Furthering the understanding of mechanism and function of HCMV IE1 chromatin association

ABSTRACT

Human cytomegalovirus (HCMV), like all herpesviruses, establishes lifelong latent infections in its host. However, the molecular mechanisms involved in HCMV latency establishment remain largely unknown. In gammaherpesviruses (eg. Kaposi's sarcoma-associated herpesvirus - KSHV), whose latency has been extensively studied, the viral genome is maintained as an episome tethered to host chromatin via a maintenance protein (MP) – for KSHV the Latency-associated nuclear antigen (LANA). MP simultaneously binds to the viral genome and host chromatin thereby tethering the viral genome to the host chromatin. While it has been shown that HCMV genomes persist in latently infected cells cells, very little is known about the mechanism of tethering and genome maintenance. The immediate early 1 (IE1) protein encoded by HCMV is known to localize to metaphase chromosomes in a pattern described as "painting of the chromosomes". The chromatin tethering domain (CTD) of IE1 was shown to mediate this localization through binding to histones, H2A and H2B. However, the functional significance of this association has not been uncovered yet. An isoform of IE1, IE1x4 protein was proposed to be the MP of HCMV and to function in a manner analogous to KSHV LANA. Additionally, HCMV genomes were shown to be tethered to host chromosomes during lytic infection and IE1 was observed to be involved in this process. The work presented in my thesis demonstrates that IE1x4 binds to mitotic chromosomes, however, the localization pattern of IE1x4 does not change in the presence of the TR region of the HCMV genome (the presumed latent origin of replication) in transfected cells as has been observed eg. for KSHV LANA. Further, IE1x4 protein was not detected during latency in infected T98G and KASUMI-3 cells, two known latency systems. Moreover, I show that in addition to the previously known painting localization pattern, IE1 also localizes as double spots (referred to as CAS – chromosome-associated spots) on mitotic chromosomes. The IE1 CAS can be observed only in some tumor cell types. The CAS formation is dependent on the IE1 core domain and is therefore independent of the CTD. The IE1 localizes to spots at the pericentromeric regions of mitotic chromosomes and recruits PML to these spots. The percentage of cells with IE1 CAS localization pattern increases in infected T98G cells, as the infection progresses from lytic to the latent phase, suggesting a role for IE1 CAS in latency or latency establishment. The IE1 CAS are reminiscent of the localization pattern of HPV8 and

HPV5 E2 proteins, which function as viral maintenance proteins, therefore I hypothesize that IE1 CAS could mediate HCMV genome tethering in T98G cells.