JOURNAL OF VIROLOGY, Apr. 1991, p. 1829–1835
0022-538X/91/041829-07$02.00/0

Vol. 65, No. 4

Down Regulation of Poliovirus Receptor RNA in HeLa Cells Resistant to Poliovirus Infection

GERARDO KAPLAN AND VINCENT R. RACANIELLO*

Department of Microbiology, College of Physicians and Surgeons, Columbia University, 701 West 168th Street, New York, New York 10032

Received 10 August 1990/Accepted 21 December 1990

A line of HeLa cells (SOFIA) was previously isolated that is resistant to poliovirus infection and does not express functional virus binding sites at the cell surface. The expression of the poliovirus receptor (PVR) gene in SOFIA cells was examined to determine the molecular basis for the failure of these cells to express PVRs. Southern blot analysis of genomic DNA revealed that the PVR gene in SOFIA cells did not contain gross alterations. However, PVR transcripts were not detected in Northern (RNA) blot analysis of SOFIA cell RNA. In vitro nuclear run-on analysis showed that transcription of PVR-specific RNA was reduced in SOFIA cells. Treatment of SOFIA cells with 5-azacytidine restored susceptibility to poliovirus infection, which correlated with the appearance of PVRs at the cell surface, as detected with anti-PVR monoclonal antibody D171. PVR RNA was detected in clones derived from 5-azacytidine-treated SOFIA cells. SOFIA cells were converted to poliovirus sensitivity at a rate of 5 to 7%, suggesting that down regulation of PVR expression involved few cellular targets. Resistance of SOFIA cells to poliovirus infection therefore appears to result from down regulation of PVR RNA, leading to lack of PVR expression at the cell surface. Methylation may play a role in regulating the expression of the PVR gene, which is not essential for survival of HeLa cells.

Poliovirus infection of primate cell cultures usually results in rapid cell death. Cell killing depends on both viral and cellular determinants, few of which have been identified. One approach to elucidating cellular requirements for virus-mediated cell killing has been to isolate cell lines that are partially or totally resistant to picornavirus infection. Although a number of such cell lines have been isolated (14, and references therein), in most cases, the mechanism of resistance to viral infection is not known.

To identify host requirements for poliovirus replication, we recently isolated HeLa cell lines that are resistant to infection with this virus (14). These cell lines were obtained by cotransfecting HeLa cells with poliovirus RNA and an in vitro-synthesized subgenomic replicon lacking sequences encoding the viral capsid proteins (15). Some cells survived the viral infection and could be propagated, despite continuous production of virus during passage. Over 6 months, the cells underwent periods of crisis accompanied by extensive cell death, followed by periods of recovery and proliferation. After 6 months, cell lines were isolated that grew stably in culture and no longer produced poliovirus but supported viral infection with different levels of efficiency. One line, SOFIA, was isolated that was resistant to poliovirus infection due to restrictions at the cell surface and within the cell (14). The nature of the intracellular block to poliovirus infection in SOFIA cells is not yet known, whereas the basis of the cell surface restriction is the topic of this study.

SOFIA cells do not adsorb poliovirus and do not express the epitope recognized by monoclonal antibody D171, directed against the cellular receptor for poliovirus (14). Here we show that poliovirus receptor (PVR) mRNA cannot be detected in SOFIA cells and that transcription of the PVR gene appears to be down regulated. Treatment of SOFIA cells with 5-azacytidine (5-azaC), a potent demethylating agent, resulted in partial restoration of susceptibility to poliovirus infection, accompanied by increased levels of PVR mRNA and cell surface reactivity with D171. These results demonstrate that SOFIA cells do not bind poliovirus because stable PVR mRNA is not present. Treatment with a demethylating agent, which restores susceptibility to infection, may activate transcription of the PVR gene or a gene required for PVR expression.

MATERIALS AND METHODS

Cells and viruses. HeLa S3 and SOFIA cells (14) were grown in either suspension cultures or monolayers. For poliovirus titration, 2×10^6 HeLa cells grown in spinner cultures were plated in 6-cm plastic cell culture dishes 24 h before use (16). For purification of genomic DNA or cellular RNA, 2×10^7 cells were plated in 15-cm plastic cell culture dishes 48 h before use and grown in Dulbecco modified Eagle medium containing 10% horse serum (16). Stocks of poliovirus P1/Mahoney were prepared in HeLa cells as described previously (16).

