LETTER TO THE EDITOR

Expression of DOK1, 2, and 3 genes in HTLV-1-infected T cells

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Received May 28, 2015; accepted April 11, 2016

Summary. – Human T-cell leukemia virus type 1 (HTLV-1) can cause an aggressive malignancy known as adult T-cell leukemia/lymphoma (ATLL). The Tax protein encoded by the pX region of the HTLV-1 genome appears to be a key element in the early stage of ATLL development. In this study, we examined the expression of the downstream of tyrosine kinase (DOK) family members DOK1, DOK2 and DOK3, recently reported to be tumor suppressors, in HTLV-1-transformed T cells (MT-2 and HUT-102) and TL-Om1 cells derived from ATLL leukemic cells. DOK2 and DOK3 expression was significantly reduced in MT-2, HUT-102, and TL-Om1 cells compared with their expression in uninfected T cells, and the expression of DOK3 was reduced by the induction of Tax expression in T cells.

Keywords: adult T-cell leukemia/lymphoma; downstream of tyrosine kinase 1; DOK1; DOK2; DOK3; human T-cell leukemia virus type; tax

The downstream of tyrosine kinase (DOK) family of proteins has seven members, DOK1 to DOK7, which are adaptor proteins that modulate tyrosine kinase signaling (1). DOK1, DOK2, and DOK3 are preferentially expressed in hematopoietic cells. DOK1 and DOK2 have been shown to inhibit BCR-ABL-driven leukemogenesis in mice (2, 3), and Dok-knockout mice have recently been used to show that DOK1, DOK2, and DOK3 contribute to tumor suppression in lung tumor and aggressive histiocytic sarcoma (4, 5).

Human T-cell leukemia virus type 1 (HTLV-1) was the first human retrovirus to be isolated. Some individuals infected with HTLV-1 develop adult T-cell leukemia/lymphoma (ATLL), an aggressive T-cell malignancy, after a long latency period of 40–60 years. HTLV-1 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 is also reported to interfere with the functions of several tumor suppressor proteins (6). In this study, we investigated the expression of the newly identified tumor suppressor genes DOK1, DOK2, and DOK3 in HTLV-1-infected cell lines and the relationship between the expression of the tax and DOK genes.

Three HTLV-1-infected T-cell lines (TL-Om1, MT-2, and HUT-102) and two HTLV-1-negative T-cell lines, Jurkat and JRX-9, were maintained in culture with RPMI1640 medium supplemented with 10% heat-inactivated fetal bovine serum at 37°C under 5% CO2. These cell lines were kindly provided by Drs T. Watanabe, M. Yamagishi, and K. Nakano of the Graduate School of Frontier Sciences, The University of Tokyo (Tokyo, Japan). Total RNA was isolated from the cells and approximately 0.2 μg of total RNA was used for reverse transcription using M-MLV-RT (Promega, Tokyo, Japan). The resulting cDNAs were used as templates for the polymerase chain reaction (PCR) and real-time PCR. The primers used for PCR and real-time PCR are shown in Table 1.
Reduced DOK gene expression in HTLV-1-infected cell lines and transient expression of tax inhibits DOK3 expression

(a) The relative mRNA expression of tax (upper, left), DOK1 (upper, right), DOK2 (lower, left), and DOK3 (lower, right) in HTLV-1-infected cell lines TL-0m1, MT-2, and HUT-102, and in an uninfected human T-cell line, Jurkat, was assessed with real-time RT-PCR. (b) JPX-9 cells were incubated with various doses of CdCl₂ for 48 hr. The relative mRNA expression of tax (upper, left), DOK1 (upper, right), DOK2 (lower, left), and DOK3 (lower, right) was assessed with real-time RT-PCR. Data represent the means ± standard errors of the means (SEM) of three separate experiments performed in duplicate.

*P < 0.05, **P < 0.01.
RNA was reverse transcribed with the ReverTra Ace (qPCR RT Master Mix kit (Toyobo Co., Ltd, Osaka, Japan). To measure DOK1, DOK2, DOK3, and HTLV-1 tax expression, real-time PCR was performed with the THUNDERBIRD SYBR qPCR Mix (Toyobo) on a Roche LightCycler 480 System II (Roche Diagnostics K.K., Tokyo, Japan). The primers for the tax region of HTLV-1 have been described previously (7). The DOK1, DOK2, and DOK3 primers were: DOK1 sense: 5′-AGCACGTTCGCTAAGGGCCAAG-3′, antisense: 5′-CCGGAGGCAGATCATAAGG-3′; DOK2 sense: 5′-CAAGGTGACAGGAGGCAGCA-3′, and antisense: 5′-TATCGCAGGCAACGAAGACA-3′; DOK3 sense: 5′-GACGACATTTGACCATGCTGGA-3′, and antisense: 5′-TTGCCTATGCTTGGAGATGG-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 human gene encoding β-actin (ACTB). The primers for ACTB were: ACTB sense: 5′-TGGCCGAGGACTTTGATTG-3′, and antisense: 5′-GTGGGTTGGCTTTTAGATG-3′.

The expression levels of tax, DOK1, DOK2, and DOK3 were analyzed with real-time RT–PCR in the HTLV-1-infected T-cell lines TL-Om1, MT-2, and HUT-102, and in the HTLV-1-negative T-cell line, Jurkat. The HTLV-1-transformed T-cell lines MT-2 and HUT-102 constitutively expressed tax mRNA, but the ATLL-derived T-cell line TL-Om1 did not (Fig. 1a, upper left panel). The relative expression of DOK2 and DOK3 in the HTLV-1-infected T-cell lines tested was significantly lower than in the HTLV-uninfected T-cell line (Fig. 1a, lower panel). The relative expression of DOK1 in MT-2 and HUT-102 cells tended to be lower than in Jurkat cells, but the difference was not significant (Fig. 1a, upper right panel). The Tax protein encoded by the pX region of the HTLV-1 genome activates the expression of many cellular genes, including those for cytokines, cytokine receptors and tumor suppressor genes (6). Next, we investigated whether Tax inhibits the expression of DOK1, DOK2, or DOK3 in T cells. Jurkat cells, a mature human leukemic cell line, phenotypically resembles resting human T lymphocytes and has been widely used to study T-cell physiology. JPX-9 cells are derived from Jurkat cells (8), but carry the viral tax gene under the control of the metallothionein gene promoter, and the addition of CdCl2 (≥ 30 μmol/l) to the medium rapidly induced tax expression (Fig. 1b, upper left panel). The expression of tax resulted in a significant reduction in DOK3 expression compared with that in non-tax-expressing cells (Fig. 1b, lower right panel). DOK1 and DOK2 expression was unaffected by tax expression (Fig. 1b).

In conclusion, these data demonstrate for the first time that the expression of DOK2 and DOK3 mRNAs is reduced in HTLV-1-infected cell lines and that tax expression inhibits DOK3 mRNA expression in T cells. These results raise the possibility that some DOK family proteins are active tumor suppressors in the development of ATLL. Tax-induced decline in DOK3 function, which precedes DOK2 dysfunction by unknown factor(s), might be the first stage in the onset of ATLL. Further studies are required of ATLL patients in various stages of the disease to confirm these important findings and to clarify the role of Tax in inhibiting the DOK family proteins in more detail.

Acknowledgements. 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.

References