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Short Communication

Reactivation of infectious simian virus 40 from normal human tissues

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In this study, 82 DNA samples of simian virus 40 (SV40)-positive human tumors and normal tissues were transfected into SV40-permissive monkey cells. SV40 wild-type strain 776 was reactivated from two DNA samples, derived from peripheral blood mononuclear cells (PBMCs) of a blood donor and from a vulvar tissue. SV40 reactivation was confirmed by obtaining rescue of SV40 from the DNA of the vulvar tissue in a second transfection experiment. This investigation indicates that infectious SV40 is present in normal human tissues and suggests that (i) PBMCs are probably vectors of SV40 to different tissues of the host and (ii) blood and sexual transmission may be routes of SV40 infection in humans, leading to (iii) virus spread in the human population. *Journal of NeuroVirology* (2004) **10,** 199–205.

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Simian virus 40 (SV40) is a neurotropic viral agent for the Asian macaque, which is its natural host. In the brain of SIV-positive monkeys, SV40 induces progressive multifocal leukoencephalopathy (PML), a fatal demyielinating disease. Accumulating data suggest that SV40 is now spreading in humans by different routes, independently from early contaminated vaccines (Barbanti-Brodano *et al*, 1998, 2004; Butel and Lednicky, 1999; Garcea and Imperiale, 2003; Jasani *et al*, 2001). Support of the diffusion of SV40 in the human population is provided by the presence of SV40 sequences in human brain tumors, other neoplasms, and normal tissues of children and adults (Barbanti-Brodano *et al*, 1998, 2004; Butel and Lednicky, 1999; Garcea and Imperiale, 2003; Jasani *et al*, 2001), of specific SV40-neutralizing antibodies in human sera (Basetse *et al*, 2002; Jafar *et al*, 1998), and of SV40 large-T antigen (Tag) antibodies in sera of mesothelioma patients (Bright *et al*, 2002). A scientific panel recently established the importance of assessing the ways of contagion and the mechanisms of SV40 transmission in humans (Ferber, 2002).

In recent years, rescue of infectious SV40 from human tissues was reported only in one case, by transfection of the SV40-positive DNA of a choroid plexus carcinoma into CV-1 cells (Lednicky *et al*, 1995). The aim of this study was the reactivation of infectious SV40 from the DNA of human specimens, found positive for the SV40 sequences in previous studies (Martini *et al*, 1996, 2002; Pacini *et al*, 1998; Dolcetti *et al*, 2003). Most of the samples of human cellular DNA analyzed in this study contain DNA sequences of both BK virus (BKV) and SV40 (Martini *et al*, 1995, 1996). Before trying reactivation of SV40, therefore, we tested whether our transfection system of human cellular DNA into CV-1 cells is selecting against BKV replication. For this purpose, BKV DNA was transfected by lipofection, in 10-fold decreasing amounts from 1 μg to 100 pg, into VERO cells, which are sensitive to BKV infection, and into

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Table 1 Transfection of CV-1 and VERO cells with BKV DNA

Transfections[a]	*Amount*	*Hemagglutinating titer at day*[b]							
		14	*17*	*21*	*25*	*31*	*39*	*45*	*60*
CV1 + BKV DNA	1 μg	–	–	–	–	–	20	40	
CV1 + BKV DNA	100 ng	–	–	–	–	–	–	80	
CV1 + BKV DNA	10 ng	–	–	–	–	–	–	–	40
CV1 + BKV DNA	1 ng	–	–	–	–	–	–	–	40
CV1 + BKV DNA	100 pg	–	–	–	–	–	–	–	–
CV1 + ssDNA	10 μg	–	–	–	–	–	–	–	–
VERO + BKV DNA	1 μg	>40	1280						
VERO + BKV DNA	100 ng	>40	640						
VERO + BKV DNA	10 ng	>40	160						
VERO + BKV DNA	1 ng	–	–	>40	2560				
VERO + BKV DNA	100 pg	–	–	>40	1280				
VERO + ssDNA	10 μg	–	–	–	–	–	–	–	–

[a]Sample of BKV DNA was brought to 10 μg with salmon sperm DNA (ssDNA).
[b]The minus sign indicates negative hemagglutination. Blank spaces indicate times at which hemagglutination was not tested.