For infectious centers assay, monolayers were infected with poliovirus at a multiplicity of infection of 10 and processed as described previously (14).

Antisera. Monoclonal antibody D171 directed against the HeLa cell PVR (21) was purified and used as described previously (14). Sheep anti-mouse immunoglobulin ^{125}I-labeled antibodies (2,000 Ci/mmol) were obtained from Amersham Co.

Genomic DNA extraction and Southern hybridization analysis. Genomic DNA from HeLa and SOFIA cells was isolated from monolayers as described previously (2). DNA was digested to completion with *Sac*I as recommended by the manufacturer (New England BioLabs), fractionated on 0.8% agarose gels, and transferred to nitrocellulose paper (Schleicher & Schuell) in 10× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) as described previously (18). Membrane-bound DNA was hybridized to a ^{32}P-labeled *Xho*I-*Eco*RI cDNA fragment of pSVL-H20A (20) containing

* Corresponding author.

nearly all the coding sequence of the PVR, prepared by oligonucleotide-primed DNA synthesis (Pharmacia) to a specific activity of 10^9 cpm/μg of DNA. The filter was washed three times (15 min each) with 2× SSC–0.1% sodium dodecyl sulfate (SDS) at room temperature and three times at 65°C. Autoradiography was for 48 h at −70°C with Kodak XAR film (Eastman Kodak Co.) and a single intensifying screen.

Isolation of RNA and Northern (RNA) hybridization analysis. Total RNA from HeLa and SOFIA cells was prepared by the guanidine thiocyanate-CsCl technique (18). Poly(A)$^+$ RNA was selected by chromatography on prepacked oligo(dT)-cellulose columns (Collaborative Research) according to the manufacturer. RNA was fractionated in 1% agarose–formaldehyde gels, transferred to nitrocellulose paper, and hybridized with a ^{32}P-labeled double-stranded DNA prepared as described above. DNA probes were either the cDNA fragment described above for Southern analysis or a 1.0-kb *Pst*I-*Xba*I cDNA fragment encoding human γ-actin (obtained from L. Kedes). Prehybridization was done for 4 h at 42°C in a solution containing 50% formamide, 5× SSCPE (1× SSCPE is 150 mM NaCl, 15 mM sodium citrate, 13 mM KH_2PO_4, 1 mM EDTA, pH 7.2), 5× Denhardt's solution (50× Denhardt's solution is 1% Ficoll DL, 1% bovine serum albumin, 1% polyvinylpyrrolidone), and 500 μg of denatured herring sperm DNA per ml. Hybridization reactions were done for 22 h at 42°C in 50% formamide–5× SSCPE–1× Denhardt's solution–100 μg of denatured herring sperm DNA per ml–10% dextran sulfate–150 ng of ^{32}P-labeled DNA probe.

In vitro nuclear transcription assay. This assay was a modification of published procedures (3, 7, 19, 24). Nuclei were isolated from 7 × 10^8 HeLa or SOFIA cells by suspending phosphate-buffered saline-washed cells in 4 ml of reticulocyte standard buffer (RSB) containing 1% Nonidet P-40 (23), incubating them for 10 min on ice, and centrifuging them for 1 min at 15,000 × *g*. The nuclear pellet was resuspended in 1 ml of RSB–1% Nonidet P-40, pelleted, and washed three times with reaction mix consisting of 20 mM Tris (pH 7.4), 140 mM KCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, and 20% glycerol. Nuclear transcription was done with 5 × 10^8 nuclei in 1 ml of reaction mix containing GTP, CTP, and ATP (1 mM each), and 1 mCi of [α-^{32}P]UTP (3,000 Ci/mmol; Dupont, NEN Research Products) at 30°C for 20 min. The reaction was terminated with 1 ml of 2× HSB (20 mM Tris [pH 7.4], 1 M NaCl, 100 mM $MgCl_2$, 4 mM $CaCl_2$) and 20 μg of DNase I per ml for 30 min at room temperature; 2.5 ml of 2× TES (20 mM Tris [pH 7.4], 20 mM EDTA, 0.4% SDS) and 300 μg of tRNA were added prior to two phenol-chloroform extractions at 65°C. RNA was precipitated from the aqueous phase with 5% trichloroacetic acid and 60 mM $Na_2P_2O_7$ and filtered through 25-mm-diameter filters (HAWP; 0.45-μm pore size; Millipore). After extensive washing with 5% trichloroacetic acid and 60 mM $Na_2P_2O_7$, filters were placed in glass vials containing 500 μl of 1× TES and incubated for 10 min at 65°C to elute RNA from the filter. This elution step was repeated until less than 0.1% of the counts remained associated with the filter. RNA was precipitated with 2 volumes of ethanol, resuspended in 50 μl of H_2O, and boiled for 3 min and placed on ice before addition to the hybridization buffer.

