

# Mystery of Human T-cell Leukemia Virus Type 1: Commentary on Two Viral Genes, Tax and HBZ

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## Summary

Two viral genes of human T cell leukemia virus type 1, Tax and HBZ, are the focus of current research that aims to understand the oncogenic mechanisms of adult T cell leukemia, which is induced by viral infection. Tax is considered to play critical roles in tumorigenesis by modulating various cellular phenotypes, and the anti-sense gene HBZ was recently reported to counteract various functions of Tax. However, HBZ itself may also play critical roles in tumorigenesis. In this short commentary, I have summarized these apparent paradoxes and discussed the limitations of the mechanisms proposed. It will be critically important to understand the protein levels of HBZ in infected cells and the mode of action of its RNA.

**Keywords:** ATL; HBZ gene; HTLV-1, NF-<kappa>B pathway; Tax gene; Wnt pathway

## Introduction to HTLV-1 and ATL

Human T cell leukemia virus type 1 (HTLV-1) is a causative agent of adult T-cell leukemia (ATL), which is a unique T cell malignancy with a CD4-positive phenotype. The molecular mechanism that allows HTLV-1 to induce ATL is a basic topic in ATL research.

The HTLV-1 genome has no oncogenes of cellular origin and primary ATL cells carry a provirus that is integrated almost randomly into regions of the host genome, when compared among patients. Thus, a unique oncogenic mechanism has been postulated. Tax (Trans-activator in X-region of HTLV-1) was the first molecular candidate to be associated with tumorigenesis in ATL and it modulates various cellular phenotypes. In 2002, the antisense gene HBZ (HTLV-1 basic leucine zipper protein) was discovered [1], and it was found to complement some of the paradoxical mechanisms proposed for Tax [2-4]. In this short commentary, I have briefly summarized the functions of Tax and HBZ, and then discuss my concerns about the mechanisms that have been proposed for these genes. Due to space limitations, the discussion and references are highly limited.

# Multiple functions of Tax in the context of ATL [2,3]

The Tax/Rex genes are located in the 3' region of the HTLV-1 genome and each is translated from a different reading frame in doubly-spliced mRNA. Tax protein is a transcriptional activator of viral gene expression and is essential for viral replication. In addition to this role, Tax has multiple functions that probably contribute to transformation of T cells in culture and to tumor induction in transgenic mice. This oncogenic property is another survival strategy of virus that amplifies its provirus via the expansion of infected cells.

Numerous molecular mechanisms have been identified, including the following.

Activation of the transcription of many cellular genes associated with cell proliferation.

Suppression of the transcription of various cellular genes associated with apoptosis or DNA repair.

Inhibition of tumor suppressor proteins such as p16ink4a in the upstream region of Rb.

Abrogation of the check point in cell cycle regulation.

Integrating these observations suggests that Tax has central roles in tumorigenesis. However, critical questions still need to be answered, as follows.

Tax does not account for the monoclonal selection of an infected cell for expansion generating malignant ATL cells.

The leukemic cells of patients carry a defective provirus in about half of ATL cases, which cannot express Tax mRNA or functional protein [5]. Thus, these leukemic cells are not explained by Tax alone.

Rex reduces the level of Tax by inhibiting viral RNA splicing, thereby lowering the level of doubly-spliced mRNA. However, this mechanism does not explain why transcription is not re-activated after the completion of inhibition.

Thus, the elucidation of an additional factor is expected to facilitate a better understanding of ATL.

### HBZ is a unique gene with novel functions

The HBZ gene is also located in the 3'-terminal region of the HTLV-1 genome, but it does not overlap with the Tax coding region. A unique feature is its transcription from the 3' LTR in an antisense direction [1]. Two isoforms, spliced and unspliced, are expressed that encode transcription factors with a basic leucine zipper (bZIP) domain, which differ slightly in the N-terminal region [6]. HBZ was first reported to down-regulate HTLV-1 transcription, which is activated by Tax, i.e., HBZ antagonizes the function of Tax (Figure 1). This mechanism may explain the persistent latency of HTLV-1 and its evasion of immune rejection.

HBZ was then reported to promote T cell proliferation in culture and was shown to be oncogenic in transgenic mice [7]. HBZ appeared to function in the opposite manner to Tax during HTLV-1 expression, but in the same manner during cell proliferation. Another notable characteristic of HBZ is its widespread expression in infected cells in vivo, irrespective of malignant and nonmalignant phenotypes [8]. The previously reported mechanisms of HBZ are as follows.

Transcriptional suppression of the HTLV-1 genome by forming inactive hetero-dimer, HBZ-CREB, counteracting Tax-induced transactivation [1] (Figure 1).

