THE CIRCADIAN TRANSCRIPTION FACTORS CLOCK AND BMAL1 ACTIVATE HIV TRANSCRIPTION THROUGH BINDING TO THE HIV LONG TERMINAL REPEAT

Stern J[1], Roche M[1], Chen W[2,3], Tumpach C[1], Gray L[2,3], Churchill M[2,3,5] and Lewin SR[1,4]

[1]The Peter Doherty Institute for Infection and Immunity, The University of Melbourne and Royal Melbourne Hospital, Melbourne
[2]School of Health and Biomedical Science, RMIT University, Melbourne
[3]Macfarlane Burnet Institute for Medical Research and Public Health, Melbourne
[4] Department of Infectious Diseases, Alfred Hospital and Monash University, Melbourne
[5] Department of Microbiology, Monash University, Melbourne

Background: In a recent clinical trial involving the administration of disulfiram as a latency reversing agent to HIV-infected individuals on antiretroviral therapy (ART), we observed time dependent variations in the expression of cell associated unspliced HIV RNA (CA-US HIV RNA) in CD4+ T cells isolated from blood. We hypothesise that circadian rhythms are exerting transcriptional control of latent HIV infection through direct interaction of the circadian transcription factors CLOCK and BMAL1 with the HIV long terminal repeat (LTR).

Methods: The epithelial cell line HEK 293T and astroglial cell line SVG were transfected with an HIV LTR-driven luciferase reporter construct in the presence and absence of CLOCK- and BMAL1-expressing constructs. Activation of HIV-1 transcription was measured as an increase in luciferase expression. HIV LTR sequences containing mutations in each of the 4 E-boxes, that could potentially bind CLOCK/BMAL1, were also assessed.

Results: Transfection of the HIV-LTR driven luciferase reporter construct into 293T resulted in high luciferase expression in the presence of Tat (20.89±2.08 fold induction). Transfection of CLOCK or BMAL1 alone failed to increase luciferase (1.35±0.07 and 1.35±0.11-fold induction, respectively), however, cotransfection of CLOCK and BMAL1 led to a substantial increase in luciferase (4.09±0.22 fold induction). Mutation of E-box 1, 3 and 4 had no notable effect on luciferase expression following transfection of CLOCK/BMAL1, however, mutation of E-box 2 led to a >50% reduction in luciferase expression. Similar results were seen in SVG cells.

Conclusion: The circadian transcription factors CLOCK/BMAL1 activate transcription from the HIV LTR, which requires the presence of at least the one intact E-box. These data are consistent with direct binding of the protein complex to the LTR upstream of the NF-κB and Sp1 binding sites. We are currently exploring the capacity of these transcription factors to activate latency in models of latent HIV infection using primary cells.

Disclosure of Interest Statement: There are no conflicts of interest to be disclosed.