Filters were spotted with 5 μg of a 1.0-kb *Xho*I-*Eco*RI cDNA fragment from plasmid pSVL-H20A (20) that contains almost all the coding sequence of the PVR, a 1.0-kb *Pst*I-*Xba*I cDNA fragment encoding human γ-actin, and *Eco*RI-linearized pUC19. Labeled RNA (5 × 10^7 cpm) from either HeLa or SOFIA cells was hybridized with a separate filter containing these three DNAs. Prehybridization and hybridization were done at 42°C for 4 h and 3 days, respectively, in 1 ml of the same solutions used for Northern hybridization. Filters were washed with 2× SSC at 65°C three times (10 min each) and once at room temperature. High background was eliminated by washing filters with 30 ml of 2× SSC containing 100 units of boiled RNase T_1 and 10 μg of boiled RNase A for 1 h at 37°C; 750 μl of 20% SDS and 150 μl of proteinase K (20 mg/ml) were added and incubated for 30 min at 37°C. Finally, filters were washed three times with 2× SSC–0.2% SDS at 65°C and exposed for 3 to 10 days as described for Southern analysis.

Treatment of cells with 5-azaC and analysis of PVR induction. To optimize the concentrations of 5-azaC suitable for our experiments, we grew cells for at least two generations in the presence of 5, 10, or 20 μM 5-azaC as described previously (8, 9); the latter concentration was found to be cytotoxic and was not used further. Monolayers grown in the presence of 5 and 10 μM of 5-azaC were infected with poliovirus at a multiplicity of infection of 10, and the kinetics of virus production were determined. There were no differences in poliovirus yield in the presence of 5 or 10 μM of 5-azaC (data not shown), and therefore the latter concentration was used for subsequent experiments. For poliovirus infection and infectious centers assay, 6-cm dishes were seeded with 10^6 HeLa or SOFIA cells containing regular growth media with 5-azaC. Cells were grown in the presence of 5-azaC for 60 h to 80% confluence and then were infected with poliovirus type 1 Mahoney at a multiplicity of infection of 10. After 60 min of adsorption, monolayers were washed extensively and either trypsinized for infectious centers assay or incubated for 48 h at 37°C for analysis of virus yield; for the latter, aliquots of medium were taken at different times and titrated on HeLa cells (16).

For detection of PVR at the cell surface, 96-well plates were seeded with 10^4 HeLa or SOFIA cells per well and grown for 60 h in growth media with or without 10 μM 5-azaC. Cells were stained with 10-fold dilutions of monoclonal antibody D171 and then with ^{125}I-labeled sheep anti-mouse immunoglobulin (1 μCi per well), as described previously (14). After extensive washing, plates were autoradiographed.

RESULTS

Analysis of PVR gene in SOFIA cells. SOFIA cells are variants of HeLa cells that are resistant to poliovirus infection. We previously showed that poliovirus cannot infect SOFIA cells because cell receptors for the virus are absent (14). As a first step toward determining the molecular basis for the lack of functional PVR in SOFIA cells, we examined the state of the PVR gene by Southern hybridization analysis. There were no differences in the patterns of *Sac*I DNA fragments observed in HeLa cells, SOFIA cells, and cloned SOFIA cell lines (Fig. 1). Similar results were obtained when other restriction enzymes were used (data not shown), suggesting that loss of PVR expression in SOFIA cells was not due to gross alterations in the PVR gene.

Down regulation of PVR RNA in SOFIA cells. In HeLa cells, a 3.3-kb PVR RNA is detected by Northern blot analysis of total cellular RNA, and in addition a 5.6-kb mRNA can be detected when poly(A)$^+$ RNA is analyzed (20). Northern hybridization analysis of total cellular RNA, using a PVR cDNA probe, revealed that SOFIA cells do not express stable levels of the 3.3-kb PVR RNA (Fig. 2).

