NATURAL VARIANTS OF EBNA1 DIFFER IN THEIR DNA BINDING AND REPLICATION PROPERTIES AND SENSITIVITY TO INHIBITORS

Vishwanath Kumble Bhat[1], Rajesh Ponnusamy[1], Elizabeth Day[1], Anis Amira Binti Aznan[1], Helen M. Webb, S. Mark Roe[1], Paul J. Farrell[2] and Michelle J. West[1]

[1] School of Life Sciences, University of Sussex, UK; [2] Imperial College Faculty of Medicine, Department of Infectious Disease, UK

m.j.west@sussex.ac.uk

Epstein-Barr virus nuclear antigen 1 (EBNA1) is a sequence specific DNA binding protein expressed in all EBV-associated tumours. EBNA1 dimers bind to the EBV latent origin of replication to direct viral genome replication, segregation and maintenance. EBNA1 trans-regulation of host genes and interactions with host proteins is also important for the growth of infected cells. High-throughput sequencing of EBV from specific geographical regions identified novel variation in EBNA1 that was independent of the usual type 1 and type 2 strain segregation. In a collection of >240 EBNA1 protein sequences from around the world, approximately 50% of sequenced EBV strains had newly-identified co-associated changes in 5 non-adjacent amino acids in the EBNA1 DNA binding domain (DBD). Thus, P476, S492, M563, V574 and T585 (PSMVT) in the B95-8 sequence become QCIGP. The QCIGP variant is present at high frequency in European Union/Australian samples (75%) and in African samples (96%). Additional variation at position 487 segregates such that QCIGP variants have either 487 L or T. In B95-8 strains this residue is either A or V, with 487V prevalent in EBV strains in Asia. We have examined how this strain variation impacts on the structure of the EBNA1 DBD, its interaction with DNA, its sensitivity to inhibitors and EBNA1 replication and genome maintenance function. We have solved the high-resolution X-ray structures of representative QCIGP 487T and QCIGP 487L variant DBDs bound to DNA as dimers and dimer-dimer complexes and identified alterations in potential druggable pockets and interfaces. DNA binding assays revealed reduced binding affinity of the QCIGP 487L variant to specific DNA sequences and small increases in DNA binding by the QCIGP 487T variant. Consistent with some enhancement in DNA binding, the QCIGP 487T variant also showed small increases in EBNA1-dependent replication. Our data therefore indicate that natural variation in the EBNA1 DBD can affect EBNA1 function and anti-EBNA1 drug sensitivity.