The endogenous HBZ interactome in ATL leukemic cells reveals an unprecedented complexity of host interacting partners involved in RNA splicing

Shallak M[1], Alberio T[2], Fasano M[2], Monti M[3], Iacobucci I[3], Mortreux F[4], Accolla RS[1], Forlani G[1]
[1]Laboratories of General Pathology and Immunology "Giovanna Tosi", Department of Medicine and Surgery, University of Insubria, Varese, Italy
[2]Laboratory of Biochemistry and Functional Proteomics, Department of Science and High Technology, University of Insubria, Busto Arsizio, Italy.
[3]Department of Chemical Sciences, University Federico II of Naples, Strada Comunale Cinthia, 26, 80126 Naples, Italy; CEINGE Advanced Biotechnologies, Naples, Italy.
[4]Laboratory of Biology and Modeling of the Cell, CNRS UMR 5239, INSERM U1210, University of Lyon, Lyon, France.

Background: Adult T-cell leukemia/lymphoma (ATL) is a T-cell lymphoproliferative neoplasm caused by the human T-cell leukemia virus type 1 (HTLV-1). Two viral proteins, Tax-1 and HBZ play important roles in HTLV-1 infectivity by altering key pathways of cell homeostasis. Tax and HBZ were recently suggested to reprogram the host cell transcriptome by perturbing the splicing landscape in T-cells**,** targeting cancer genes that are also altered in ATL patients. To investigate in detail the involvement of HBZ in deregulation of cell homeostasis we analyzed the endogenous HBZ interactome in leukemic cells.

Methods: The investigation of the HBZ interactome was carried out by immunoprecipitation with the 4D4-F3 anti-HBZ mAb in ATL-2 leukemic cells, followed by tandem mass spectrometry analyses. RNA seq analysis was performed to decipher the differential gene expression and the alternative splicing modifications upon HTLV-1 infection. The DEAD-Box RNA helicases DDX5-p68 and DDX17-p72 and their interaction with HBZ were confirmed by immunofluorescence followed by confocal microscopy analysis and by co-immunoprecipitation assay.

Results: The HBZ interactome of ATL-2 cells identified three main nodules corresponding to protein families mainly involved in mRNA splicing, nonsense-mediated RNA decay (NMD) and JAK-STAT signaling pathway. Here we analyzed RNA splicing. RNAseq analysis showed that HBZ specifically altered the transcription of many genes including crucial oncogenes by affecting different splicing events. Consistently, the two RNA helicases, members of the RNA splicing family, DDX5 and its paralog DDX17, which has been recently shown to be involved in alternative splicing of cellular genes after NF-κB activation by HTLV-1 Tax-1, interacted and partially co-localized with HBZ.

Conclusion: For the first time, a complete picture of the endogenous HBZ interactome was elucidated. The wide interaction of HBZ with molecules involved in RNA splicing and the subsequent transcriptome alteration strongly suggests an unprecedented complex role of the viral oncogene in the establishment of the leukemic state.

Disclosure of Interest Statement: none