to be a key molecule in the development of ATLL. Tax also interferes with the functions of several tumor suppressor proteins [3, 7, 15, 16]. To investigate the pathogenic role of Tax in vivo, my research group created a transgenic (Tg) mouse model of HTLV-I using the distal promoter of Lck to express tax in mature thymocytes and peripheral T lymphocytes [10]. Over 2 years, 28.1% of the Tax-expressing Tg (Tax-Tg) mice developed mature T cell leukemia/lymphoma compared with only 1% of their non-Tg littermates [9]. A few aged Tax-Tg mice developed HAM/TSP-like disease with symmetrical paraparesis of the hind limbs, which was caused by the infiltration of bone-marrow-derived histiocytic sarcoma (HS) cells [12]. The downstream of tyrosine kinase (DOK) family of proteins has seven members, DOK1–DOK7, which are adaptor proteins that modulate tyrosine kinase signaling [5]. DOK1, DOK2 and DOK3 are preferentially expressed in hematopoietic cells, and DOK1 and DOK2 inhibit BCR-ABL-driven leukemogenesis in mice [8, 17]. DOK-knockout mice were recently used to show that DOK1, DOK2 and DOK3 contribute to tumor suppression in lung tumor and aggressive HS [1, 6]. We have also reported that DOK2 and DOK3 expression is significantly reduced in HTLV-I-infected cell lines in vitro [11]. In this study, we investigated the expression of the DOK1, DOK2 and DOK3 genes in Tax-Tg mice with leukemia or HS (HAM/TSP-like disease) and the relationship between the expression of the tax and DOK genes in vivo.

The generation of Tg mice expressing the tax gene under the control of the distal Lck promoter is described elsewhere [9, 10]. The Tax-Tg mice and the littermate control mice were maintained under specific pathogen-free conditions in laminar-flow benches at 22 ± 2°C with a 12 hr light/dark cycle. Food (CE-2; CLEA, Tokyo, Japan) and water were supplied ad libitum. Over a 2-year period, the Tax-Tg mice spontaneously developed T-cell leukemia with an incidence of approximately 20% and HS with an incidence of only 5%. T-cell leukemia and HS were evaluated based on the pathological findings and a flow-cytometric analysis of the mouse spleenocytes (Fig. 1b). Four of the mice with T-cell leukemia mice and three of mice with HS were used in this study. This study was carried out in strict accordance with the Guidelines for the Proper Conduct of Animal Experiments of the Science Council of Japan. All procedures involving animals and their care were approved by the Animal Care Committee of Kumamoto University in accordance with the regulations for animal experiments outlined by Kumamoto University. Single-cell suspensions were prepared from the spleens of healthy Tax-Tg mice, those with T cell leukemia/lymphoma or HS (HAM/TSP-like), and their non-Tg littermates, which were used as controls. The splenic cell populations

*Correspondence to: Ohsugi, T., Department of Laboratory Animal Science, School of Veterinary Medicine, Rakuno-Gakuen University, 582 Bunkyodai-Midorimachi, Ebetsu, Hokkaido 069-8501, Japan. e-mail: ohsugi@rakuno.ac.jp