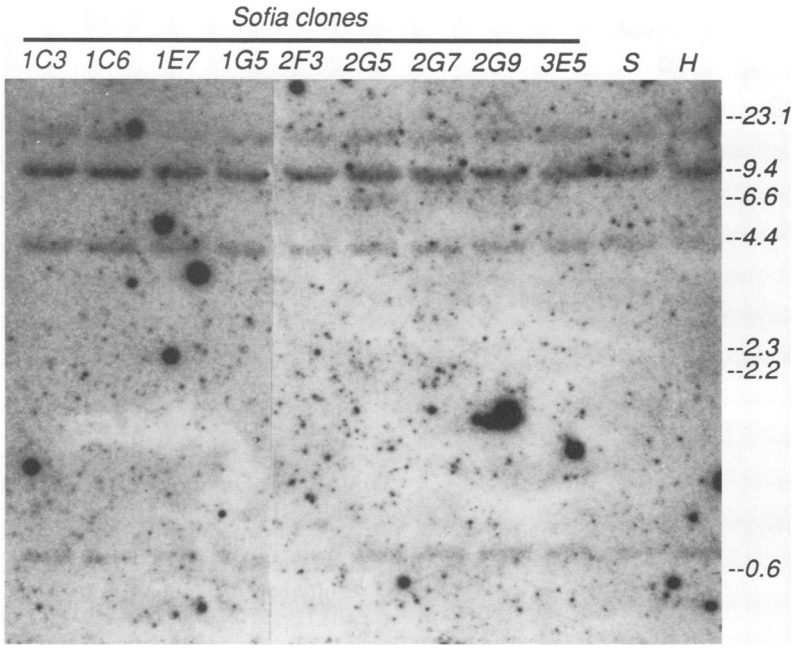

FIG. 1. Southern blot analysis of PVR gene in SOFIA cells. DNA samples (10 μg) of HeLa cells (H), uncloned SOFIA cells (S), and several single-cell clones derived from SOFIA cells (1C3, 1C6, 1E7, 1G5, 2F3, 2G5, 2G7, 2G9, and 3E5) were digested with *Sac*I, fractionated in a 0.8% agarose gel, transferred to nitrocellulose, and hybridized with a ^{32}P-labeled cDNA probe representing almost all the coding sequence of the PVR gene. Positions of marker DNAs (*Hin*dIII fragments of bacteriophage λ DNA) are indicated, with sizes in kilobase pairs.

Northern analysis of poly(A)$^+$ RNA from one SOFIA clone, 2G9, confirmed the absence of stable 3.3-kb mRNA and in addition demonstrated absence of the 5.6-kb mRNA as well (Fig. 3).

To determine whether the absence of PVR mRNA in SOFIA cells was due to transcriptional down regulation or to posttranscriptional events such as degradation, we determined the transcription rate of the PVR gene in HeLa and SOFIA cells by in vitro nuclear run-on analysis. Similar amounts of ^{32}P-labeled RNA probes isolated from in vitro nuclear run-on transcription assays were hybridized to nitrocellulose filters containing PVR cDNA, human γ-actin cDNA as an internal standard, and pUC18 as negative control. Transcription of the PVR gene is very low in HeLa cells compared with γ-actin (Fig. 4). In SOFIA 1E7 and 2G9 clones, transcription of the PVR gene was barely detectable. Scanning of the filters indicated that transcription of the PVR gene was three- to fivefold lower in 1E7 and 2G9 cells than in HeLa cells. The transcription rate of γ-actin was similar in all three cell lines.

