EFFECTS OF RAD21 AND CTCF ON EBV REACTIVATION FROM LATENCY

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CTCF, a host insulator protein that demarcates chromatin boundaries and mediates three-dimensional chromatin loops, is frequently utilized by DNA viruses to regulate viral gene expression (1). Cohesin, a ring-shaped complex comprised of the subunits SMC1, SMC3 and RAD21, essential for chromatid segregation, is also a global regulator of transcription (2). CTCF and cohesin bind at distinct sites on herpesvirus genomes, including HSV, EBV and KSHV. Both CTCF and RAD21 act as potent host cell repressors of KSHV lytic replication by modulating viral gene transcription (3, 4). We investigated the roles of CTCF and RAD21 in regulating EBV lytic replication. Infectious EBV virus yield was found to be moderately decreased by depletion of CTCF or RAD21 in epithelial cells, in contrast to the findings in KSHV. Surprisingly, based on RNA-sequencing analysis, depletion of CTCF and RAD21 led to global increases in EBV gene transcript levels and viral DNA quantity. Further, RAD21 knockdown, but not CTCF knockdown, activated expression of EBV transactivator Zta and triggered EBV lytic replication, leading to a 20-fold increase in EBV lytic transcription.

We next performed reciprocal ChIP-seq analysis after CTCF or RAD21 depletion to determine the interdependence of CTCF and RAD21 occupancy on the EBV genome. RAD21 knockdown led to loss of RAD21 binding at the Zta promoter region, thus triggering Zta expression whereas CTCF knockdown did not. These results suggest that although CTCF and RAD21 may restrict early stages of EBV lytic replication, other factors in the later stages of viral lytic replication constrain enhanced virus production despite loss of CTCF and RAD21. We therefore measured viral encapsidation by electron microscopy and extracellular viral DNA by qPCR. Encapsidation and virus egress were not found to be significantly enhanced when CTCF and RAD21 were depleted. Further, late protein expression was not enhanced by CTCF or RAD21 loss, suggesting that despite increased viral transcription and DNA replication, bottlenecks in late protein production limit the effect of CTCF and RAD21 depletion on infectious virus production.

In summary, CTCF and particularly cohesin, may inhibit maximal early EBV transcription and DNA replication but do not significantly restrict later steps of EBV virus production. Based on these results, we examined the replication of EBV in EBV-transformed B lymphocytes from patients with Cornelia de Lange syndrome, who suffer from genetic defects in cohesin function (5). Lymphoblastoid cell lines from three different individuals, with mutations mapping to three genes implicated in such cohesinopathies, demonstrated greater entry of EBV into the lytic replicative cycle compared to normal controls, indicating that EBV lytic replication control by cohesin occurs in vivo and is physiologically significant. Rescue of RAD21 by lentiviral transduction in these cells inhibited EBV lytic replication, supporting the repressive role of Rad21 in EBV reactivation.

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