On the other hand, formation of hetero-dimer, HBZ-c-Jun suppresses basal transcription of HTLV-1 [9].

Enhancement of its own transcription by forming a hetero-dimer, HBZ-JunD [10].

Selective inhibition of the classical NF-<kappa>B pathway, which is activated by Tax, via the binding of HBZ to p65, but without affecting the alternative pathway, which is also activated by Tax [11] (Figure 1).



Selective inhibition of the canonical Wnt signal, which is activated by Tax, via the binding of HBZ to TCF/LEF, as well as selective

Selective inhibition of the canonical Wnt signal, which is activated by Tax, via the binding of HBZ to TCF/LEF, as well as selective augmentation of the non-canonical Wnt pathway [12] (Figure 1). The latter is located downstream of TGF $\beta$  and is activated by HBZ binding to the smad2/3-p300 complex [13].

# Tax and HBZ: expectations and limitations

Tax and HBZ have both been shown to promote cell growths in culture and to be oncogenic in transgenic mice, although HBZ basically antagonizes the effects of Tax. How is our understanding of ATL improved by the determination of the antagonistic effects of HBZ on Tax?

First, HBZ or Tax cannot explain the monoclonal selection of an infected cell and its subsequent expansion generating malignant ATL cells. The widespread expression of HBZ in infected cells may explain the promotion of the multi-clonal proliferation of infected cells, which is a typical phenotype of carriers, but an additional mechanism is required to select an infected cell to promote malignant proliferation.

# Activity suppression by HBZ protein

Initially, the function of HBZ was reported to be the counteraction of the Tax-mediated activation of HTLV-1 transcription [1]. This effect of HBZ appears to be unique because it does not directly bind to or compete with Tax, but instead it binds to CREB proteins. HTLV-1 transcription is mediated by Tax binding to the homo-dimer of CREB protein, but HBZ forms an inactive heterodimer and sequesters CREB from the active homo-dimer. This mechanism is non-catalytic, thus effective suppression requires sequestration of most of the CREB protein, i.e., the level of HBZ protein must be at least equivalent to that of CREB. Otherwise, the excess CREB would form the active homodimer and mediate Tax activation catalytically, even if the Tax protein level is lower than that of HBZ. Conversely, if the level of HBZ is sufficient, even a high level of Tax would not be capable of activating HTLV-1 transcription. HTLV-1 transcription is also mediated by c-Jun and suppressed by HBZ via the formation of the inactive heterodimer HBZ-c-Jun [9]. This mechanism is not a target of Tax-mediated activation, but it may explain "no re-activation of HTLV-1 transcription" in the absence of Tax, since the HBZ is expressed widespread. A sufficient level of HBZ expression relative to c-Jun is also required in this scenario.

However, the expression of HBZ protein was reported to be "scarcely detectable" in vivo by western blotting [14]. Furthermore, some infected cell lines that expressed HBZ RNA at rather high levels did not express the protein at significant levels [14]. Whereas, in vivo expression was guaranteed by the detection of serum antibodies against HBZ in carriers and ATL patients [15], as well as by the detection of HBZ-specific CTL [14]. However, the prevalence of the antibody is somewhat limited to a subset of the infected population, i.e., about 10%. This low prevalence may reflect the low immunogenicity of HBZ or the low sensitivity of the technique used, but observations do not suggest that the level of HBZ protein is significantly high in vivo. The protein levels of CREB and c-Jun in T cells have also not been determined quantitatively, thus the in vivo impact of HBZ-mediated suppression is uncertain. It will be necessary to confirm the protein levels of HBZ, CREB, and c-Jun to understand their in vivo impact.

Nevertheless, it was reported that a HBZ-negative HTLV-1 mutant resulted in a higher provirus load in a rabbit model [16]. Does this result contradict the discussion above? It could be argued that a rabbit model may differ from the natural host or that a different mechanism may be involved. Another mechanism for latency has been proposed, i.e., HBZ inhibits the function of Rex [17], which suppresses splicing and enhances the nuclear export of intron-containing viral mRNAs for Gag, Pol, and Env. This model appears to be interesting but elucidation of this inhibitory mechanism is required in further studies. The inhibition of Rex may have a risk resulting in the higher expression of Tax/Rex mRNA, thereby leading to more activation.

