The Human T-cell Leukemia Virus type I basic leucine zipper factor upregulates the expression of the antioxidant Heme Oxygenase I

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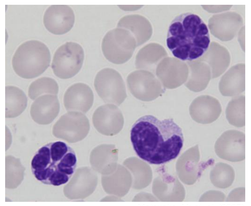
Abstract

Adult T-cell Leukemia/Lymphoma (ATLL) is a resilient lymphoproliferative disease of CD4⁺ T-cells infected by the Human T-cell Leukemia Virus type I (HTLV-1), for which there are no effective treatments. Mounting evidence supports that the overexpression of antioxidants contributes to drug resistance in many types of cancer. One such antioxidant is the iron-recycling enzyme Heme Oxygenase (HMOX-1), which has been shown to enhance cancer cell survival upon exposure to stress-inducing agents. HMOX-1 expression is regulated by the small Maf AP1 proteins, which control transcription from promoter antioxidant response elements (AREs). A previous report, confirmed by our laboratory, shows that the HTLV-1 antisense-encoded basic leucine zipper factor, HBZ, interacts with small Mafs for recruitment to AREs *in vitro*. We questioned whether HBZ and small Mafs regulate the expression of antioxidants like HMOX-1 as a pro-survival strategy in ATLL cells. Our results show that HMOX-1 is overexpressed in ATLL cells in a manner dependent upon both HBZ and the small Mafs. These proteins were found to be present at an ARE in the promoter of HMOX-1 *in vivo*, and HBZ expression was observed to promote ARE transactivation in a small Maf-dependent manner. HMOX-1 is thought to be the main mediator of iron metabolism and functions in a cytoprotective capacity during oxidative stress. We observed that ATLL cells, as well as HBZ-expressing cells, exhibited resistance to iron-induced cytotoxicity, which was attenuated upon inhibition of HMOX-1 enzyme activity. Furthermore, HBZ expression was found to be important for maintaining ATLL cell redox state, as well as for maintaining cell viability in response to iron exposure. These findings support the possibility that HBZ and small Mafs may upregulate transcription at AREs to positively regulate some antioxidant response genes in ATLL cells, wherein these gene products may have cytoprotective effects in response to oxidative stress and may contribute to anti-cancer drug resistance.

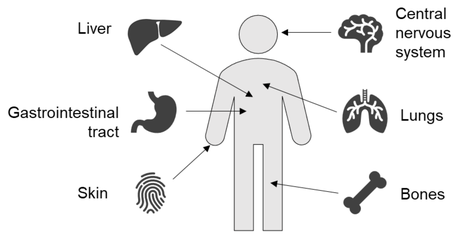
HTLV-1 is the causative agent of Adult T-cell Leukemia/Lymphoma

Leukemic HTLV-1⁺ CD4⁺ T-cells



Matsuoka, M; *et. al.* (2005)

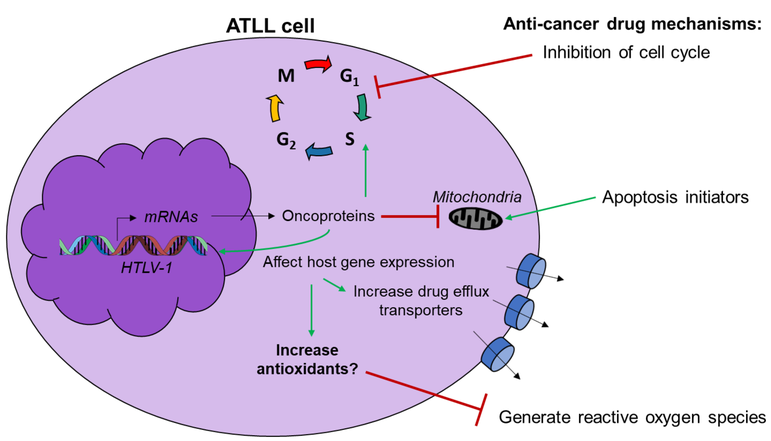
Common sites of ATLL cell infiltration



Adult T-cell Leukemia/Lymphoma (ATLL):

- HTLV-1-infected CD4⁺ T-cells [1] aggressively proliferate and infiltrate into solid tissues.
- Chemotherapeutics are ineffective; Average survival time from diagnosis is 13 months.

HTLV-1 activates pro-survival mechanisms in ATLL cells to resist anti-cancer treatments



Hypothesis: HTLV-1-encoded, pro-oncogenic transcriptional regulators upregulate the expression of antioxidants to promote ATLL cell resistance to oxidative stress-induced cell death.