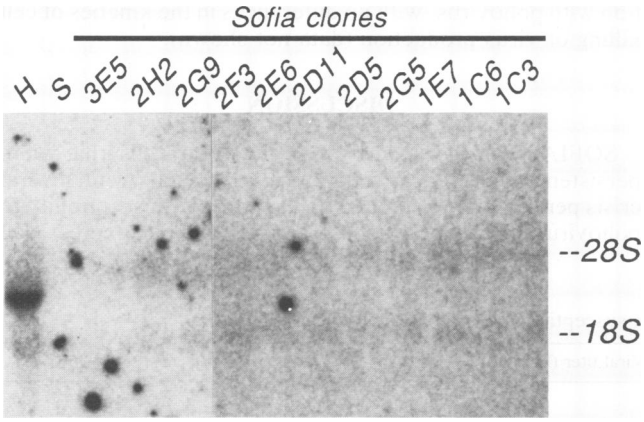

FIG. 2. Northern blot analysis of total cell RNA from SOFIA cells. Samples (10 μg) of total cellular RNA from HeLa cells (H), uncloned SOFIA cells (S), and several single-cell clones derived from SOFIA cells (1C3, 1C6, 1E7, 2G5, 2D5, 2D11, 2E6, 2F3, 2G9, 2H2, and 3E5) were fractionated in a 1% agarose–formaldehyde gel, transferred to nitrocellulose, and hybridized with a ^{32}P-labeled cDNA probe representing almost all the coding sequence of the PVR gene. Positions of 28S and 18S rRNA markers are indicated.

5-azaC-treated SOFIA cells regain susceptibility to poliovirus infection. Many silent genes can be activated by treatment of cells with the demethylating agent 5-azaC (4). Therefore, it was of interest to determine whether susceptibility of SOFIA cells to poliovirus infection could be restored by treatment of SOFIA cells with 5-azaC.

The effect of 5-azaC treatment on poliovirus yield in two single-cell clones derived from SOFIA cells (1E7 and 2G9) and in HeLa cells was determined (Table 1). Poliovirus titers increased 10^5-fold in 5-azaC-treated SOFIA cells after 24 h of infection, whereas very little increase in viral titer was observed in untreated cells compared with untreated HeLa cells. Virus yield in HeLa cells was not affected by 5-azaC treatment. There was no further increase in poliovirus yield at 72 h postinfection in SOFIA cells, and most of the SOFIA cells did not show cytopathic effect. In contrast, HeLa cells were completely destroyed at 24 h postinfection. Mouse L cells, which do not express PVR, remained resistant to poliovirus infection after treatment with 5-azaC (data not shown).

An infectious centers assay was used to determine the fraction of SOFIA cells that regained susceptibility to poliovirus infection after 5-azaC treatment. The 1E7 and 2G9

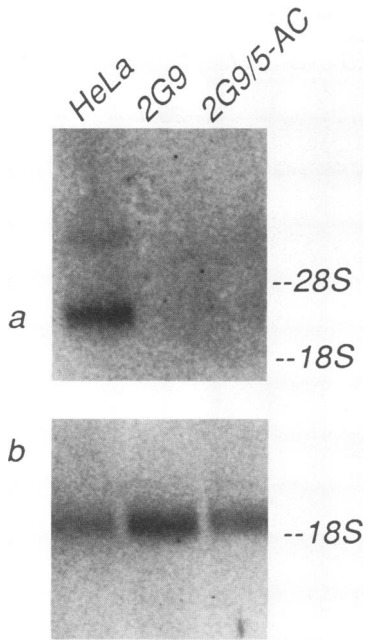

FIG. 3. Northern blot analysis of poly(A)$^+$ RNA from clone 2G9 of SOFIA cells. Samples (10 μg) of oligo-dT-selected RNA from HeLa cells (H), clone 2G9 cells (2G9), and clone 2G9 cells treated with 10 μg of 5-azaC per ml for 48 h (2G9/5-AC) were fractionated in a 1% agarose–formaldehyde gel and blotted onto nitrocellulose paper. (a) The filter was hybridized to a ^{32}P-labeled PVR cDNA probe. (b) The filter was stripped of probe and rehybridized to a ^{32}P-labeled γ-actin cDNA probe and autoradiographed for 24 h. Positions of 28S and 18S rRNA markers are indicated.

clones of SOFIA cells and HeLa cells were treated with 10 μM of 5-azaC for 60 h, infected with poliovirus at a multiplicity of infection of 10, dispersed with trypsin, and seeded onto monolayers of HeLa cells. Only 1 in 10^3 1E7 and 2G9 cells appeared to produce infectious virus, while each HeLa cell produced infectious progeny (Table 2). However, since previous results indicate that SOFIA cells do not produce infectious poliovirus (14), it is likely that virus detected in the infectious centers assay represented particles that adhered nonspecifically to the cells. Treatment with 5-azaC increased the number of SOFIA cell infectious centers by 100-fold or more; 7.6% of 1E7 cells and 5.5% of 2G9 cells became susceptible to poliovirus infection after 5-azaC treatment.

