Hsp90 inhibitors:
A potential treatment for latent EBV infection?

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Epstein-Barr virus (EBV) is a human herpesvirus that causes infectious mononucleosis, and is associated with B-cell and epithelial cell malignancies.¹ Only one viral protein, EBNA1, is essential for sustained latent EBV infection of host cells. EBNA1 collaborates with the cellular synthetic machinery to replicate the EBV genome, and tethers the replicated virus to mitotic chromosomes during cellular division.² Therefore, inhibiting EBNA1 expression, or its functions, is an attractive strategy for treating EBV-related diseases. However, the development of drugs which inhibit EBNA1 has remained an elusive goal. Here, we discuss a recent finding that Hsp90 inhibitors decrease expression of EBNA1 in EBV-infected cells, and prevent the growth of EBV-infected malignant cells via an EBNA1-dependent mechanism.³

Hsp90 is an evolutionarily conserved molecular chaperone that is necessary for folding, stabilization, and functions of client proteins. Geldanamycin and its less toxic analogues (17-AAG and 17-DMAG) bind to the ATP-binding motif of Hsp90 and inhibit its activity as a protein chaperone, resulting in misfolding and/or degradation of client proteins.³⁵ Only a limited subset of cellular proteins are Hsp90 clients, and Hsp90 inhibitors are relatively nontoxic to normal cells. However, Hsp90 inhibitors, by inducing the misfolding and/or degradation of essential viral proteins, may provide a novel means for treating certain types of viral infections. For example, the poliovirus capsid protein, P1, requires Hsp90 for proper folding, and 17-AAG inhibits viral replication in poliovirus-infected mice.⁶ Hsp90 inhibitors are also highly toxic to some tumor cell types, reflecting not only the ability of these drugs to induce degradation of certain oncoproteins, but the fact that tumor cells have a higher level of the particular Hsp90 conformation that binds to geldanamycin analogues.⁵⁷

While EBV-positive tumors universally express EBNA1, several different types of viral latency can be found within tumor cells.¹ We found that Hsp90 inhibitors decrease expression of EBNA1 independent of the viral latency type, and that this effect occurs in both B-cells and epithelial cells.³ Furthermore, Hsp90 inhibitors decrease EBNA1 expression in plasmid-based assays performed in EBV negative cells.³ Although we initially hypothesized that EBNA1 itself is an Hsp90 client protein, our subsequent results indicated that this is unlikely the case. The drug effect on EBNA1 was not reversed by either proteosomal inhibitors or autophagy inhibitors, and the half life of EBNA1 was not decreased by the drugs.³ In addition, we did not find that Hsp90 and EBNA1 interact directly.³
These unexpected findings prompted us to ask whether EBNA1 translation is attenuated in the presence of Hsp90 inhibitors. EBNA1 contains an unusual internal Gly-Ala repeat domain that inhibits both EBNA1 translation and proteasomal pathway-mediated degradation. The Gly-Ala repeat domain ensures that EBNA1 is rarely translated in cells, but is highly stable once made. We found that geldanamycin inhibits the translation of EBNA1 in vitro, while not affecting translation of another viral protein expressed in the same vector. Furthermore, an EBNA1 mutant missing the Gly-Ala repeat domain was highly resistant to the effect of Hsp90 inhibitors both in vitro and in vivo. These results indicated that the Gly-Ala repeat domain of EBNA1 mediates much of the Hsp90 inhibitor effect.

Although the detailed mechanism(s) by which Hsp90 inhibitors reduce EBNA1 expression in cells have yet to be fully unraveled, our results suggest that one or more cellular Hsp90 client proteins are required for efficient translation of EBNA1 through the Gly-Ala repeat domain (Fig. 1). Consistent with this, certain ribosomal proteins are known Hsp90 client proteins. Interestingly, the poor translation efficiency of the Gly-Ala repeat domain is due to the purine-rich nature of the corresponding mRNA, rather than the protein sequence per se. While not required for the replicative functions of EBNA1 in vitro, the Gly-Ala repeat domain, by decreasing EBNA1 translation, reduces presentation of EBNA1-derived peptides on MHC class I and decreases its recognition by virus-specific T cells. Since EBV strains missing this domain have yet to be isolated, it may be required for persistence of the virus in humans.

What is the evidence that Hsp90 inhibitors might be useful for treating EBV-induced diseases in humans? We found that Hsp90 inhibitors prevent EBV transformation of primary B cells, induce killing of established EBV-transformed B cells, and effectively inhibit the growth of EBV-induced lymphoproliferative disease in SCID mice when given at low nontoxic doses. Although Hsp90 inhibitors reduce the expression of a variety of cellular proteins (such as NFkappaB) that are likely important for the viability of EBV-transformed B cells, the effect of low dose 17-DMAG on EBV-transformed B cells was substantially reversed by expression of the EBNA1 mutant missing the Gly-Ala repeat domain. Thus, at the low doses used in our experiments, the drug killing effect appears to be at least partially mediated through EBNA1 loss.

Clearly, Hsp90 inhibitors will need to be tested in humans before concluding that such drugs are useful for treating EBV-induced diseases at non-toxic doses in patients. Geldanamycin analogues are currently in clinical trials for various types of cancers. In addition, other classes of drugs in development, such as the purine-scaffold Hsp90 inhibitor PU-H71, show promise as being more potent, and less toxic, Hsp90 inhibitors. Of the various serious EBV-associated illnesses in humans, EBV-positive lymphoproliferative disease and fulminant mononucleosis are the two that most clearly require the continued presence of the EBV genome, and thus might be the best candidates for testing the therapeutic effect of Hsp90 inhibitors.

Acknowledgments

This work was supported by NIH grant P01 CA022443. We thank Bill Sugden for reviewing the manuscript.

References

Figure 1.
A model for the Hsp90 inhibitor effect on EBNA1. The Gly-Ala (GA) repeat domain of EBNA1 inhibits its translation. One or more cellular Hsp90 client proteins are required for efficient translation of EBNA1 through the GA repeat domain. Hsp90 inhibitors repress the chaperoning activity of Hsp90, reducing the functions of Hsp90 clients and therefore resulting in decreased translation of EBNA1 through the GA domain.

*Cell Cycle. Author manuscript; available in PMC 2010 June 2.*