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Original article

Interaction of HTLV-1 Tax protein with calreticulin: Implications for Tax nuclear export and secretion

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Abstract

Human T cell leukemia virus type 1 (HTLV-1) is the etiologic agent of adult T cell leukemia (ATL) and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). The HTLV-1 transcriptional transactivator protein Tax plays an integral role in virus replication and disease progression. Traditionally, Tax is described as a nuclear protein where it performs its primary role as a transcriptional transactivator. However, recent studies have clearly shown that Tax can also be localized to the cytoplasm where it has been shown to interact with a number of host transcription factors most notably NF-κB, constitutive expression of which is directly related to the T cell transforming properties of Tax in ATL patients. The presence of a functional nuclear export signal (NES) within Tax and the secretion of full-length Tax have also been demonstrated previously. Additionally, release of Tax from HTLV-1-infected cells and the presence of cell-free Tax was demonstrated in the CSF of HAM/TSP patients suggesting that the progression to HAM/TSP might be mediated by the ability of Tax to function as an extracellular cytokine. Therefore, in both ATL and HAM/TSP Tax nuclear export and nucleocytoplasmic shuttling may play a critical role, the mechanism of which remains unknown. In this study, we have demonstrated that the calcium binding protein calreticulin interacts with Tax by co-immunoprecipitation. This interaction was found to localize to a region at or near the nuclear membrane. In addition, differential expression of calreticulin was demonstrated in various cell types that correlated with their ability to retain cytoplasmic Tax, particularly in astrocytes. Finally, a comparison of a number of HTLV-1-infected T cell lines to non-infected T cells revealed higher expression of calreticulin in infected cells implicating a direct role for this protein in HTLV-1 infection.

Keywords: HTLV-1 Tax; Nucleocytoplasmic shuttling; Calreticulin

1. Introduction

HTLV-1 is the etiologic agent of two major diseases; a progressive lymphoma designated adult T cell leukemia (ATL) and a debilitating neurological disease known as HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). The HTLV-1 oncoprotein Tax has been studied extensively with respect to its role in regulating cellular and viral gene expression during the course of HTLV-1 infection [1–3]. Specifically, within the context of the viral life cycle, Tax interacts with host transcriptional machinery, especially the ATF/CREB pathway, to facilitate the binding of selected cellular transcription factors to the viral long terminal repeat (LTR) within the nucleus. In addition to the ATF/CREB pathway, Tax also interacts with the NF-κB signaling pathway in the cytoplasm to induce NF-κB translocation to the nucleus by binding to IKKγ, a subunit of the IKK complex [4–7]. The ability of Tax to induce

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the NF-κB pathway has been intensely studied because of its possible role in cellular transformation and the onset of ATL in HTLV-1-infected individuals [4–6,8]. It is unknown whether Tax that interacts with NF-κB in the cytoplasm represents newly synthesized protein resident in the cytoplasm, or whether it represents protein shuttled from the nucleus back to the cytoplasm. If it is the latter, or a combination of the two, the regulation of Tax nucleocytoplasmic shuttling is important since the differential availability of Tax to the NF-κB as compared to ATF/CREB pathway could be expected to have different effects on cellular metabolism and progression of HTLV-1-associated pathogenesis. In addition to its intracellular functions, Tax is also known to cause a variety of effects on cells as an extracellular protein particularly in the context of HAM/TSP. The presence of antibodies against Tax is demonstrated in a majority of HTLV-1-infected individuals [9,10]. Recently, cell-free Tax has been detected in the CSF of HAM/TSP patients [11]. These observations, along with a number of studies including our own, demonstrating the effects of extracellular Tax on both lymphoid and non-lymphoid cells, suggest that Tax is available for immune recognition and can function as an extracellular cytokine [12–21]. However, it is not yet certain whether the cell-free Tax was the result of apoptosis or necrosis of HTLV-1-infected cells or if it was secreted from the infected cell populations. Consistent with the concept of cellular secretion, the presence of a leucine-rich NES between amino acids 188–200 of Tax has been reported [22]. The secretion of Tax has also been reported from HTLV-1-infected and Tax-transfected cells [16,23]. At any given time, an HTLV-1-infected cell may contain a rate-limiting amount of Tax. Thus, an alteration in the balance of Tax nucleocytoplasmic shuttling may have functional impact on the Tax-induced signaling pathways in both the nucleus and cytoplasm. Furthermore, we hypothesize that Tax may localize to the cytoplasm as an intermediary step during the course of its release from HTLV-1-infected cells, as demonstrated by the presence of Tax in cytoplasmic secretory-like vesicles [23]. Therefore, understanding the mechanism(s) of Tax nuclear export and nucleocytoplasmic shuttling will be critical in clarifying the overall processes associated with HTLV-1-associated immune and central nervous system disease.

