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LETTER TO THE EDITOR

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

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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 JPX-9, were maintained in culture with RPMI1640 medium supplemented with 10% heat-inactivated fetal bovine serum at 37°C under 5% CO_2. 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

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Abbreviations: ATLL = adult T-cell leukemia/lymphoma; DOK = downstream of tyrosine kinase; HTLV-1 = human T-cell leukemia virus type 1

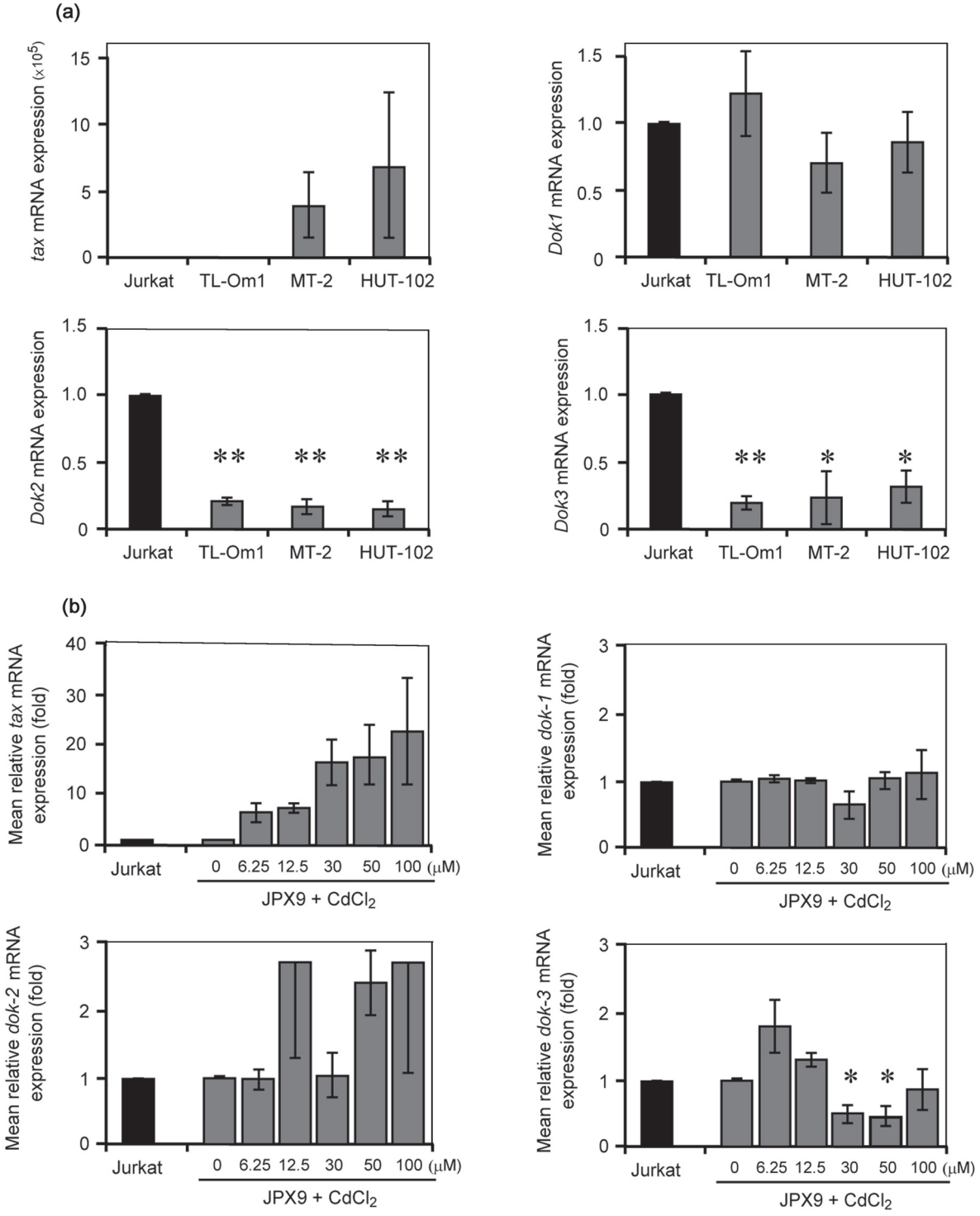

Fig. 1

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-Om1, 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 $CdCl_2$ 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'-AGCAGTTGCTGAAGGCCAAG-3', antisense: 5'-CCCGAGGCAGATCATAAAGG-3'; *DOK2* sense: 5'-CAAAGTGACAGAGGCAGCAGA-3', and antisense: 5'-TATCAGCCCCAACGAAGACA-3'; *DOK3* sense: 5'-GACGACATTGATGCTTGTGGA-3', and antisense: 5'-TTGGCTATGCTTGGGATTGG-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'-GTGGGGTGGCTTTTAGGATG-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 $CdCl_2$ (≥ 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.

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