HAUSP/USP7 as an Epstein–Barr virus target

M.N. Holowaty and L. Frappier
Department of Medical Genetics and Microbiology, University of Toronto, Toronto, Canada

Abstract

USP7 (also called HAUSP) is a de-ubiquitinating enzyme recently identified as a key regulator of the p53–mdm2 pathway, which stabilizes both p53 and mdm2. We have discovered that the Epstein–Barr nuclear antigen 1 protein of Epstein–Barr virus binds with high affinity to USP7 and disrupts the USP7–p53 interaction. The results have important implications for the role of Epstein–Barr nuclear antigen 1 in the cellular immortalization that is typical of an Epstein–Barr virus latent infection.

Introduction

EBV (Epstein–Barr virus) is a γ-herpes virus with potent growth-transforming ability, which is carried by most of the population as a lifelong, latent infection [1,2]. EBV is causally associated with a number of malignancies, including Burkitt’s lymphoma, nasopharyngeal carcinoma and post-transplant lymphoproliferative disorders. EBNA1 (Epstein–Barr nuclear antigen 1) is a viral DNA-binding protein required for the replication and segregation of EBV DNA episomes in dividing cells and, as such, is essential for viral persistence. In addition, EBNA1 can enhance the transcription of other viral latency genes and repress its own transcription. Several lines of evidence also suggest that EBNA1 plays a direct, but currently unknown, role in cellular immortalization. First, EBNA1 is required for the efficient immortalization of EBV-infected primary human B-cells in culture. Secondly, EBNA1 is the only EBV protein expressed in endemic Burkitt’s lymphoma and some post-transplant lymphoproliferative disorders, and the only viral protein consistently expressed in all EBV-associated tumours. Finally, expression of EBNA1 in two separate systems resulted in tumorigenicity in mice [3,4].

It is supposed that EBNA1 directs these functions by binding to human cellular proteins; however, until recently, few such interacting partners were identified. Since all previous EBNA1–protein interaction studies used yeast one- or two-hybrid systems, we recently adopted two biochemical approaches. Using EBNA1 affinity chromatography and TAP (tandem affinity purification) tagging in human cells, we isolated several new EBNA1-interacting proteins, as well as verifying previously reported EBNA1 interactions [5]. One of the specific protein interactions identified in both approaches was with the cellular de-ubiquitinating enzyme, USP7 (also known as HAUSP).

USP7/HAUSP

USP7 was first identified by virtue of its interaction with another herpes virus protein, the ICP0 protein of herpes simplex virus [6]. ICP0 is a promiscuous transactivator, which appears to function primarily through its E3 ubiquitin ligase activity [7]. Although targeted disruption of the ICP0–USP7 interaction results in a reduction in the ability of ICP0 to stimulate lytic replication in cell culture [8], these studies did not reveal the cellular function of USP7. However, the fact that proteins from two different herpes viruses targeted this protein, suggested that USP7 probably serves an important cellular role.

Insight into the cellular function of USP7 was provided by Li et al. [9], when they reported that USP7 specifically binds and de-ubiquitinates p53 and, therefore, stabilizes this key tumour suppressor. In keeping with these findings, overexpression of USP7 resulted in p53-mediated apoptosis [9]. A previous report also linked USP7 to programmed cell death, as USP7 was found to be cleaved in primary thymocytes undergoing apoptosis [10]. However, the role of USP7 in p53 regulation was recently shown to be more complicated than originally thought, as ablation of USP7 expression resulted in p53 accumulation as opposed to the expected destabilization of p53 [11,12]. This effect has been traced to the ability of USP7 to stabilize mdm2, an ubiquitin ligase that promotes the degradation of p53. Therefore USP7 appears to play multiple roles in regulating the p53–mdm2 pathway.