CV-1 cells. Hemagglutination of human type 0 erythrocytes was then carried out at different times after transfection with the cell homogenate of infected cells suspended in their culture medium. The results are shown in Table 1. The hemagglutinating titer was 2560 and 1280 at 25 days after transfection of VERO cells with 1 ng and 100 pg, respectively, of BKV DNA. At 25 days after transfection, CV-1 cells did not show any hemagglutinating activity. VERO cells were completely lysed at 31 days after transfection with 1 ng and 100 pg of BKV DNA, whereas the first hemagglutinating activity in CV-1 cells (hemagglutinating titer equal to 20) appeared at 39 days after transfection with 1 μg of BKV DNA. At 60 days after transfection, CV-1 cells transfected with 100 pg of BKV DNA were still negative for hemagglutinating activity and CV-1 cells transfected with any amount of BKV DNA, from 1 μg to 100 pg, did not show cytopathic effect (CPE), whereas they were lysed at 3 to 4 weeks after transfection with 10 femtograms (fg) of SV40 DNA. These results indicate that CV-1 cells, which are very sensitive to SV40, are insensitive to BKV infection and will select against BKV replication upon a double transfection with BKV and SV40 DNA.

Human DNA (10 μg) from 82 different human tumors and normal tissues, was transfected into two SV40-permissive monkey kidney cell lines, CV-1 and COS-7, with the polycation reagents Lipofectin, Lipofectamin (Invitrogen, Milan, Italy), or Superfect (Qiagen, Milan, Italy), as indicated by the manufacturers. COS-7 cells were used in these transfection experiments because they constitutively express an endogenous SV40 Tag and could therefore complement human DNA samples containing SV40 DNA defective for the Tag coding sequences. Based on semiquantitative polymerase chain reaction (PCR) analysis of the human DNAs used in previous studies (Martini *et al*, 1998; Dolcetti *et al*, 2003), we estimated that by transfection of 10 μg of human cellular DNA, an amount of SV40 DNA between 20 attograms and 20 fg would be potentially introduced into recipient cells. In reconstruction experiments, we reproducibly obtained infectious SV40 after transfection of CV-1 cells with an amount of SV40 DNA down to 10 fg and transfected cells lysed 3 to 4 weeks after transfection showing a typical SV40 CPE. Thus, it is possible that a fraction of the 82 human DNA samples used in this study contains SV40 DNA in amounts that are below the threshold of the sensitivity of our transfection assay.

The 82 DNA samples were from 8 primary brain tumors (3 glioblastomas, 2 astrocytomas, 1 ependymoma, 1 oligodendroglioma, 1 medulloblastoma), 2 classic Kaposi's sarcomas, 2 labial and 2 genital tumors, 1 vulvar dysplasia, 1 human papillomavirus 16 (HPV-16)–infected vulvar tissue showing a normal histological pattern, 9 papillary thyroid carcinomas, 5 brain tumor–derived cell lines (4 from glioblastoma and 1 from astrocytoma), 10 bone tumor–derived cell lines (6 from osteosarcoma and 4 from giant cell tumor of bone), 5 peripheral blood mononuclear cell (PBMC) samples, 11 sperm fluids, and 26 buffy coats from healthy individuals. Positive controls were carried out by transfection of DNA from SV40 strains wild-type (wt) reference strains 776 and VA45-54-A1.

Transfected cell monolayers were monitored for 2 months to detect the appearance of SV40 CPE. Two out of the 82 SV40-positive samples, one from PBMCs of a blood donor and one from a HPV-16–infected vulvar tissue, 3 and 4 weeks after transfection, respectively, showed the typical SV40 CPE in the transfected cells, characterized by the appearance of cytoplasmic vacuoles, ordered as pearl necklaces around the nuclear membrane (Figure 1B, C). Electron microscopic analysis revealed the accumulation of viral particles in infected cells, with the morphology of polyomavirus virions (data not shown). Viral infectivity of the two isolates and of the two SV40 strains 776 and VA 45-54-A1, used as controls, was neutralized by a specific anti-SV40 goat serum diluted 1:50 (Huang *et al*, 1999). In parallel control experiments, viral infectivity was not blocked by a normal goat serum. This result indicates that the reactivated