Induction of PVR after treatment of SOFIA cells with 5-azaC. To determine whether treatment of SOFIA cells with 5-azaC induced cell surface expression of PVR, we

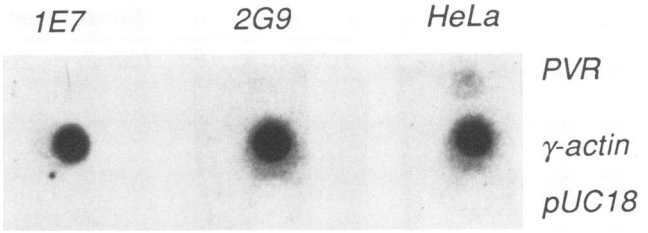

FIG. 4. In vitro nuclear transcription. Nuclei from 7×10^8 HeLa cells or SOFIA cell clones 1E7 and 2G9 were isolated. RNA chains were allowed to elongate in the presence of [^{32}P]UTP and used to probe nitrocellulose filters containing PVR cDNA, γ-actin cDNA, and pUC18. After washing and treatment with RNases, the filters were exposed to X-ray film.

assayed for the presence of the receptor using antireceptor monoclonal antibody D171 (21). Cells were grown in 96-well plates in the presence or absence of 10 μM 5-azaC and incubated first with D171 and then with ^{125}I-labeled anti-mouse antibody. Treatment of SOFIA clones with 5-azaC resulted in the induction of PVR compared with untreated cells (Fig. 5). Thus, 5-azaC appears to restore sensitivity of SOFIA cells to poliovirus infection by restoring cell surface expression of PVR.

It was not possible to detect PVR RNA by Northern hybridization analysis of total RNA (data not shown) or poly(A)$^+$ (Fig. 3, compare lanes 2G9 and 2G9/5-azaC) in 5-azaC-treated SOFIA cells. This result was not unexpected, since only 5 to 7% of 5-azaC-treated SOFIA cells became susceptible to poliovirus infection (Table 2), and the levels of PVR transcripts in these cells are lower than in HeLa cells (see below and Fig. 5). To circumvent this problem, SOFIA cells were treated with 5-azaC and cloned, and poliovirus-susceptible and -resistant clones were identified. Northern hybridization analysis demonstrated that poliovirus-resistant clones did not contain stable PVR RNA, while different levels of the 3.3-kb PVR RNA were observed in the poliovirus-susceptible cell lines (Fig. 6). Although the amount of PVR RNA detected was very low, except in clone 13 which expressed levels comparable to those in HeLa cells, all the clones displayed complete cytopathic effect 24 h after infection with poliovirus, with no differences in the kinetics of cell killing or virus production (data not shown).

DISCUSSION

SOFIA cells were derived from HeLa cells that were persistently infected with poliovirus and underwent several crisis periods before stabilization and loss of susceptibility to poliovirus infection (14). SOFIA cells do not express cell

TABLE 1. Effect of 5-azaC treatment on susceptibility of cells to poliovirus

Hour postinfection	Viral titer (PFU/ml)[a]					
	SOFIA				HeLa	
	1E7		2G9			
	−	+	−	+	−	+
0	$<2 \times 10^2$	$<2 \times 10^2$	$<2 \times 10^2$	$<2 \times 10^2$	$<2 \times 10^2$	$<2 \times 10^2$
24	2×10^3	2.5×10^7	2.5×10^3	1.8×10^7	3.6×10^8	4×10^8
72	1.5×10^3	2.3×10^7	2×10^3	2×10^7	3.2×10^8	3.8×10^8

[a] −, without 5-azaC; +, with 5-azaC.

TABLE 2. Infectious center assay of 5-azaC-treated cells

5-azaC[a]	No. of cells/infectious center		
	SOFIA		HeLa
	1E7	2G9	
−	1,300	3,600	1
+	13	18	1

[a] +, monolayers were treated with 10 μM 5-azaC for 60 h and then infected with poliovirus; −, no 5-azaC treatment.

receptors for poliovirus. Here we demonstrated that stable PVR mRNA is absent from SOFIA cells and that treatment of SOFIA cells with 5-azaC restores PVR expression and sensitivity to poliovirus infection. These results indicate that PVR expression is not essential for viability of HeLa cells.

