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Foxp3-dependent transformation of human primary CD4+ T lymphocytes by the retroviral protein tax

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ABSTRACT

The retroviral Tax proteins of human T cell leukemia virus type 1 and 2 (HTLV-1 and -2) are highly homologous viral transactivators. Both viral proteins can immortalize human primary CD4+ memory T cells, but when expressed alone they rarely transform T cells. In the present study, we found that the Tax proteins displayed a differential ability to immortalize human CD4+Foxp3+ T cells with characteristic expression of CTLA-4 and GITR. Because epidermal growth factor receptor (EGFR) was reportedly expressed and activated in a subset of CD4+Foxp3+ T cells, we introduced an activated EGFR into Tax-immortalized CD4+Foxp3+ T cells. We observed that these modified cells were grown independently of exogenous IL-2, correlating with a T cell transformation phenotype. In Tax-immortalized CD4+Foxp3- T cells, ectopic expression of Foxp3 was a prerequisite for Tax transformation of T cells. Accordingly, treatment of the transformed T cells with erlotinib, a selective inhibitor of EGFR, induced degradation of EGFR in lysosome, consequently causing T cell growth inhibition. Further, we identified autophagy as a crucial cellular survival pathway for the transformed T cells. Silencing key autophagy molecules including Beclin1, Atg5 and PI3 kinase class III (PI3KC3) resulted in drastic impairment of T cell growth. Our data, therefore, unveiled a previously unidentified role of Foxp3 in T cell transformation, providing a molecular basis for HTLV-1 transformation of CD4+Foxp3+ T cells.

1. Introduction

Human T cell leukemia virus type 1 and type 2 (HTLV-1 and HTLV-2 respectively) are two related human retroviruses of bloodborne pathogens. HTLV-1 is the etiological factor that causes adult T cell leukemia and lymphoma (ATL) in 20 million of HTLV-1-infected patients [1,2]. Although HTLV-2 is detected in CD8 T cells from a patient with hairy cell leukemia, a rare type of leukemia that affects B cells [3], a causative link between HTLV-2 infection and hairy cell leukemia has not been established. HTLV-1 viral genome is known to encode two oncogenic products, Tax-1 and HBZ, an antisense gene product that is constitutively transcribed in ATL cells [4,5]. Tax-1 is the first recognized viral component that displays oncogenic ability in human primary CD4+ T lymphocytes, humanized mouse model and transgenic mice [4,6]. Increasing evidence also supports the important role of HBZ during leukemogenesis of HTLV-1 [7,8].

The underlying mechanism of HTLV-1-mediated T cell transformation remains incompletely understood. Neither Tax nor HBZ is able to transform human primary lymphocytes. It is well recognized that expression of Tax is essential for initiation of HTLV-1-associated oncogenesis [4,6]. This concept is based on several experimental findings. The molecular clone of HTLV-1 with the disrupted *tax* gene has no capacity to transform human T cells [9,10]. In addition, Tax, not HBZ, induces immortalization of human CD4+ memory T cells, a crucial step leading to T cell malignancy [11]. Further, the ability of Tax in imitating T cell activation signaling, in promoting cell cycle progression and in causing genomic damage correlates with its role in oncogenic initiation [4,12,13]. Although it is not clear if HBZ is required during the early stage of oncogenesis, this viral protein was found constitutively

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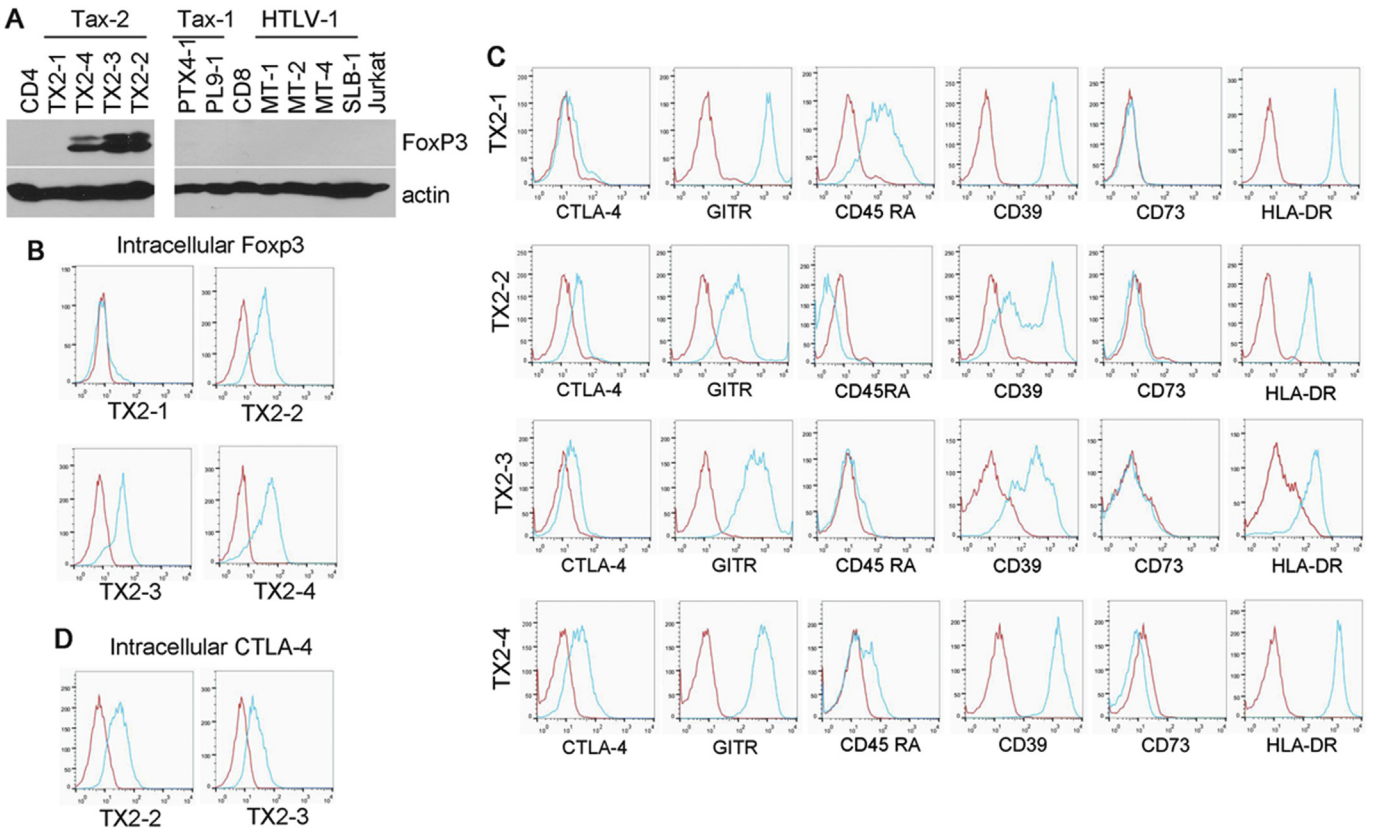

