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Induction of OX40, a Receptor of gp34, on T Cells by Trans-acting Transcriptional Activator, Tax, of Human T-Cell Leukemia Virus Type I

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gp34, which we had identified as a target molecule of the trans-activation by Tax of human T-cell leukemia virus type I (HTLV-I), has been found to bind OX40, a member of the tumor necrosis factor receptor family, resulting in growth stimulation of activated T cells. We here demonstrate that not only gp34 (OX40L), but also OX40 can be transcriptionally activated by Tax. Three Tax-producing human T-cell lines carrying the HTLV-I genome expressed OX40 on their surfaces. Furthermore, Tax-induced transcriptional activation of OX40 was shown in Tax-inducible JPX-9 cells. These results demonstrate that both OX40 and its ligand (gp34) are constitutively expressed on the surfaces of Tax-expressing T lymphocytes, suggesting that the OX40L/OX40 system contributes to growth stimulation of the virus-infected T cells.

Key words: HTLV-I — Tax — OX40 — OX40 ligand

Human T-cell leukemia virus type I (HTLV-I) was the first identified human retrovirus, and is etiologically related to a human T-cell malignancy, adult T-cell leukemia (ATL), and a demyelinating disease, tropical spastic paraparesis/HTLV-I-associated myelopathy.[1] Although the precise mechanisms of occurrence of these diseases after HTLV-I infection are not well understood yet, immortalization of normal T cells in co-culture with HTLV-I producing cells can be observed as one of the oncogenic characteristics of HTLV-I. HTLV-I has no typical oncogene, unlike other animal retroviruses, but it has a unique gene designated as pX, of which the product, named Tax, initially found to be a trans-acting transcriptional activator for its own viral promoter,[2–8] has been shown to activate various cellular genes, such as c-*fos*,[9,10] interleukin 2 (IL-2),[11,12] the α and γ subunits of the IL-2 receptor (IL-2R)[13,14] and so on. Among them, IL-2 and the IL-2R subunits are known to play a pivotal role in T cell growth, suggesting that their constitutive expression induced by Tax may contribute to immortalization of HTLV-I-infected T cells. Furthermore, transfection of the pX gene induced transformation of mouse and rat fibroblastoid cells[15] and immortalization of human T cells,[16] and transgenic mice expressing Tax developed mesenchymal tumors[17] and leukemia.[18] All these observations support the possibility that Tax is involved in immortalization and malignant transformation of T cells, resulting in ATL.

gp34 was initially identified by the use of a monoclonal antibody (mAb) specifically reacting with HTLV-I-infected cells.[19] Molecular cloning studies revealed that gp34 is a cellular gene product belonging to the tumor necrosis factor (TNF) family with the characteristics of type 2 transmembrane molecules.[20] The gp34 gene has been shown to be trans-activated by Tax.[20] On the other hand, OX40, expressed on activated T cells, was initially identified as a member of the TNF receptor family.[21,22] The ligand for OX40 was demonstrated to be gp34 (OX40L).[23,24] Proliferation of T cells expressing OX40 was stimulated in the presence of gp34 expressed on COS-7 or CV-1/EBNA transfectant cells, suggesting that ligand binding to OX40 induces signals for growth stimulation in activated T cells.[23,24] In the present study, we demonstrate that not only gp34 (OX40L), but also OX40 is a target for trans-activation by HTLV-I Tax.

We first examined the expression of OX40 and gp34 by immunofluorescence flow cytometry on the surfaces of six human T cell lines carrying HTLV-I, which included three Tax-positive cell lines (MT-2, HUT102 and TL-Su) and three Tax-negative cell lines (TL-Hir, TL-Oml and MT-1). Expression of Tax on these cell lines, which were the same cultures as used for examination of expression of OX40 and gp34, was confirmed by fluorescence microscopy of cells smeared on a slide glass and stained with rabbit anti-Tax serum (300-fold dilution)[10] and fluorescein-labeled goat anti-rabbit IgG (100-fold dilution, Zymed, San Francisco, CA). Logarithmically growing cells were incubated with a mixture of human serum

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Table I. Expression of OX40, gp34 and Tax on HTLV-I-infected Cell Lines[a)]