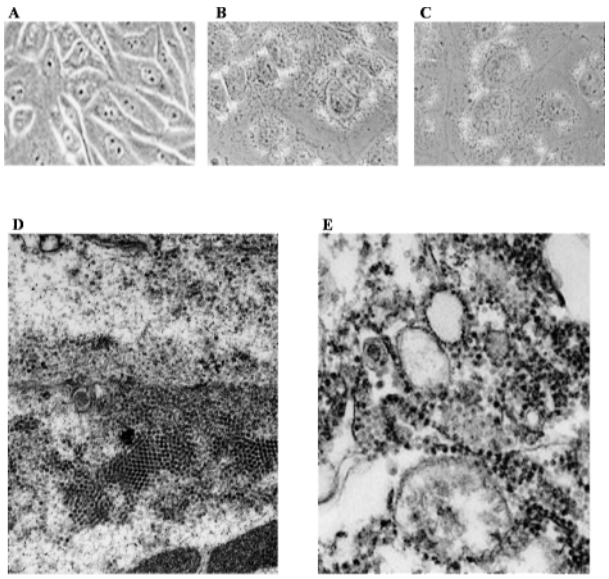

Figure 1 (**A**) Normal, nontransfected CV-1 cells. (**B**, **C**) CV-1 cells transfected with human DNA from PBMCs (**B**) and from vulvar tissue (**C**), which yielded infectious SV40 in the first transfection experiment. The initial CPE appeared at 3 to 4 weeks after transfection and was characterized by typical cytoplasmic vacuoles, ordered around the nuclear membrane. Magnification: 150×. (**D**, **E**) Electron micrographs of CV-1 cells transfected with human DNA from vulvar tissue in the second transfection experiment, where the SV40 reactivation was repeated. Virions of 42 to 45 nm in size are assembled in crystal-like structures in the nucleus (**D**) or closely associated with membranes around vacuoles in the cytoplasm (**E**). Magnification: 30,000×.

viruses have the antigenic structure of SV40. None of the 80 human DNA samples, which gave negative results in CV-1 cells, produced a SV40 viral progeny upon transfection of COS-7 cells, indicating that the Tag complementation did not work to help SV40 replication in these transfection experiments. The low frequency of virus reactivation, 2/82 SV40-positive human samples, can be ascribed to the low viral DNA load (Martini *et al*, 1998; Dolcetti *et al*, 2003) or to the SV40 DNA molecules found mostly as defective genomes in human DNA samples (Martini *et al*, 2002).

The two DNA samples yielding infectious SV40 were analyzed by PCR for the SV40 early, late, and regulatory regions (Table 2), and the amplified viral DNAs were sequenced. In addition, the entire genome of the two rescued viruses was amplified by long PCR (data not shown) and sequenced. These analyses assessed the SV40 specificity of the sequences and showed that the two isolates were not distinguishable from the wt strain 776 (Figure 2A–D), the only exception being a silent point mutation a nucleotide (nt) 948 of the late region, detected both in the original SV40 DNA of the vulvar sample and in the DNA of the corresponding rescued SV40 progeny. Our results do not seem to be a consequence of laboratory contamination, because we used as control, together with the SV40 strain 776, the SV40 strain VA 45-54-A1 and the recombinant plasmid pSV3E containing three 72-bp repeats in the regulatory region (Lednicky and Butel, 1997; Martini *et al*, 2002). Indeed, none of the fragments amplified by PCR from the SV40 regulatory region of the two human DNA specimens and of the two rescued viruses exhibited three 72-bp repeats (Figure 2B) or DNA sequences of the SV40 strain VA 45-54-A1. Likewise, in previous investigations (Martini *et al*, 1996, 1998, 2002; Pacini *et al*, 1998), none of the other 80 samples of human DNA, which are part of this panel of specimens, exhibited three 72-bp repeats when analyzed by PCR. Moreover, none of the SV40 strains sequenced until now in different laboratories showed the point mutation detected in this study at nt 948 of the late viral region.