Fig. 1. The retroviral Tax proteins differentially immortalize human CD4+Foxp3+ T cells. (A) The expression status of Foxp3 in Tax-immortalized T cell lines and HTLV-1-transformed T cell lines as determined by anti-FoxP3 immunoblot. (B) Intracellular staining using APC-conjugated anti-FoxP3 antibody. (C) Immunophenotypes of Tax-2-immortalized T cell lines, TX2-1, TX2-2, TX2-3 and TX2-4, as measured by FACS. (D) Intracellular expression of CTLA-4 in TX2-2 and TX2-3 cell lines as detected by the intracellular staining method with APC-conjugated anti-CTLA-4 antibody.

expressed at the leukemia stage, and silencing HBZ led to growth inhibition of the leukemia cells [7,14]. A consensus view for HTLV-1-mediated T cell transformation is that Tax-1 by itself is inadequate to fully transform mature CD4+ T cells and it requires cooperation with HBZ to overcome Tax-1-induced cell senescence, thereby promoting oncogenesis [15].

HTLV-1-transformed T cells demonstrate a CD4+Foxp3+ immunophenotype [16]. HBZ, not Tax, is able to upregulate Foxp3 expression [17]. Foxp3 is a master regulator of immunity in lymphoid tissues and a specific marker of regulatory T cells (Treg) [18–20]. The Treg cells function as a potent immune suppressor that restrains the activity of self-reactive cytotoxic T cells and inhibits proliferation of effector T cells. Loss of Treg cells is associated with autoimmune disease and conversely, hyper-activation of Treg cells may facilitate tumor growth and metastasis, probably by suppressing anti-tumor immunity [21,22]. The Foxp3+ tumors imitate Treg's immune suppressive function, which is associated with poor prognosis in certain types of cancer [23,24]. In HTLV-1-associated ATL, the immunodeficiency phenotype manifests at certain stage of leukemia development [25]. However, in HTLV-1-associated neurological diseases, the presence of hyper reactive T cells to viral antigens suggested that HTLV-1-infected Foxp3 cells could be converted into Th1-like cells [26]. Yet, it is still not clear about the role of Foxp3 in HTLV-1-mediated transformation of T cells. In the present study, we report our new finding that Foxp3 is a prerequisite for Tax transformation of human differentiated CD4+ T cells. In addition, we have found that autophagy molecules play a key role in promoting survival and proliferation of Tax-transformed T cells.

2. Materials and methods

2.1. Cell cultures and antibodies

TX2-1, TX2-2, TX2-3, TX2-4, PTX4-1 and PL9-1 cell lines were described previously [27,28], and were cultured in RPMI1640 medium containing 10% fetal bovine serum and 100 units/ml of recombinant IL-2. MT-2, MT-4 and Jurkat cells were obtained from AIDS research and reference reagent program (NIAID, National Institutes of Health). SLB-1 and MT-1 cell lines were described previously (28). Primary human CD4+ and CD8+ T cells were isolated from healthy blood donors and were enriched using anti-CD4 and anti-CD8 magnetic beads (Invitrogen).

Antibodies for Foxp3, EGFR and GFP were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-phospho-Tyr(PY99) antibody was from BD Transduction Lab (San Diego, CA). DMSO, MG-132, chloroquine and erlotinib were purchased from Sigma (St Louis, MO).

2.2. Lentivirus vectors and lentivirus production

Lentivirus constructs for Tax-GFP and the CD3-EGFR chimera were described previously [27,35]. Lentivirus vectors for Beclin1- and Atg5-specific shRNAs were purchased from Open Biosystems (Pittsburgh, PA). Lentivirus vector for PI3KC3-specific shRNA was purchased from Thermo Scientific (Grand Island, NY, USA). The Foxp3 cDNA was PCR-cloned, constructed in the lentivirus vector and sequence-verified. The procedure for lentiviral production and concentration was described previously [27].