Traditionally, nuclear export of proteins containing a leucine-rich NES has been shown to be mediated by CRM-1 nuclear export receptor via the nuclear pore complex [24–26]. Previous observations have indicated that the Tax NES (tNES) was shown to function in a CRM-1-dependent manner when fused as an isolated element directly to green fluorescent protein (GFP), the same was not true when the tNES was within the context of full-length Tax [22]. The nucleocytoplasmic localization of full-length Tax-GFP was not altered following treatment of transfected cells with leptomycin B (LMB), an inhibitor of CRM-1 nuclear export. Similarly, studies investigating the nuclear export of a leucine-rich NES containing protein, protein kinase inhibitor (PKI), demonstrated that in a system with CRM-1-depleted cytosol, nuclear export of PKI was retained [27]. These studies have strongly suggested that there are additional export receptors present in mammalian cells that can mediate a CRM-1-independent mechanism for nuclear export. Recently the calcium-binding protein implicated in protein folding in the ER lumen, calreticulin, was demonstrated to act as a nuclear export protein [25,27,28]. Calreticulin was demonstrated to facilitate the nuclear export of PKI by a pathway independent of CRM-1 [25]. Several investigators have reported the presence of calreticulin in the nucleus and the cytoplasm [29,30]. Both CRM-1 and calreticulin bind to transport substrates that contain an NES, and require export receptor-substrate-RanGTP complex for export [25]. It has been shown that like HTLV-1 Tax, nuclear export of PKI was not inhibited by LMB. The results presented herein begin to examine the role of calreticulin in Tax nuclear export. Specifically, this investigation demonstrated that calreticulin and Tax interact and co-localize at or near the nuclear membrane in baby hamster kidney (BHK-21) cells, indicating nuclear transport of Tax. Additionally, four HTLV-1-infected T lymphocyte cell lines were demonstrated to contain high levels of calreticulin expression as compared to non-infected Jurkat T lymphocytes. The difference between cells with high levels of cytoplasmic Tax versus those with low levels may be explained by the expression level or relative availability of calreticulin. Therefore, it has been hypothesized that the balance of Tax import and calreticulin-dependent export from the nucleus provides the cell with a mechanism that may play an important role in regulating Tax biological activity and HTLV-1 induced disease progression.

2. Materials and methods

2.1. Cell culture

293T and BHK-21 cells were grown in Dulbecco's modified Eagle's media. U-87 MG and HeLa cells were grown in Eagle's minimum essential medium. U-937, Jurkat, and HTLV-1-infected T cell lines MT-2, C8166, HuT 102, and SLB-1 were grown in RPMI 1640. Growth media for each cell line was supplemented with 10% fetal bovine serum, antibiotics (penicillin, streptomycin, and kanamycin at 0.04 mg/ml each), L-glutamine (0.3 mg/ml), and sodium bicarbonate (0.05%). Cell lines were maintained at 37 °C in 5% CO_2 at 90% relative humidity.

2.2. Fusion protein construction and plasmid DNA purification

Double-stranded oligonucleotides encoding a six-histidine tag was inserted into the carboxy-terminus of the Tax coding sequence using the QuickChange site-directed mutagenesis kit as described by the manufacturer (Stratagene, La Jolla, CA). The cDNA coding sequence of calreticulin (kindly provided by Dr. Bryce Paschal, University of Virginia School of Medicine) was cloned into the 3xFLAG vector (Sigma, St. Louis, MO) using PCR and calreticulin-specific primers (FLAG-CRT). HTLV-1 Tax constructs for fluorescence microscopy were constructed by cloning full-length Tax cDNA coding sequence into a green fluorescent protein construct