ONCOGENIC PROPERTIES OF THE EPSTEIN BARR VIRUS LARGE TEGUMENT PROTEIN.

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The Epstein Barr Virus (EBV) is a human tumorigenic virus known to transform B cells from activation of its latency programme. However, not much is known about the oncogenic properties of the viral proteins present in the infectious particle itself. We previously found that the tegument protein BNRF1 induce chromosomal instability and centrosomal amplification [1]. More recently, BNRF1 has been found to downregulate expression of SMC5/6, a protein crucial for faithful chromosome segregation [2].

We now show that the large tegument protein, BPLF1, induces nuclear abnormalities and mitotic abnormalities in host cells. We made use of co-immunoprecipitation coupled with mass spectrometry to identify binding partners of the full length BPLF1. The Sentrin-specific protease 6 (SENP6) was found to precipitate with BPLF1 in three independent experiments. SENP6 possesses SUMO2/3 deconjugase activity and BPLF1 interaction effectively attenuates its activity in the nucleus. Domain deletions and subsequent pull-down experiments were performed to identify the interacting regions of BPLF1 and SENP6. A novel SENP6 interacting region of approximately 1200 amino acids is described and was further verified through the cloning of a recombinant version of the isolated domain. Through western blot and immunostaining studies, we found that this interaction inhibits SENP6 activity, thereby increasing the total SUMOylation status of SENP6 targets in HEK293 cells expressing BPLF1 and in B cells exposed to virus particles.

Components of the inner kinetochore are known substrates of SENP6 and, upon its depletion, become hyperSUMOylated and disassemble from the centromeres. Chromosomal abnormalities consequently accumulate within the host cells, which is a hallmark of cancer. In U2OS cells expressing BPLF1, and B cells infected with EBV, we observe a progressive reduction of CENPA at the centromeric regions when compared to controls, coupled with increased rates of aneuploidy.

These results further document EBV infection's early deleterious effects on B cells linked to lytically encoded proteins enacting on the cellular machinery prior to establishment of latency. They identify a deSUMOylase activity encoded by BPLF1, next to the previously identified deubiquitinase and deneddylase activities [3, 4]. Therefore, BPLF1 appears to be a master regulator of post-translational modifications with multiple consequences for the host, ranging from modulating the host nuclear environment to favour EBV genome replication evading the host cell's innate immune TLR receptor responses [5], to the development of genetic abnormalities.

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