Mechanism and Therapeutic Implications of Host Cell Telomerase Modulation by Human Cytomegalovirus

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Abstract

We and others have observed telomerase activation by Human Cytomegalovirus in fibroblasts, yet the details of this interaction remain uncharacterized. In understanding this relationship, one goal is to inhibit HCMV replication through telomerase modulation. To do this we are investigating the influence of pharmaceutical telomerase inhibition and stage-specific viral gene expression on the dynamic between HCMV and telomerase.

Introduction

Human Cytomegalovirus (HCMV) is a double-stranded, linear DNA beta-herpesvirus that infects a large proportion of the global population. Though often clinically insignificant in immunocompetent patients, HCMV can cause serious morbidity and mortality in immunocompromised patients and remains a leading cause of congenital neurological defects[1]. Telomeres are conserved repetitive DNA sequences that cap the ends of chromosomes, providing protection to coding DNA and stability to the chromosome. Telomeres are maintained by the reverse transcriptase telomerase, which is regulated by various proteins and RNAs[2]. We have confirmed the previous observation[3] that active HCMV infection of laboratory and clinical HCMV strains upregulates telomerase in fibroblasts, and now seek to elucidate the yet undescribed mechanism and clinical implications of this interaction.

Results

Telomerase Activity Increases with Dose and Time Following HCMV Infection

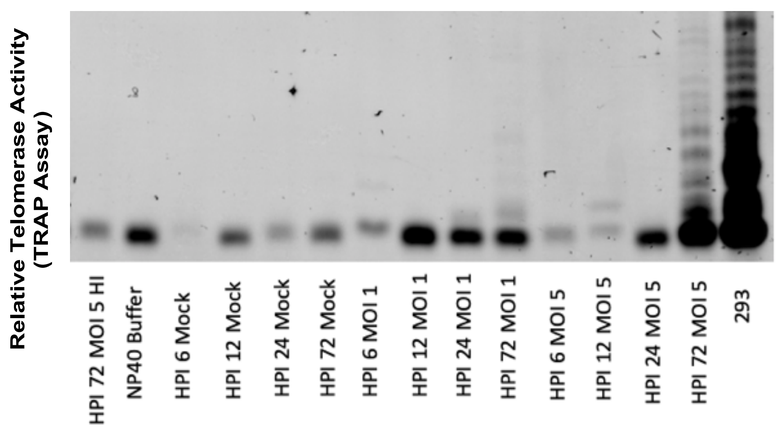

Figure 1: Dose and Time-Dependent Increase in Telomerase Activity Following HCMV Infection. Human foreskin fibroblast (HFF) cells were infected with HCMV strain AD169 at Multiplicity of Infection (MOI) 1 or 5, and harvested 6, 12, 24, and 72 hours post infection. **(A)** Non-radioactive telomerase repeat amplification protocol (TRAP) assay of protein extracts prepared from mock-infected or HCMV AD169 infected HFF cells, 24, 48 or 72 hours post infection (hpi). HI = Heat Inactivated negative control, NP40 Buffer = negative control and 293 (HEK293) = positive control.

Pharmaceutical Telomerase Inhibition Reduces HCMV Viral Titer in Laboratory and Clinical HCMV Strains

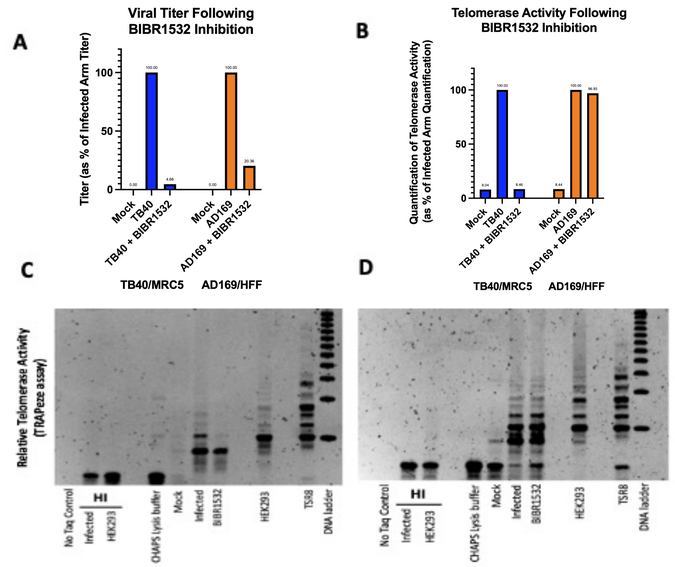

Figure 2. Reduction in HCMV replication following treatment with BIBR1532. **(A)** Quantification of viral titer by IE1 Fluorescent Focus Assay following treatment of TB40-Infected MRC5 cells with non-cytotoxic dose of 20 µm BIBR1532 and AD169-infected HFF cells with non-cytotoxic dose of 15 µm BIBR1532. Values represented as percent of infected arm quantification. **(B)** Quantification of relative telomerase activity using ImageJ. Values represented as percent of infected arm quantification. **(C, D)** Relative telomerase activity for TB40-infected MRC5 cells **(C)** and AD269-infected HFF cells **(D)**, determined by TRAPeze assay. No Taq control = negative control. HI = Heat Inactivated (negative control). HEK293 = positive control. TSR8 = TRAPeze assay positive control.