©2017 The Japanese Society of Veterinary Science

This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial No Derivatives (by-nc-nd) License. (CC-BY-NC-ND 4.0: https://creativecommons.org/licenses/by-nc-nd/4.0/)
were examined with flow cytometry [9, 10, 12]. The spleen samples were treated with ammonium chloride to lyse the red blood cells. The cells were then stained with fluorescein isothiocyanate-conjugated anti-CD45 (30-F11; eBioscience, San Diego, CA, U.S.A.), anti-CD3 (145-2C11; eBioscience), anti-CD19 (MB19-1; eBioscience), anti-CD11b (M1/70; eBioscience), anti-Ly-6G (Gr-1; RB6-8C5; eBioscience), anti-Mac-2 (M3/8; BioLegend, San Diego, CA, U.S.A.), anti-CD68 (FA-11, BioLegend) or anti-F4/80 antibody (BM8, BioLegend). Flow cytometry was performed with a FACSCalibur (Becton Dickinson; Franklin Lakes, NJ, U.S.A.) or Guava easyCyte 6HT/2L flow cytometer (Merck Millipore, Billerica, MA, U.S.A.) with FlowJo software (Tree Star, Ashland, OR, U.S.A.). Total RNA was isolated from the spleens of the T cell leukemia/lymphoma mice, HS (HAM/TSP-like) mice, age-matched healthy Tax-Tg mice and non-Tg mice. Approximately 0.2 µg of total RNA was reverse transcribed with the ReverTra Ace qPCR RT Master Mix kit (Toyobo, Osaka, Japan). Real-time PCR was used to measure DOK1, DOK2, DOK3 and HTLV-1 tax expression on an ABI Prism 7700 Sequence Detection System (Applied Biosystems, Foster City, CA, U.S.A.) or a Roche LightCycler 480 System II (Roche Diagnostics, Indianapolis, IN, U.S.A.). The primers used to amplify the tax region of HTLV-1 are described elsewhere [13]. The murine DOK1, DOK2 and DOK3 primers used were: DOK1 sense, 5′-TGTCCCTGTATGCCTCTGGT-3′ and antisense, 5′-TGGTCATCCCTTGACTCCTC-3′; DOK2 sense, 5′-ACCATAACGACCCACAGAAC-3′ and antisense, 5′-GAAACTGTAGGGCCAGTCA-3′; and DOK3 sense, 5′-TCCACCTGAGGGACACATCTC-3′ and antisense, 5′-CAGCCTCAAAGAGAACACA-3′. The real-time PCR cycling parameters were: 1 min at 95°C and then 40 cycles of 15 sec at 95°C and 1 min at 60°C. The expression levels of the target genes were normalized to the expression of the murine gene encoding β-actin (ACTB). The primers for ACTB were: ACTB sense, 5′-TGTCCCTGTATGCCTCTGGT-3′ and antisense, 5′-TGTCCCTGTATGCCTCTGGT-3′ and antisense, 5′-TGGTCATCCCTTGACTCCTC-3′; DOK2 sense, 5′-ACCATAACGACCCACAGAAC-3′ and antisense, 5′-GAAACTGTAGGGCCAGTCA-3′; and DOK3 sense, 5′-TCCACCTGAGGGACACATCTC-3′ and antisense, 5′-CAGCCTCAAAGAGAACACA-3′. The expression levels of tax, DOK1, DOK2 and DOK3 were analyzed by real-time reverse transcription (RT)-PCR in the T cell leukemia/lymphoma mice, HS (HAM/TSP-like) mice, age-matched healthy Tax-Tg mice (the same litters) and non-Tg mice. The expression levels of tax in the spleens of the healthy and leukemic mice were not significantly different, whereas the expression of DOK1, DOK2 and DOK3 was significantly lower in the Tax-Tg mice at 5–6 months of age, and expression of DOK2 remained constantly low after this time. These results suggest that the expression of the DOK genes inhibited the onset of myelogenous leukemia or aggressive HS [6, 8, 17]. These reports suggest that the expression of the DOK genes inhibited the onset of hematopoietic and lymphoid tumors, including leukemia/lymphoma and HS, in mice. Therefore, the DOK genes might also be associated with the onset of T-cell leukemia and HS caused by Tax of HTLV-I.

The Tax-Tg mice showed that the expression of Tax significantly reduced DOK2 expression before the development of any disease, compared with that in the non-Tg mice (Fig. 1c). We investigated the changes in DOK gene expression in the healthy Tax-Tg at 5–13 months of age before disease onset (Fig. 2). DOK2 expression was already low in the Tax-Tg mice at 5–6 months of age, and expression of DOK2 remained constantly low after this time. These results suggest that downregulation of DOK2 expression is the first step in the development of disease characterized by low expression of DOK genes. By contrast, the expression of DOK1 and DOK3 was not significantly reduced at 5–13 months (Fig. 2). DOK1, DOK2 and DOK3 are preferentially expressed in immune cells. DOK1 mRNA is expressed in B and T cells, in myeloid cells, such as macrophages and neutrophils, and in CD4 CD8+ thymocytes and pro-B (B220 CD43 IgM) lymphocytes. Similarly, DOK2 mRNA is preferentially expressed in T cells and myeloid cells, with little or no expression in B cells. By contrast, DOK3 mRNA is preferentially expressed in myeloid cells and B cells, with little or no expression in T cells [5]. The Tax-Tg mice, in which tax was expressed from the distal Lck promoter, express Tax in mature thymocytes and peripheral T cells [9, 10], so Tax might first inhibit the expression of the DOK2 gene in CD3+ T cells. Furthermore, Lck is also expressed in a subset of B cells known as ‘B-1’ cells [2, 4, 14], so the expression of DOK1 and DOK3 might be slightly reduced in Tax-Tg mice.