The viral oncoprotein HTLV-1 basic leucine zipper factor (HBZ) enhances the expression of some antioxidants

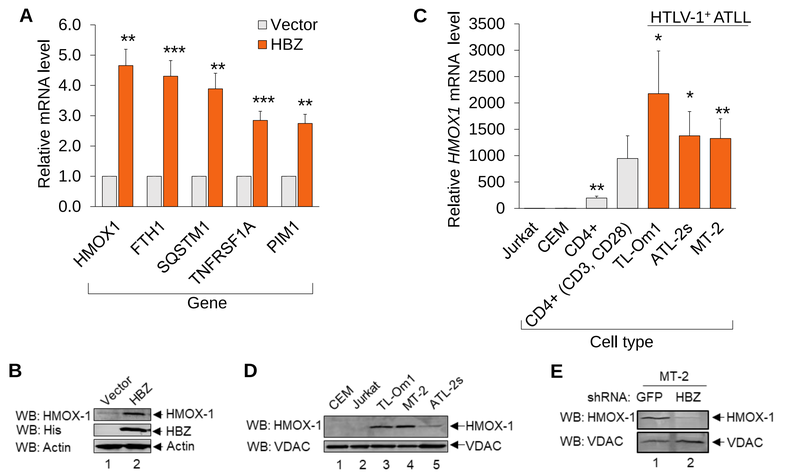


Figure 1: (A) Expression of a set of oxidative stress-response genes is upregulated in the presence of HBZ. qRT-PCR was used to quantify relative mRNA levels for the indicated genes in HeLa clonal cell lines expressing wild-type HBZ (HBZ WT, and in a cell line containing the empty expression vector. *HMOX1* values are averages from three independent experiments; *FTH1*, *SQSTM1*, *TNFRSF1A*, and *PIM1* values are averages from four independent experiments. Data were normalized to the vector sample (set to 1). Error bars represent the SEM (two-tailed Student's t-test, *p≤0.05, **p≤0.01, ***p≤0.001). **(B)** HBZ upregulates HMOX-1 protein levels. Levels of the indicated proteins were evaluated in 30 µg of whole cell extract from each of the HeLa cell lines. The indicated antibodies were used for the Western blot analysis. **(C)** *HMOX1* transcript levels are elevated in HTLV-1-infected T-cells compared to in uninfected T-cells. qRT-PCR was used to quantify relative transcript levels in uninfected T-cell lines (Jurkat and CEM), primary resting and activated CD4⁺ T-cells, and in HTLV-1-infected T-cell lines (TL-Om1, ATL-2s, and MT-2). Data are an average of seven independent experiments, with the exception of primary CD4⁺ data, which are an average of three independent experiments. Error bars represent the SEM (two-tailed Student's t-test, **p≤0.01). **(D)** HMOX-1 protein levels are elevated in HTLV-1-infected T-cells compared to in uninfected T-cells. HMOX-1 and VDAC protein levels were evaluated in 60 µg of the membrane-bound fraction from the indicated T-cell lines. The indicated antibodies were used for the Western blot analysis. **(E)** shRNA-mediated knockdown of HBZ reduces HMOX-1 expression. HMOX-1 and VDAC protein levels were evaluated in 60 µg of membrane-bound fractions from MT-2 shHBZ and from MT-2 shGFP. The indicated antibodies were used for the Western blot analysis.

Heme Oxygenase I is an essential iron-recycling enzyme

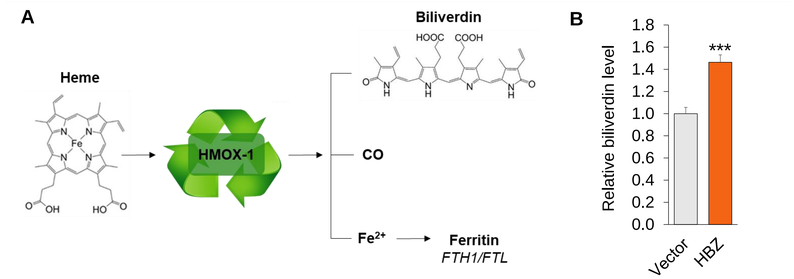


Figure 2: (A) The schematic shows heme metabolism by HMOX-1. HMOX-1 cleaves the protoporphyrin ring of heme, creating biliverdin, carbon monoxide (CO), and free ferrous iron (Fe2+). Ferrous iron is scavenged by Ferritin (FTH1/FTL). **(B)** Higher HMOX-1 levels in HBZ-expressing cells is associated with increased HMOX-1 enzymatic activity in these cells. Biliverdin production was quantified as a measure of HMOX enzymatic enzyme activity in the indicated HeLa cell lines. Cells were homogenized and lysates were incubated with the HMOX substrate, hemin, and bilirubin oxidase as described in the Materials and Methods. Data are an average of three independent experiments. Error bars represent the SEM (two-tailed Student's t-test, **p<0.01).

