SIZE AND TRANSCRIPTIONAL ACTIVATION DIFFERENCES OF B95-8 ZTA VERSUS AKATA AND P3HR-1 ZTA MAP TO SPECIFIC AMINO ACIDS IN THE TRANSACTIVATION DOMAIN

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The EBV Zta protein (ZEBRA, EB1, Z) encoded by the BZLF1 gene, is a lytic DNA binding transactivator that is a key factor in lytic replication and reactivation from latency. Zta binds preferentially to methylated promoters containing Z response elements and activates many EBV lytic cycle promoters as well as modulating cellular gene expression. Experiments using a gastric carcinoma cell line AGS, infected with a recombinant Akata EBV [1] revealed an unexpected finding of differences between the B95-8 and Akata Zta proteins. We had made a cell line (AGSiZ) [2] in which the latent EBV can be efficiently induced to enter lytic replication by transducing AGS-Akata with a doxycycline-inducible Zta gene. Deep sequencing of RNA from induced AGSiZ revealed polymorphism at several sites in the Zta gene. Further analysis of the two Zta sequences present revealed that both B95-8 Zta from the transgene and Akata Zta from the Akata virus were expressed at high levels in these cells when the B95-8 Zta was induced with doxycycline. Immunoblotting showed that the Akata Zta is slightly larger by SDS-PAGE. Comparison of the function of each Zta demonstrated that Akata Zta was a more potent activator of lytic genes and resulted in higher expression of other EBV lytic genes when transfected into cells with Zta-KO EBV [3]. Akata Zta was also more effective in enhancing DNA replication and virus production that B95-8 Zta.

We next asked whether the size difference and activity mapped together to specific amino acid differences between the two strains. The Zta protein contains an N-terminal trans-activation domain (aa 1-166), a DNA binding domain (aa 178-194), and a coiled-coil dimerization domain (195-221). Chimeras between Akata and B95-8 Ztas indicated that the difference in both larger apparent size on SDS-PAGE and transcriptional activation mapped in the transcriptional activation domain between aa 100-175. Comparison with P3HR-1 Zta revealed that P3HR-1 Zta behaves like Akata Zta in size and activation function. This region contains 7 aa differences between Akata and B95-8. The relevant region in B95-8 contains the Akata polymorphism at 4 of these amino acids, and a P3HR-1 amino acid at the other 3, allowing us to focus on these 4 amino acids. Further analysis by site directed mutagenesis of these four amino acids indicated that no single aa change could transfer the Akata phenotype but that a combination of Akata aa 124(P) and 146(A) resulted in both a larger Zta and greater transcriptional activation function.

Zta is post-translationally modified by phosphorylation, acetylation, SUMOylation. Neither of the Akata amino acids identified in this study are known to be post-translationally modified by these mechanisms. Further analysis of the mechanism by which these polymorphisms contribute both to apparent larger molecular mass as well as enhanced transactivation is ongoing and will be presented. Such strain variations may contribute to differences in biological behavior.

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