It has been reported that the heterozygous loss of DOK2 can promote tumorigenesis. Moreover, DOK1, DOK2 and DOK3 have overlapping functions and can cooperate in tumor suppression [1, 6, 8, 17]. Further studies are required to evaluate whether DOK1 and DOK3 are key factors in the development of leukemia and HS in Tax-Tg mice or ATLL patients.

ACKNOWLEDGMENTS. This work was supported in part by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (No. 24500493) and the Japan Leukemia Research Fund. Part of the animal experiment in this study was performed at the Division of Microbiology and Genetics, Institute of Resource Development and Analysis, Kumamoto University, Japan.
Fig. 1. Reduced DOK gene expression in Tax-Tg mice with leukemia or HS. (a) Relative mRNA expression of tax in the splenocytes of non-Tg (Tax[−]) mice (n=5), healthy Tax-Tg (Tax[+]n=4) mice, Tax-Tg mice with T cell leukemia (leukemia) (n=4) and Tax-Tg mice with histiocytic sarcoma (HS) (n=3). The expression of tax in the spleens of the healthy Tax-Tg mice was arbitrarily set to 1.0. (b) Relative spleen cell populations in Tax (−) (n=5), Tax (+) (n=4), leukemia (n=4) and HS mice (n=3) were assessed with flow cytometry. (c) Relative mRNA expression of DOK1, DOK2 and DOK3 in the spleens of Tax (−) (n=5), Tax (+) (n=4), leukemia (n=4) and HS mice (n=3). The expression of each DOK gene in the spleens of Tax (−) mice was arbitrarily set to 1.0. Data are presented as the mean ± standard error of the mean. Statistical analysis was performed with Dunnett’s test. *P<0.05, **P<0.01 and ***P<0.001 vs non-Tg mice.

Fig. 2. DOK2 gene expression is reduced in Tax-Tg mice before disease onset. (a) DOK1 expression in non-Tg (Tax[−]) and healthy Tax-Tg (Tax[+]n=4) mice between 5 and 13 months of age (n=5 per group). (b) DOK2 expression in Tax (−) and Tax (+) mice between 5 and 13 months of age (n=5 per group). (c) DOK3 expression in Tax (−) and Ta (+) mice between 5 and 13 months of age (n=5 per group). Data are presented as the mean ± standard error of the mean. Statistical analysis was performed with Student’s t-test. *P<0.05 vs non-Tg mice.
REFERENCES

1. Berger, A. H., Niki, M., Morotti, A., Taylor, B. S., Socci, N. D., Viale, A., Brennan, C., Szoke, J., Motoi, N., Rothman, P. B., Teruya-Feldstein, J., Gerald, W. L., Ladanyi, M. and Pandolfi, P. P. 2010. Identification of DOK genes as lung tumor suppressors. Nat. Genet. 42: 216–223. [Medline] [CrossRef]

2. Dal Porto, J. M., Burke, K. and Cambier, J. C. 2004. Regulation of BCR signal transduction in B-1 cells requires the expression of the Src family kinase Lck. Immunity 21: 443–453. [Medline] [CrossRef]

3. Grassmann, R., Aboud, M. and Jeang, K. T. 2005. Molecular mechanisms of cellular transformation by HTLV-1 Tax. Oncogene 24: 5976–5985. [Medline] [CrossRef]

4. Majolini, M. B., D’Elios, M. M., Galieni, P., Boncristiano, M., Lauria, F., Del Prete, G., Telford, J. L. and Baldari, C. T. 1998. Expression of the T-cell-specific tyrosine kinase Lck in normal B-1 cells and in chronic lymphocytic leukemia B cells. Blood 91: 3390–3396. [Medline]