Cell lines	HTLV-I genome	Expression		
		OX40	gp34	Tax
MT-2	+	+	+	+++
HUT102	+	+	+	++
TL-Su	+	+	+	+
TL-Hir	+	−	−	−
TL-OmI	+	−	−	−
MT-1	+	−	−	−

a) Cells were analyzed by immunofluorescence flow cytometry with Ber-ACT35 mAb specific for human OX40[22)] and TAG34 mAb specific for human gp34[19)] and also by fluorescence microscopy with a rabbit antiserum specific for Tax as described previously.[20)]

and Par3 (IgG_1), a mouse monoclonal antibody specific for parvovirus B19,[25)] for 30 min on ice to block non-specific binding and then incubated with biotinylated-TAG34 or Ber-ACT35 for 30 min, and further incubated with streptavidin-conjugated PE (Becton Dickinson, San Jose, CA) or fluorescein isothiocyanate-conjugated anti-mouse IgG for 30 min, respectively. The cells were analyzed with a flow cytometer (FACScan, Becton Dickinson Immunocytometry Systems, Mountain View, CA). The three Tax-positive cell lines (MT-2, HUT102 and TL-Su) expressed OX40 as well as gp34 (Table I).[19)] On the other hand, the three Tax-negative cell lines showed little or no expression of OX40 and gp34 (Table I). These results suggest that not only gp34, but also its receptor, OX40, is a target for the trans-acting transcriptional activator, Tax.

To demonstrate directly the Tax-induced expression of OX40, we used JPX-9 cells, in which Tax is expressed from Tax gene driven by a metallothionein promoter upon treatment with $CdCl_2$.[10, 26)] JPX-9 cells were cultured in the presence of 10 μM $CdCl_2$, and checked for expression of OX40, as well as gp34 and IL-2Rα, by flow cytometry (Fig. 1). Expression of OX40 was induced within 2 days after addition of $CdCl_2$ and then reached a plateau level within 4 days after the treatment. Induction of IL-2Rα expression was also detected with similar kinetics to that of OX40 expression. On the other hand, expression of gp34 was delayed, being undetectable until day 4, but becoming strong by day 6 after addition of $CdCl_2$. Expression of Tax was also detected by intracellular immunofluorescence staining on day 4 (data not shown).

We further examined the kinetics of Tax-induced expression of the OX40 mRNA transcript by Northern blot analyses with JPX-9 cells (Fig. 2). As shown previously,[10, 20)] Tax mRNA was induced within 1 day after treatment with $CdCl_2$ and gradually increased until day 6. Under this condition, induction of OX40 mRNA was detected within 2 days, and reached a plateau level within 4 days after the treatment, which is in agreement with the kinetics of induction of OX40 antigen on the cell surface. gp34 mRNA also became detectable within 4 days after $CdCl_2$ treatment, which again correlated with induction of the gp34 antigen on the cell surface. These results suggest that expression of Tax leads to induction of OX40, in addition to gp34.

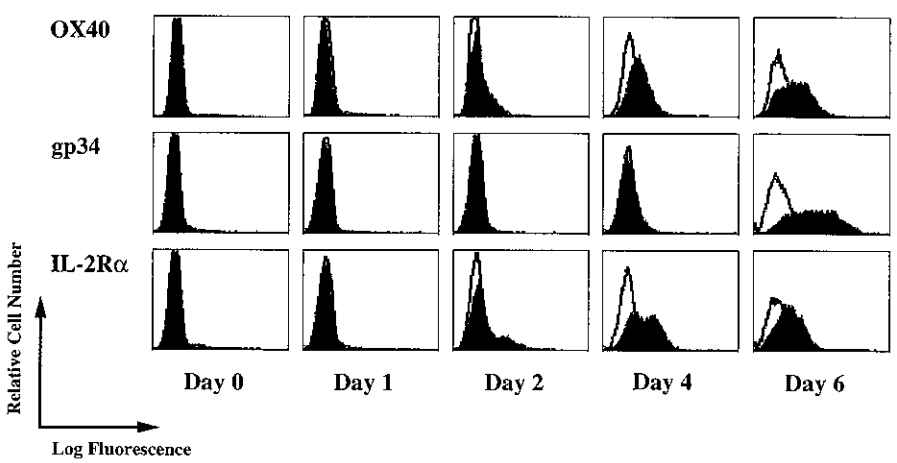

Fig. 1. Effects of Tax on expression of OX40, gp34 and IL-2Rα in JPX-9 cells. JPX-9 cells were cultured in the presence of 10 μM $CdCl_2$ and harvested on the indicated days. Cells were separately stained with Ber-ACT35 mAb specific for human OX40,[22)] TAG34 mAb specific for human gp34,[19)] and H-31 mAb specific for the human IL-2R α chain (IL-2Rα),[27)] and subjected to FACScan.