Acknowledgements and References

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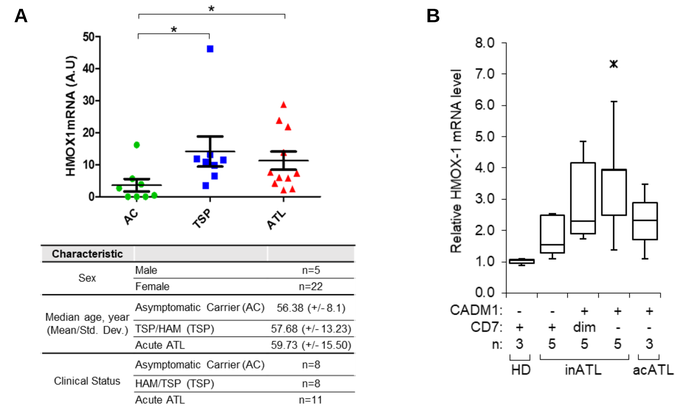
Elevated *HMOX1* expression may correspond to ATLL disease progression



Characteristic		
Sex	Male	n=5
	Female	n=22
Median age, year (Mean/Std. Dev.)	Asymptomatic Carrier (AC)	56.38 (+/- 8.1)
	TSP/HAM (TSP)	57.68 (+/- 13.23)
	Acute ATL	59.73 (+/- 15.50)
Clinical Status	Asymptomatic Carrier (AC)	n=8
	HAM/TSP (TSP)	n=8
	Acute ATL	n=11

Figure 3: (A) HMOX1 transcript levels are elevated in the PBMC from HSM/TSP and ATL patients compared to the PBMC from asymptomatic HTLV-1 carriers. qRT-PCR was used to quantify relative HMOX1 transcript levels in CD8+ T-cell-depleted PBMCs isolated from asymptomatic HTLV-1 carriers (AC), HAM/TSP (TSP) patients and acute ATL (ATL) patients. Error bars represent the standard deviation (two-tailed Student's t-test, *p<0.05). Subject/patient information is shown below the graph. **(B)** Increasing levels of the HMOX1 transcript may correlate with ATL disease progression. The graph was generated from published microarray data [2, 3] and shows HMOX1 transcript levels partitioned by CADM1 and CD7 expression in healthy donors (HD) and patients with acute (acATL) or indolent ATL (inATL). Data obtained using GEOR from the GSE55851 series are represented as a Tukey boxplot where the ends of each whisker are set to 1.5 times the interquartile range above the third quartile and below the first quartile (ж, outlier).

HMOX-1 promotes resistance to heme-induced oxidative stress in ATLL cells

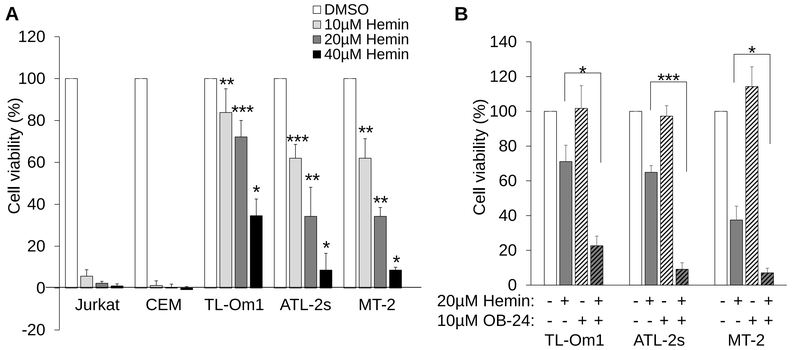


Figure 4: (A) HTLV-1-infected T-cells exhibit resistance to the cytotoxic effects of hemin. A cell viability dye, alamarBlue was used to quantify the relative viability of uninfected (Jurkat and CEM) and HTLV-1-infected (TL-Om1, MT-2, and ATL-2s) T-cells cultured in low-serum medium (0.5% serum) with 10, 20 or 40 µM hemin or the vehicle control (DMSO). For each experiment, cell viability was determined from 12 replicates for each treatment of each cell line. Data are the average of three independent experiments and error bars represent SEM (two-tailed Student's t-test, *p≤0.05, **p≤0.01, ***p≤0.001). **(B)** Inhibition of HMOX-1 sensitizes HTLV-1-infected cells to the cytotoxic effects of hemin. The alamarBlue cell viability dye was used to quantify the relative viability of uninfected (Jurkat and CEM) and HTLV-1-infected (TL-Om1, MT-2, and ATL-2s) T-cells cultured in low-serum medium (0.5% serum) with the HMOX-1 inhibitor, OB-24 (10 µM), and/or 20 µM hemin. For each experiment, cell-viability was determined from eight replicates for each treatment of each cell line. Data are the average of three independent experiments. Error bars represent the SEM (two-tailed Student's t-test, *p≤0.05, **p≤0.01, ***p≤0.001).

Conclusions and Future Directions

1. The antioxidant enzyme Heme Oxygenase I is overexpressed in some ATLL cell lines, as well as in a small group of ATLL patients.
   - Screen larger pools of patients.
2. HMOX-1 upregulation occurs trough the transcription regulatory activities of the viral oncoprotein HBZ.
   - Is HBZ regulating other antioxidant genes through a similar mechanism?
3. HMOX-1 protects against heme-induced oxidative stress in ATLL cell lines.
   - Will pharmacologic inhibition of HMOX-1 enhance sensitivity to anti-cancer drugs?