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ScienceDirect
Virology 370 (2008) 255–263
VIROLOGY
www.elsevier.com/locate/yviro

Role of SV40 ST antigen in the persistent infection of mesothelial cells

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Received 10 January 2007; returned to author for revision 12 February 2007; accepted 7 September 2007
Available online 23 October 2007

Abstract

Viral DNA is maintained episomally in SV40 infected mesothelial cells and virus is produced at low but steady rates. High copy numbers of the viral DNA are maintained in a WT infection where both early antigens are expressed. In the absence of ST, cells are immortal but non-transformed and the infected cells maintain only a few copies of episomal viral DNA. We show that ST expression is necessary for the maintenance of high copy numbers of viral DNA and that the PP2A binding ability of ST plays a role in genome maintenance. Interestingly, an siRNA to the virus late region downregulates virus copy number and virus production but does not prevent the anchorage-independent growth of these cells. Furthermore, addition of virus neutralizing antibody to culture media also decreases copy numbers of viral DNA in WT-infected cells, suggesting that virus production and re-infection of cells may play a role in maintaining the persistent infection.

Keywords: SV40 small-t; Mesothelial cells; Episomal genome; Persistent infection

Introduction

SV40 virus is known to replicate productively in monkey kidney cells in which large amounts of virus are produced and cells are killed by the infection. In contrast, the viral DNA does not replicate in rodent cells but may become integrated into the cellular genome, resulting in cellular transformation. Infection of human fibroblasts causes a third type of virus–host interaction, termed semi-permissive. Once these cells are infected, a small fraction of the cells becomes permissive at any given time. These cells show high levels of viral DNA replication and virion production and die of the infection releasing virus to culture media. Most of the cells show only a low level of viral DNA and late protein synthesis. As first described by Carbone (Bocchetta et al., 2000), human mesothelial cells define a fourth type of infection in which all cells remain persistently infected over long periods of time and none of the cells show evidence of cytopathic effect. As for other DNA tumor viruses, such as Human Papilloma Virus (HPV) and Epstein–Barr Virus (EBV) (Adams and Lindahl, 1975; LaPorta and Taichman, 1982), the SV40 DNA is maintained as an episome in mesothelial cells and very low levels of virus are chronically produced. Whereas the relationship between the expression of viral proteins and maintenance of viral DNA has been more thoroughly examined with HPV and EBV, little is known about the maintenance of the viral genome in persistently infected mesothelial cells. Examining the state and the maintenance of the viral genome in these cells is the subject of this report.

The SV40 large-T (LT) antigen is essential to the replication of viral DNA in permissive and semi-permissive primate and human cells. LT binds the origin of replication and interacts with cellular proteins necessary for the initiation of replication (Fanning, 1992; Prives et al., 1980; Stadlbayer et al., 1996). It is likely that this is essential for persistence in mesothelial cells because some mesothelial cell lines transformed by SV40 virus infection displayed reduced viability after downregulation of LT (Bocchetta et al., 2000).

Another SV40 protein, small-t (ST) antigen, can also play a critical role in the transformation of cells. This is especially the case in less proliferative cells, most likely due to the ability of ST to stimulate mitogenic and anti-apoptotic proteins and down-regulate proteins that inhibit cell cycling (Howe and Tan, 1977; Porras et al., 1999; Sontag et al., 1993; Watanabe et al., 1996). ST is essential for the transformation of several human cell types,

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0042-6822/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2007.09.008

in conjunction with LT (Hahn et al., 2002; Yu et al., 2001), even in the absence of oncogenes such as EJ-ras. The primary effects of ST occur through its interaction with protein phosphatase 2A (PP2A) in which it displaces many regulatory subunits of this trimeric enzyme. The deregulation of PP2A activity and altered intracellular localization serve to stimulate cells to enter the cell cycle through sustained phosphorylation of key cell cycle related proteins (Howe et al., 1998; Sontag et al., 1993; Watanabe et al., 1996).

Recently, we established a series of mesothelial cell lines that were immortalized by LT and the cellular telomerase protein h-TERT. These cells have now been in culture for several years and show little evidence of transformation. When these cells express ST, introduced either by virus infection or transfection, the cells lose their contact inhibition and become anchorage independent. The differences in the behavior of these cells and the fact that ST can influence viral DNA replication (Cicala et al., 1994; Lin et al., 1998; Virshup et al., 1989) made it of interest to examine the state of the viral genome in these cells and the role ST might play in genome maintenance. In the experiments described here, we show that high levels of viral DNA are maintained in cells infected with WT SV40 while viral DNA levels in ST mutant infections are extremely low. ST is also required to maintain higher copy numbers of superinfecting genomes, and this ability is disrupted when ST does not have a fully functional PP2A binding domain. Finally, once anchorage-independent cells are obtained, high levels of viral DNA or infectious virus are not required for cells to remain transformed. These are dramatically reduced by growth of cells in the presence of SV40 neutralizing antibody, yet these cells continue to show anchorage-independent growth.