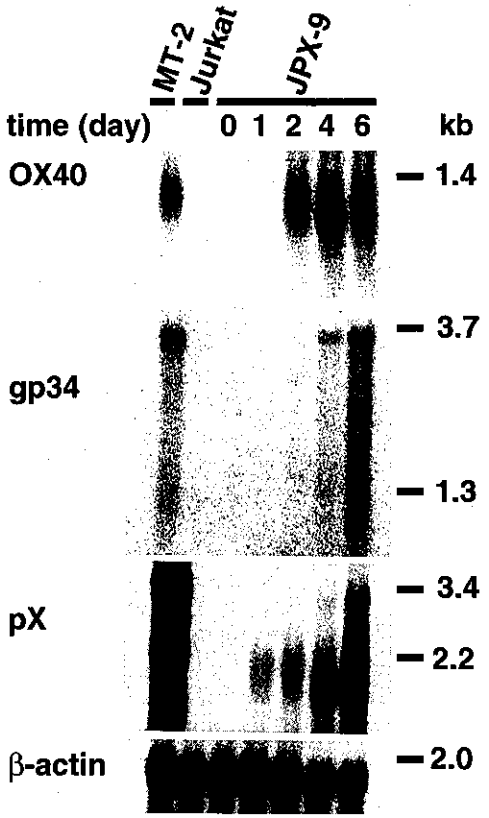

Fig. 2. Northern blot analysis of OX40 and gp34 transcripts in JPX-9 cells after induction of Tax. JPX-9 cells were cultured in the presence of 10 μM $CdCl_2$ and harvested on the indicated days. Total cellular RNA was isolated from cells by extraction using the guanidine thiocyanate-cesium chloride method. Total RNA (20 μg) was applied to a 1% denaturing agarose gel, electrophoresed and transferred to nitrocellulose filters (Hybond N, Amersham, Buckinghamshire, England). Subsequently, blotted nitrocellulose membranes were hybridized overnight with probes for the OX40, gp34, Tax (pX) and β-actin genes at 42°C in 50% formamide-containing solution, and washed in 2× SSC (300 mM sodium chloride and 30 mM sodium citrate (pH 7.0)) containing 0.1% sodium dodecyl sulfate (SDS) twice and 0.2× SSC containing 0.1% SDS twice. Membranes were autoradiographed with an imaging plate and targeted mRNAs were visualized with a bioimage analyzer (BAS2000: Fuji Film, Tokyo). The OX40 probe was a 1.2-kb pair *Xho*I fragment from pKU2-OX40-11.[22] The probes for gp34 and Tax (pX) were described previously.[20] The β-actin probe was purchased from Wako Chemical (Osaka). These probes were pre-labeled with a Quick-prime Labeling Kit (Pharmacia P-L Biochemical Inc., Milwaukee, WI).

IL-2Rα expression has been considered to be mediated by NF-κB, which is directly activated by Tax in HTLV-I-infected cells.[28,29] Although the kinetics of OX40 induction by Tax is very similar to that of IL-2Rα induction, it is not known whether the Tax-mediated induction of OX40 is mediated by the same mechanism as the induction of IL-2Rα. Since the OX40 induction was delayed for at least 1 day after the Tax induction, one may suppose that Tax contributes to the production of some humoral factor(s) that is capable of inducing OX40 expression. To examine this possibility, the supernatant of JPX-9 cells treated with $CdCl_2$ for 4 days was tested for activity to induce OX40. Little expression of OX40 was detected on the surface of Jurkat cells treated with the supernatant, indicating that no humoral factor(s) participates in the Tax-mediated OX40 induction (data not shown). It is also possible that Tax induces *de novo* protein synthesis, which is involved in OX40 induction. Therefore, further study of the OX40 promoter region is required for clarification of the mechanism of the Tax-mediated OX40 induction.

The time course of gp34 induction was delayed for 2 days as compared with our previous study.[20] The level of Tax induction seems critical for gp34 induction, because Tax induction in JPX-9 cells reached the maximum level within a day after treatment with $CdCl_2$ in our previous study,[20] whereas it gradually increased until day 6 in the present study. The kinetics of gp34 induction is significantly different from that of OX40 induction, suggesting that the Tax-mediated inducing mechanisms are not the same for OX40 and gp34.

In the OX40/gp34 system, OX40 interacting with gp34 is known to induce intracellular signals for [^{3}H]thymidine uptake of T cells pre-activated with phytohemagglutinin, phorbol 12-myristate 13-acetate and calcium ionophore or anti-CD3 antibody, and for secretion of IL-2 and IL-4 from $CD4^+$ T cells stimulated with anti-T cell receptor $\alpha\beta$ antibody, suggesting that OX40 which is expressed on activated T cells participates in cell growth promotion.[22,23] On the other hand, gp34 has been shown to be expressed on B cells activated with both anti-IgD antibody and CD40 ligand, and stimulation of the activated B cells with OX40-immunoglobulin chimeric protein increased their [^{3}H]thymidine uptake.[30] These results suggest that not only OX40, but also gp34 has the ability to transduce cell growth signals via their interactions. Since OX40 and gp34 are induced in HTLV-I-infected human T cells expressing Tax, they may interact with each other. Although we have not obtained yet any direct evidence for involvement of OX40 and gp34 in growth promotion of HTLV-I-infected T cells, the OX40/gp34 system may play an important role in the process of immortalization and malignant transformation of HTLV-I-infected T cells.

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