Although the PVR gene in SOFIA cells does not appear to be grossly altered, it was possible that undetected mutations were present which result in loss of PVR function. The ability to induce functional PVR with 5-azaC demonstrated the absence of such mutations in SOFIA cells. The doses of 5-azaC used in this study are only slightly mutagenic (17, 22), and therefore it is unlikely that restoration of poliovirus sensitivity to SOFIA cells results from 5-azaC-induced mutations. The high frequency at which SOFIA cells were converted to poliovirus susceptibility also makes this possibility unlikely.

Our data suggest that 5-azaC restores transcription of PVR RNA in SOFIA cells. Stable PVR mRNAs were not detected in SOFIA cells, and nuclear run-on experiments indicated a reduced level of transduction of the PVR gene. The rate of PVR transcription in SOFIA cells was reduced three- to fivefold compared with HeLa cells. Although this difference is small, it results in barely detectable transcription in SOFIA cells. This difference in transcription rate could account for the failure to detect PVR mRNA in these cells, if this mRNA was very unstable. One species of PVR mRNA contains a consensus mRNA destabilizing sequence in the 3′-noncoding region (20). Our results showed that the level of PVR mRNA observed in HeLa cells is apparently not required for susceptibility to poliovirus infection, since several poliovirus-susceptible 5-azaC-treated SOFIA cell clones expressed low but detectable levels of PVR mRNA (Fig. 6).

The mechanism by which 5-azaC restores transcription of the PVR gene is not known. The cytidine analog 5-azaC is a potent demethylating agent that causes permanent alterations of gene expression via incorporation at multiple sites in DNA and by inhibition by hemimethylases (for a recent review, see reference 4). Activation of genes by 5-azaC treatment has been correlated with changes in DNA methylation (5, 6, 11, 12), but 5-azaC also induces phenotypic changes in organisms with unmethylated genomes (1, 25) and genetic changes such as mitotic recombination, chromosome decondensation, and chromatid exchanges (10, 26). There-