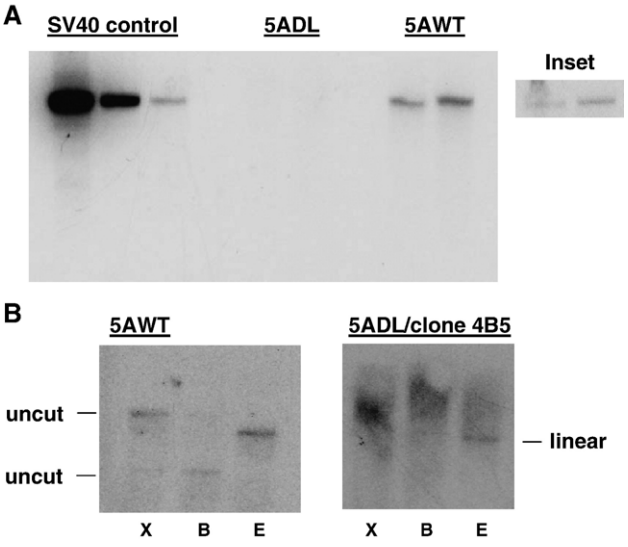

Fig. 1. Detection of viral DNA in mesothelial cell lines. Total genomic DNA was isolated from 5ADL and 5AWT cells as described in Materials and methods. DNA from approximately 5×10^5 cells was linearized with *Eco*RI and run on an agarose gel for analysis by Southern blotting. Viral DNA was detected by incubation with a probe made from *Hin*dIII-digested SV40 DNA. (A) The membrane was exposed until signal was detected in 5AWT samples. Control lanes are loaded with 25, 2.5 or 0.25 ng of linear SV40 plasmid. This represents a range between 5×10^9 and 5×10^7 total copies of SV40. *Inset*: a ten-times longer exposure of the 5ADL lanes shown in panel A. (B) Genomic DNA was prepared from 5AWT cells or a clone of 5ADL, clone 4B5. DNA was digested with *Xba*I (X) or *Bgl*II (B), enzymes that do not cut SV40, or with *Eco*RI (E), which cuts SV40 once. The positions of form I and nicked circular SV40 DNA are labeled as uncut. The position of linear SV40 DNA is also shown. After digestion, 5AWT DNA was diluted 20-fold before running on the same agarose gel as the DNA from the 5ADL clone.

Table 1
Real-time PCR analysis of low molecular weight DNA from persistently infected mesothelial cells[a]

Cells	SV40 copies/cell
Experiment 1	
5AWT pool	217.9
5ADL clone 4C10	1.5
5ADL clone 5C2	2.1
5ADL clone 4B5	13.6
5ADL clone 4E2	0.5
5ADL clone 4C4	1.6
Experiment 2	
5AWT pool	622.0
5ADL pool	4.4
5AWT/si	1.7

[a] SV40 low molecular weight DNA was isolated with a Genelute Plasmid Miniprep Kit (Sigma) and analyzed as described in Materials and methods.

Results

As we have shown previously (Yu et al., 2001), TERT-expressing mesothelial cells become immortalized, but not transformed, when persistently infected with DL888, a virus that does not produce ST. In contrast, cells infected with WT virus were both immortalized and transformed as indicated by anchorage independence. The pools described in this report are referred to as 5ADL and 5AWT, respectively; additional pools have also been made and show behaviors identical to those of 5ADL and 5AWT. Both the 5ADL and 5AWT cell lines express LT at similar levels, as determined by Western blotting (data not shown), so differences in anchorage-independent growth reflect only the absence of ST in 5ADL cells.

It has been reported that the SV40 genome is maintained as an episome in mesothelial cells infected with WT SV40 (Carbone et al., 2003) and low levels of virus are continuously produced. We have found that the levels of virus produced in the absence of ST are far lower than levels produced from WT-infected cultures. For example, a 6 cm dish of 5AWT cells (over 10^6 cells) produces a total of 10^3–10^4 pfu in a 1-week period. In contrast, a 6 cm dish of 5ADL cells produces fewer than 10 pfu, although virus can always be detected by plaque assay or co-cultivation with permissive monkey kidney cells (data not shown).

The state of the SV40 genome was determined in 5ADL and 5AWT cells that had been in culture for several months. Total genomic DNA was isolated from 5ADL and 5AWT cells, linearized with *Eco*RI, then analyzed by Southern blotting. 5AWT cells contain easily detectable genome-length copies of SV40 DNA (Fig. 1A). The intensity of the episomal DNA from 5AWT was consistent with these cells containing at least 100 copies per cell. More accurate quantitation was obtained using