IDENTIFYING G-QUADRUPLEXES AND THEIR FUNCTIONAL ROLE IN THE REGULATION OF MIRNAS AND HUMAN HOMOLOGUES ENCODED BY HUMAN HERPESVIRUSES

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Guanine rich nucleic acids can fold into four stranded secondary structures called G-quadruplexes. In the human genome, G-quadruplexes are predominantly found in telomeres and promoters of oncogenes. These nucleic acid secondary structures can regulate crucial cellular processes including transcriptional and translational regulation, telomere maintenance and recombination. Recent studies have shed light on the biological significance of G-quadruplexes in viruses particularly herpesviruses, highlighting their role in regulating transcription, translation, virus latency, virus recombination, virus packaging and replication. Herpesviruses differ from other viruses by their ability to establish latency in the infected host. Since herpesviruses replicate in the host nucleus, these viruses have acquired host genes as well as their regulatory elements over the course of evolution as they share a long-term relationship with the host. Herpesvirus encoded miRNAs and homologues (mimics) of human proteins work together in a close-knit manner to efficiently fine tune the life cycle of a herpesvirus (lytic and latent phase). We therefore sought to investigate the upstream regulatory regions of all known herpesvirus miRNAs and human genes mimicked by herpesviruses as well as their human counterparts for putative G-quadruplex forming sequences (PQS). We performed extensive biophysical characterization to determine the stability of the G-quadruplexes formed in a subset of PQS motifs identified. G-quadruplexes found upstream of miRNAs encoded by Kaposi's sarcoma-associated Herpesvirus (KSHV) and Human Cytomegalovirus (HCMV) were able to significantly modulate miRNA expression in transfected HEK293T cells. Another aspect of our study highlights the prevalence of PQS motifs in regulatory regions of human herpesvirus homologues and their human counterparts. Moreover, G-quadruplexes flanking viral Bcl-2 homologues namely KSHV KS-Bcl-2 and EBV BHRF1 (structural and functional mimics of anti-apoptotic human Bcl-2 protein), were found to significantly upregulate viral Bcl-2 expression. Intriguingly, the role of G-quadruplexes in regulation of virus-encoded
Bcl-2 expression was found to be diametrically opposite to that of G-quadruplexes in the host bcl-2 promoter. We also observe that promoter G-quadruplexes may facilitate virus-encoded transcription factors mediated regulation of vBcl-2. In sum, our findings highlight previously unknown mechanisms of regulation of herpesvirus-encoded miRNAs and shed light on new roles for G-quadruplexes in herpesvirus biology. Our findings might expand our understanding of G-quadruplex linked regulation of critical host defense pathways upon herpesvirus infection.