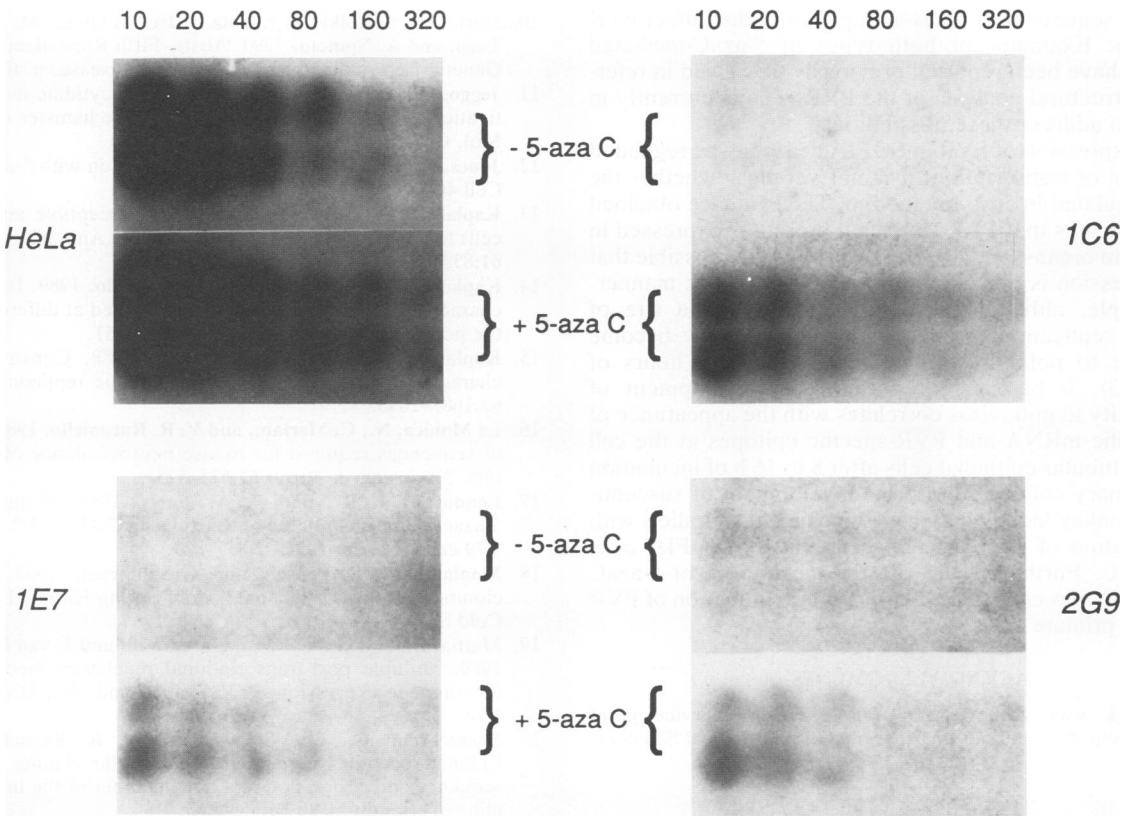

FIG. 5. Induction of PVR at the cell surface of SOFIA cells treated with 5-azaC. HeLa cells and SOFIA cell clones 1C6, 1E7, and 2G9 were grown in 96-well plates for 48 h in the presence (+ 5-azaC) or absence (− 5-azaC) of 10 μM 5-azaC. Growth medium was removed, and monolayers were treated with dilutions of anti-PVR monoclonal antibody D171 (1/10, 1/20, 1/40, 1/80, 1/160, and 1/320), extensively washed, and stained with ^{125}I-labeled sheep anti-mouse immunoglobulin (1 μCi per well). Plates were exposed to X-ray film in the presence of an intensifying screen for 5 days. Concentrations of monoclonal antibody D171 are expressed as the reciprocal of the corresponding dilution.

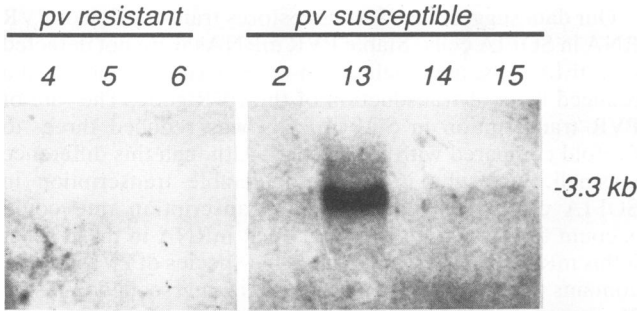

FIG. 6. Induction of PVR-specific RNA in SOFIA cells treated with 5-azaC. Northern blot analysis of total RNA from single-cell clones of SOFIA cells treated with 5-azaC was performed as described in the legend to Fig. 2. Three clones were resistant to poliovirus infection (4, 5, and 6), whereas four other clones were susceptible (2, 13, 14, and 15). The position of 3.3-kb PVR mRNA is indicated.

fore, we cannot rule out the possibility that mechanisms other than demethylation are involved in the effect of 5-azaC on SOFIA cells. Whatever the mechanism by which 5-azaC induces the expression of PVR in SOFIA cells, the high frequency of conversion to poliovirus sensitivity is consistent with a small number of targets required for activation of this gene.

If hypomethylation is involved in restoration of PVR expression in SOFIA cells, the target of 5-azaC might be *cis*-acting sequences of the PVR gene itself or, alternatively, regulatory sequences of *trans*-acting factors that affect PVR expression. Examples of both types of 5-azaC-mediated activation have been reported previously (reviewed in reference 4). Structural analysis of the PVR gene is currently in progress to address these possibilities.

While expression of PVR in SOFIA cells may be regulated at the level of transcription, it is not yet clear whether the gene is regulated in any way in vivo. The evidence obtained to date indicates that PVR RNA and protein is expressed in most human organs (9a, 20). However, it is still possible that PVR expression is regulated in a cell-type specific manner. For example, although monkey kidney is not a site of poliovirus replication, primary cultures of kidney become susceptible to poliovirus infection after several hours of culture (13). It has been reported that development of susceptibility to poliovirus correlates with the appearance of PVR-specific mRNA and PVR-specific epitopes at the cell surface of tubular epithelial cells after 8 to 16 h of incubation of the primary culture (10a). The development of susceptibility in monkey kidney may have mechanistic parallels with the restoration of poliovirus susceptibility in SOFIA cells with 5-azaC. Further studies on the mechanism of 5-azaC action on SOFIA cells might be relevant to induction of PVR activity in primate tissues.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant GM38155 from the National Institute of General Medical Sciences.

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