In analogy to HBZ and CREB, the levels of specific proteins can be discussed for other inhibitory mechanisms. For example, HBZ binds to p65 during the suppression of the classical NF-<kappa>B signaling which is activated by the Tax [11]. Here, relative protein levels of HBZ and p65 would be critical. HBZ binds to TCF/LEF during the inhibition of canonical Wnt signaling [12], the HBZ levels are expected to be more than equivalent to the TCF/LEF to facilitate effective suppression in vivo. These systems should be examined to clarify the physiological impact of this mechanism in vivo.

# Enhancement activities of HBZ protein

HBZ was shown to counteract the functions of Tax, but not all of them. HBZ counteracts Tax only in a subfamily of the NF-<kappa>B and Wnt signaling pathways. These selective effects are interesting and they suggest more important roles for the selected pathways, which have been discussed by others [18]. For example, the selective activation of NF-<kappa>B subfamily has been described in pancreatic cancers [18] and ATL cells [19]. With respect to the combined effect of HBZ and Tax, it has been described that the alternative NF-<kappa>B pathway is dominantly activated in infected cells as a consequence of the Tax-mediated activation of both NF-<kappa>B pathways followed by HBZ-mediated selective inhibition of the classical pathway [11]. Two different explanations may account for this mechanism, i.e., selective suppression is critical for preventing apoptosis [17] or dominant activation is significant for promoting cell growth. If the latter is the case, question is whether HBZ can affect the alternative NF-<kappa>B pathway in the absence of Tax, which is the most common status in vivo? Thus, various possibilities should be examined.

In the case of the Wnt pathway, HBZ itself enhances the noncanonical pathway by activating TGF $\beta$  signalling, which is suppressed by Tax. The binding of HBZ to Smad2/3 catalytically enhances TGF $\beta$ expression, which may affect the cellular phenotypes more dynamically. Thus, understanding the effect of HBZ via TGF $\beta$  may help to elucidate its roles in cell proliferation.

# Function of HBZ RNA

Another striking feature of HBZ is its functions at both the RNA and protein levels. A mutant where the ATG codon required for translation was deleted from HBZ was still active in promoting T cell proliferation, but not in the suppression of HTLV-1 transcription [8]. Furthermore, a missense mutant that possibly disrupted the RNA secondary structure was inactive in promoting T cell proliferation and it was concluded that "the growth-promoting effect of HBZ depended on RNA structure" [8]. The missense mutant was expected to produce HBZ protein, which suggests that the HBZ protein is not involved in tumorigenesis. If this is the case, selective modulation of NF-<kappa>B or Wnt signals are meaningless? The protein expression by the mutant should be confirmed.

The function of the RNA has not been characterized fully, but a report suggested that HBZ mRNA is localized mostly in the nucleus [20]. This nuclear localization may suggest other roles of HBZ mRNA in addition to its translation in the cytoplasm. The role of the RNA in growth promotion is not well understood but it would be clearly different from those of siRNAs or miRNAs, and its interaction with specific nuclear factors may be expected.

However, it should be noted that the HBZ RNA has only been detected in vivo by PCR and never by blot analysis. In situ hybridization can detect the RNA in infected cells in vivo [21], but the frequency of detection is rather low, and this technique does not indicate the expression levels. However, it is not unreasonable to assume a rather low expression level, and the mechanism of the RNA remains intriguing.

Another interesting approach for elucidating the roles of HBZ is a comparison with APH in HTLV-2. Originally, it was reported that HTLV-2 lacks HBZ due to the absence of the bZIP domain [22]. This finding was linked to the nonpathogenic property of HTLV-2, thereby supporting the importance of HBZ protein in tumorigenesis. However, APH corresponds to HTLV-2 HBZ, and it was found to interact with CREB via its N-terminal domain, thereby counteracting the activation by Tax-2 in a similar manner to HBZ [23]. Therefore, it may be useful to examine the RNA function of APH.

# **Beyond Tax and HBZ**

A combination of Tax and HBZ is not sufficient to explain the overall molecular mechanisms that lead to ATL. Thus, it is necessary to identify an additional factor/event that facilitates the monoclonal selection of an infected cell to promote malignant cell expansion.

Tax and HBZ have important effects, i.e., strong effects with Tax and sustained effects with HBZ, but they also have limitations, i.e., infrequent expression with Tax and low expression with HBZ. It is possible that the counteracting mechanisms of Tax and HBZ may not operate concurrently, but it would be extremely difficult to determine

J Leuk ISSN:2329-6917 JLU, an open access journal where, when, and to what extent these functions occur at significant levels. It will be useful to determine the HBZ protein levels and those of its cellular partners, as well as understanding the mode of action of HBZ RNA. In particular, the roles of HBZ RNA in tumorigenesis may provide new insights that facilitate novel approaches to studying growth regulation and tumorigenesis.

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