The EBNA1–USP7 interaction affects EBNA1 function

The region of EBNA1 responsible for the USP7 interaction was mapped by mutational analysis to within amino acids 395–450 [5]. This sequence, which had not previously been functionally characterized, falls just N-terminus to the DNA-binding domain (residues 459–607) (Figure 1A). TAP-tagging experiments performed with an EBNA1 mutant lacking this sequence (A395–450) in human cells showed that this deletion disrupted binding to USP7 without affecting other cellular protein interactions.
USP7 was shown to de-ubiquitinate EBNA1 detectably affected by the same mutation [5]. While purified was slightly decreased and the segregation function was not type EBNA1, whereas the transcriptional activation activity based plasmids four times more efficiently than did wild-

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in vivo

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395–450 mutation had no effect on the stability of EBNA1 in vitro [5,13]. The results suggest that USP7 binding inhibits the ability of EBNA1 to activate DNA replication. The reason for this inhibition is not yet clear but could indicate that (i) EBNA1 activity is regulated by ubiquitination, (ii) USP7 recruitment to the origin through EBNA1 affects the ubiquitination state and activity of cellular proteins at the origin or (iii) USP7 binding to EBNA1 physically inhibits the interaction of EBNA1 with the origin DNA or with cell-

Functional studies in human cells showed that the Δ395–450 EBNA1 mutant supported the replication of EBV-based plasmids four times more efficiently than did wild-type EBNA1, whereas the transcriptional activation activity was slightly decreased and the segregation function was not detectably affected by the same mutation [5]. While purified USP7 was shown to de-ubiquitinate EBNA1 in vitro, the Δ395–450 mutation had no effect on the stability of EBNA1 in vitro [5,13]. The results suggest that USP7 binding inhibits the ability of EBNA1 to activate DNA replication. The reason for this inhibition is not yet clear but could indicate that (i) EBNA1 activity is regulated by ubiquitination, (ii) USP7 recruitment to the origin through EBNA1 affects the ubiquitination state and activity of cellular proteins at the origin or (iii) USP7 binding to EBNA1 physically inhibits the interaction of EBNA1 with the origin DNA or with cell-

Domain structure of USP7
Partial proteolysis was used to define the structural organization of USP7 and identified four domains [13,14] (Figure 1B). The catalytic domain is composed of amino acids 208–560, and the crystal structure of this domain was recently solved [14]. The N-terminal 200 amino acids are predicted to form a TRAF domain [15], and have been found to bind p53 [9]. By passing a mixture of USP7 domains over an EBNA1 affinity column, we found that this N-terminal domain was also responsible for the EBNA1 interaction [13]. The C-terminal half of USP7 is comprised of two structural domains, and we found that one of these (residues 599–801) mediates the interaction with ICP0 [13]. It is not yet known which of the USP7 domains is responsible for the interaction with mdm2.

EBNA1 effectively competes with p53 for USP7 binding
Our finding that EBNA1 interacts with the same region of USP7 as p53 suggests that EBNA1 might affect the ability of USP7 to bind and de-ubiquitinate p53 [13]. To investigate further this possibility, we used tryptophan fluorescence to quantify the binding of the EBNA1 395–450 peptide to the USP7 N-terminal domain, and compared the binding affinity with that of p53 peptides spanning the 357–382 sequence previously shown to be sufficient for the USP7 interaction [14]. The EBNA1 peptide bound USP7 with 10-fold higher affinity than the p53 peptides and was subsequently shown by gel filtration analysis to compete effectively with p53 sequences for USP7 binding [13]. These results indicate that EBNA1 and p53 bind the same or overlapping sites in the USP7 N-terminal domain and that EBNA1 is capable of sequestering USP7 from p53.

Conclusion
These results are the first indication of a biochemical link between the EBNA1 protein of EBV and p53. On the basis of our current results, we propose that EBNA1 may interfere with the normal regulation by USP7 of the p53–mdm2 pathway. Our in vitro results suggest that EBNA1 could disrupt the USP7–p53 interaction in vivo, which would be expected to decrease p53 levels, resulting in increased cell survival and proliferation, as is seen in most forms of EBV-latent infection. Depending on the region of mdm2 that interacts with USP7, sequestration of USP7 by EBNA1 may also result in decreased mdm2 levels, which should increase p53 levels, leading to its downstream apoptotic effects. Thus it is possible that the delicate interplay between EBNA1 and USP7 may contribute to either cell death or cellular immortalization under different circumstances.

References
8 Everett, R.D., Meredith, M. and Ori, A. (1999) J. Virol. 73, 417-426

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