CHAPTER 5

The Significance of the CtBP – AdE1A Interaction during Viral Infection and Transformation

Roger J.A. Grand,* Claire Baker, Paola M. Barral, Rachel K. Bruton, Julian Parkhill, Tadge Szestak and Philip H. Gallimore

Abstract

C-terminal binding protein (CtBP) associates with adenovirus early region 1A (AdE1A) proteins through a highly conserved PXDLS motif located very close to its C-terminus in conserved region 4. To try to understand the importance of this interaction for the virus a point mutation in the CtBP binding site of Ad12E1A (P→S at amino acid 255) was engineered. The mutant Ad12E1A DNA (Ad12E1A6f) encoded a protein temperature sensitive (ts) for transformation of baby rat kidney cells when in combination with Ad12E1B. At 33°C transformation frequency was comparable to *wt*. At 37° and 38.5° transformants appeared as larger epithelioid cells and colonies senesced relatively rapidly. When the Ad12 6f AdE1A was incorporated into a mutant virus it caused a marked reduction in its ability to replicate with only Ad12E1A and Ad12E1B19K being expressed at early times. It was observed that 6fE1A bound to CtBP very inefficiently. Ad12E1 transformed rat cell lines, carrying the 6f mutation were established from the 33°C transformants but failed to express the Ad12E1B54K protein. After a number of weeks in culture the cells developed a mesenchymal character; expression of proteins such as E-cadherin, P-cadherin and γ catenin was much reduced and expression of fibronection increased. These observations are consistent with inhibition of CtBP activity in *wt* Ad12E1 transformants but not in the 6f transformed cells. In a complementary study the effect of down-regulation of CtBP expression (using siRNA protocols) was examined. Consistent with results obtained with the 6f virus it was observed that reduction in expression of CtBP1 and CtBP2 facilitated viral infection and this effect was enhanced when expression of C-terminal interacting protein (CTIP) was also reduced.

Introduction

Adenovirus early region 1A (AdE1A) is the first viral protein to be expressed following viral infection and is essential for Ad-mediated transformation of mammalian cells in culture.[1,2] AdE1A's primary, although not only, role is as a regulator of transcription and it is through this activity that it can drive expression of other viral early region genes and usurp the cellular mechanisms of growth control.[3,4]

*Corresponding Author: Roger J.A. Grand—Cancer Research U.K. Institute for Cancer Studies, University of Birmingham, Birmingham B15 2TT U.K. Email: R.J.A.Grand@bham.ac.uk.

CtBP Family Proteins, edited by G. Chinnadurai.

As AdE1A appears to possess no enzymic activity and is unable to bind to DNA it is likely that all its activities are dependent upon interaction with cellular proteins.[1,5,6] The binding sites for these are distributed throughout the E1A molecule although most are concentrated within the N-terminal region and those amino acid sequences which are most highly conserved between AdE1As from different virus serotypes.[7-9] There are considered to be four conserved regions (CRs), with, for example, CR1 and CR2 containing binding sites for the Rb family of proteins;[10,11] CR1, together with the N-terminal region, interacting with CBP/p300[12] and CR3 binding to a variety of proteins involved in transcriptional activation such as TBP, transcription factors and TAFs.[13] Conserved region 4 has, in the past, been most notable for interaction with C terminal binding protein (CtBP) but more recently it has been shown to contain binding sites for Dyrk and $p27^{kip1}$.[14,15] The N-terminal region is involved in the association of AdE1A with regulatory components of the proteasome,[16] with p400 and TRRAP-containing complexes[17,18] and with TBP.[19] Space precludes a detailed consideration of the multiple interactions of AdE1A[1,2] but it is notable that binding to certain partners can be associated with particular biological properties of E1A. For example, interaction with the Rb family and CBP/p300 is necessary for initiation of cell cycle progression during viral infection and for cellular transformation.[20-22] Similarly binding to transcription factors and the basic transcriptional machinery through CR3 is required for the expression of other viral early region genes (reviewed see ref. 13). A further host cell binding protein which appears to be of considerable significance in determining the activities of AdE1A is CtBP-a transcriptional corepressor which interacts with a highly conserved motif occurring in CR4 very close to the C-terminus of virtually all AdE1As.[8,9,14,23]

CtBP was first isolated on the basis of its ability to bind to exon 2 of AdE1A.[14] The essential site of interaction on AdE1A comprises a short amino acid sequence, PXDLS, now known to be widespread in CtBP binding proteins.[23-26] However, considerable effort has been devoted to understanding whether amino acids outside the PXDLS sequence can contribute to the binding motif. Data derived from a study of synthetic peptides and AdE1A protein domains certainly suggest that this might be the case. For example it has been shown that substitution of amino acids outside the PXDLS site causes changes in Kd of peptides for CtBP.[27] Similarly, synthetic peptides with identical PXDLS motifs but with different surrounding sequences can have different K_i for the inhibition of Ad12E1A binding to CtBP1.[28] Furthermore it appears that full-length Ad12E1A will bind more strongly to CtBP than a polypeptide encompassing exon 2 (amino acids 190-266) and this, in turn, binds with higher affinity than a synthetic peptide comprising only 20 amino acids, but still containing PVDLS.[29] In addition it has been reported that mutations in exon 2 of AdE1A, outside the PXDLS motif, produce biological effects generally attributed to inhibition of CtBP interaction (compare refs. 14,30,31) CtBP appears to function primarily as a transcriptional corepressor. This may be through interaction with other coregulating proteins such as members of the human polycomb family,[32] *Drosophila* short range and long range repressors, such as Knirps, Snail and Hairy,[33,34] and/or through direct binding to histone deacetylases (discussed in more detail in refs. 24,26). It has been noted that HDAC-4 and HDAC-7 and perhaps HDAC-5 contain PXDLS motifs which are probably sites for CtBP binding but that HDAC-1, HDAC-2 and Sin3 interact in a PXDLS-independent manner.[15,35]

Two CtBP genes (1 and 2) have been mapped in mammals and these are approximately 80% homologous. Relatively little difference has been observed between the CtBP1 and CtBP2 proteins at the biochemical level but knock-out animals have appreciably different life expectancies.[36] CtBP1-/- mice are fertile but approximately 30% smaller than *wt* animals.[36] The CtBP2-/- mice die in utero at E10.5, possibly due to incorrect development of the placenta. It appears that these embryos also have defects in heart and neural development. A third CtBP protein (CtBP3) has been isolated from rat brain and been shown to possess acyl transferase activity.[37] This protein is, in fact, the product of alternate splicing of the CtBP1 gene, such that two proteins are coexpressed, differing only in the 12 N-terminal amino acids, in human,