5. Mashima, R., Hishida, Y., Tezuka, T. and Yamanashi, Y. 2009. The roles of Dok family adapters in immunoreceptor signaling. Immunol. Rev. 232: 273–285. [Medline] [CrossRef]

6. Mashima, R., Honda, K., Yang, Y., Morita, Y., Inoue, A., Arimura, S., Nishina, H., Ema, H., Nakauchi, H., Seed, B., Oda, H. and Yamanashi, Y. 2010. Mice lacking Dok-1, Dok-2, and Dok-3 succumb to aggressive histiocytic sarcoma. Lab. Invest. 90: 1357–1364. [Medline] [CrossRef]

7. Matsuoka, M. and Jeang, K. T. 2007. Human T-cell leukaemia virus type 1 (HTLV-1) infectivity and cellular transformation. Nat. Rev. Cancer 7: 270–280. [Medline] [CrossRef]

8. Niki, M., Di Cristofano, A., Zhao, M., Honda, H., Hirai, H., Van Aelst, L., Cordon-Cardo, C. and Pandolfi, P. P. 2004. Role of Dok-1 and Dok-2 in leukemia suppression. J. Exp. Med. 200: 1689–1695. [Medline] [CrossRef]

9. Ohsugi, T. and Kumasaka, T. 2011. Low CD4/CD8 T-cell ratio associated with inflammatory arthropathy in human T-cell leukemia virus type I Tax transgenic mice. PLoS ONE 6: e18518. [Medline] [CrossRef]

10. Ohsugi, T., Kumasaka, T., Okada, S. and Urano, T. 2007. The Tax protein of HTLV-1 promotes oncogenesis in not only immature T cells but also mature T cells. Nat. Med. 13: 527–528. [Medline] [CrossRef]

11. Ohsugi, T., Wakamiya, M., Morikawa, S. and Fujita, M. 2016. Expression of DOK1, 2, and 3 genes in HTLV-1-infected T cells. Acta Virol. 60: 211–213. [Medline] [CrossRef]

12. Ohsugi, T., Wakamiya, M., Morikawa, S., Matsuura, K., Kumar, J. M., Kumasaka, T. and Yamaguchi, K. 2013. Invasion of histiocytic sarcoma into the spinal cord of HTLV-1 tax transgenic mice with HTLV-1-associated myelopathy/tropical spastic paraparesis-like disease. Oncol. Res. 20: 403–410. [Medline] [CrossRef]

13. Ohsugi, T., Kumasaka, T., Ishida, A., Ishida, T., Horie, R., Watanabe, T., Umezawa, K. and Yamaguchi, K. 2006. In vitro and in vivo antitumor activity of the NF-kappaB inhibitor DHMEQ in the human T-cell leukemia virus type I-infected cell line, HUT-102. Leuk. Res. 30: 90–97. [Medline] [CrossRef]

14. Ouli, C., Valensin, S., Majolini, M. B., Matthews, R. J. and Baldari, C. T. 2003. Normal B-1 cell development but defective BCR signaling in Lck-/- mice. Eur. J. Immunol. 33: 441–445. [Medline] [CrossRef]

15. Verdonck, K., González, E., Van Dooren, S., Vandamme, A. M., Vanham, G. and Gotuzzo, E. 2007. Human T-lymphotropic virus 1: recent knowledge about an ancient infection. Lancet Infect. Dis. 7: 266–281. [Medline] [CrossRef]

16. Watanabe, T. 1997. HTLV-1-associated diseases. Int. J. Hematol. 66: 257–278. [Medline] [CrossRef]

17. Yasuda, T., Shirakata, M., Iwama, A., Ishii, A., Ebihara, Y., Osawa, M., Honda, K., Shinohara, H., Sudo, K., Tsuji, K., Nakauchi, H., Iwakura, Y., Hirai, H., Oda, H., Yamamoto, T. and Yamanashi, Y. 2004. Role of Dok-1 and Dok-2 in myeloid homeostasis and suppression of leukemia. J. Exp. Med. 200: 1681–1687. [Medline] [CrossRef]