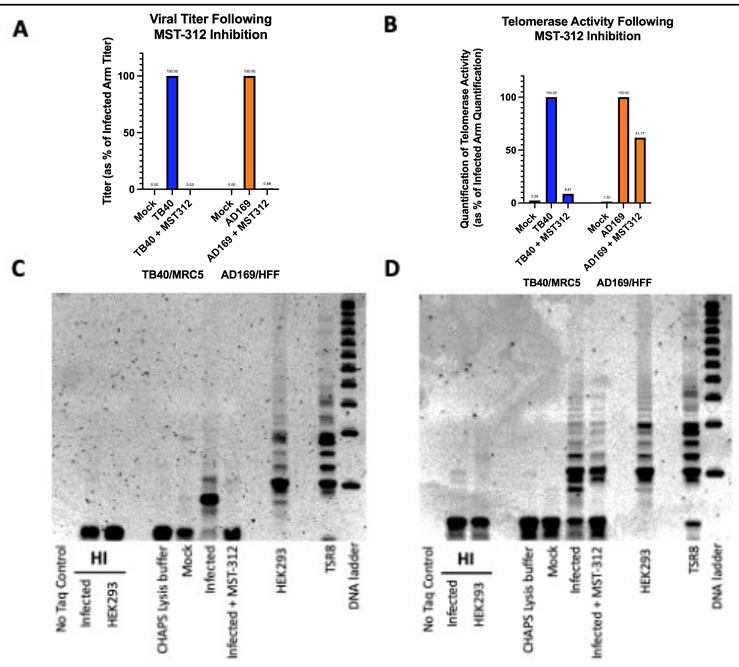

Figure 3. Reduction in HCMV replication following treatment with MST-312. **(A)** Quantification of viral titer by IE1 Fluorescent Focus Assay following treatment of TB40-Infected MRC5 cells with non-cytotoxic dose of 0.5 µm MST-312 and AD169-infected HFF cells with non-cytotoxic dose of 0.25 µm MST-312. Values represented as percent of infected arm quantification. **(B)** Quantification of relative telomerase activity using ImageJ. Values represented as percent of infected arm quantification. **(C, D)** Relative telomerase activity for TB40-infected MRC5 cells **(C)** and AD269-infected HFF cells **(D)**, determined by TRAPeze assay. No Taq control = negative control. HI = Heat Inactivated (negative control). HEK293 = positive control. TSR8 = TRAPeze assay positive control.

HCMV-mediated telomerase increase requires newly synthesized viral proteins

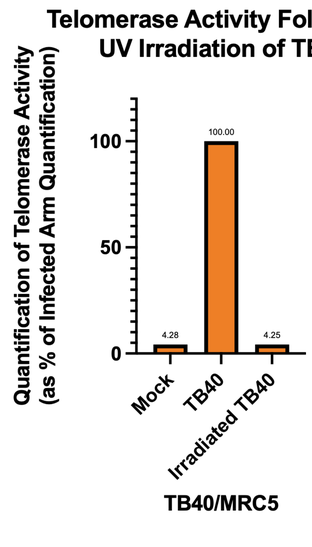

Figure 4. Abrogation of telomerase activity increase following UV-irradiation of TB40 prior to infection of MRC5 cells. Quantification of Mock, Infected, and Irradiated Infected lanes as percent of Infected arm by ImageJ (values are averages of three experiments).

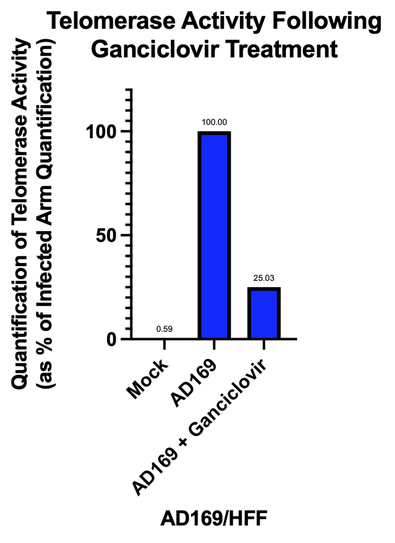

Figure 5. Reduction of telomerase activity increase following non-cytotoxic Ganciclovir treatment of AD169-infected HFF cells. Quantification of Mock, Infected, and Infected + Ganciclovir treated lanes as percent of Infected arm by ImageJ.

Summary and Future Directions

- We have thus far identified that HCMV-mediated telomerase upregulation is dose- and time-dependent and requires newly synthesized viral proteins. Additionally, we have observed that pharmaceutical telomerase inhibition with multiple telomerase inhibitors sharply reduces HCMV viral titer, though curiously in AD169-infected HFF cells treated with BIBR1532 this did not require significant reduction in telomerase activity, suggesting the potential for pleiotropic or more general reverse transcriptase effects.
- We aim to test viral infection and replication following genetic telomerase inhibition models in laboratory and clinical HCMV strains.
- We also aim to Investigate the mechanism of action and significance of telomerase activation during HCMV infection via: lentiviral overexpression of IE genes UL123 and UL122, and binding assays of these proteins and others previously implicated[3] in the interaction of HCMV and telomerase.

Acknowledgements

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