The SV40 rescue from human DNA appears to be a rare event in our experiments. To confirm the positive results and rule out an accidental contamination with the SV40 laboratory strain used as a reference in transfection control samples, the rescue experiment was repeated with the DNA from vulvar tissue. The DNA from PBMCs was exhausted because it had been used in the first transfection experiment and in previous studies (Martini *et al*, 1996). In this second experiment, we avoided to make any transfection control of CV-1 cells with purified reference SV40 DNA, in order to eliminate, as a possible source of contamination, any circulation of infectious virus produced by positive transfection controls. Three weeks after transfection, monolayer cultures of CV-1 cells started to show vacuoles in the cytoplasm and were lysed in a few days with a typical SV40 CPE. Electron microscopy of transfected cells with CPE showed viral factories in the nucleus with virions of 42 to 45 nm in size assembled in crystal-like structures (Figure 1D) and virions closely associated with membranes around vacuoles in the cytoplasm of lysing cells (Figure 1E). PCR analysis for the SV40 early, late, and regulatory regions and long PCR for the entire genome, followed by nucleotide sequence analysis of the amplified products, confirmed the SV40 specificity of the sequences detected in CV-1–transfected cells, whereas normal, nontransfected CV-1 cells, kept during all the period of the experiment as negative controls, were negative for SV40 sequences (data not shown). Nucleotide sequence analysis of the entire genome of the rescued virus showed it to be identical to the SV40 strain rescued from the same vulvar specimen in the first transfection experiment. The silent mutation, at nt 948 of the late region changing the third nucleotide of the codon 316 AGG (Arg) to AGA (Arg), detected in the rescued virus from the first transfection experiment with the DNA of the vulvar sample, was detected also in the DNA of the reactivated SV40 strain of the second transfection experiment. This result indicates that the two SV40 strains, rescued from the same vulvar specimen in the two distinct transfection experiments, are identical to each other,

Table 2 Oligonucleotides and oligoprobes employed in PCR and long-PCR analysis, filter hybridization, and DNA sequencing

SV40 DNA regions	*Oligonucleotides and oligoprobes*	*Reference positions*[a]	*T*[b]	*Size*[c]
Tag N:	SV.for2: 5′-CTTTGGAGGCTTCTGGGATGCAACT-3′	nt 4945–4921	55	575
	SV.rev: 5′-GCATGACTCAAAAAACTTAGCAATTCTG-3′	nt 4372–4399		
	PYV.for: 5′-TAGGTGCCAACCTATGGAACAGA-3′	nt 4429–4407	58	172
	PYV.rev: 5′-GAAAGTCTTTAGGGTCTTCTACC-3′	nt 4403–4425		
	SV probe: 5’-ATGTTGAGAGTCAGCAGTAGCC-3′	nt 4452–4473		
Tag C:	TA1: 5′-GACCTGTGGCTGAGTTTGCTCA-3′	nt 3070–3048	58	441
	TA2: 5′-GCTTTATTTGTAACCATTATAAG-3′	nt 2630–2652		
	T probe: 5′-AACCTCTACAAATGTGGTATGGCT-3′	nt 2741–2764		
VP1:	LA1: 5′-GGGTGTTGGGCCCTTGTGCAAAGC-3′	nt 2251–2274	60	294
	LA2: 5′-CATGTCTGGATCCCCAGGAAGCTC-3′	nt 2545–2522		
	L probe: 5′-TTAACAGGAGGACACAGAGGGTGGATGG-3′	nt 2433–2460		
Regulatory:	RA3: 5′-GCGTGACAGCCGGCGCAGCACCA-3′	nt 358–336	52	483
	RA4: 5′-GTCCATTAGCTGCAAAGATTCCTC-3′	nt 5119–5142		
	RA1: 5′-AATGTGTGTCAGTTAGGGTGTG-3′	nt 266–245	55	314
	RA2: 5′-TCCAAAAAAGCCTCCTCACTACTT-3′	nt 5195–5218		
	R probe: 5′-TTAGTCAGCCATGGGGCGGAGA-3′	nt 29–50		
Long PCR:	4BB: 5′-AGTTGCATCCCAGAAGCCTCCAAAC-3′	nt 4921–4945	57	4806
	5BB: 5′-GTGATGATGAGGCTACTGCTGACTC-3′	nt 4483–4459		
	SV40 probe: full-length DNA of strain 776	nt 1–5243		
Tag-Reg:	SVTR.for: 5′-AACACCAGGATTTAAGGAAGA-3′	nt 4888–4908	55	714
	RA3: 5′-GCGTGACAGCCGGCGCAGCACCA-3′	nt 358–336		
Reg-VP2:	SVRV.for: 5′-ACCAAGTTCCTCTTTCAGAGGT-3′	nt 302–823	55	628
	SVRV.rev: 5′-AAATCTACAGCCATTCCTGGT-3′	nt 929–909		
VP2-VP3:	SVVV.for: 5′-CTGGGATCACAAAGTTTCTACTGTT-3′	nt 870–894	55	369
	VP2: 5′-GGCCTAATGGGAGACAAAGTAGAG-3′	nt 1238–1215		
VP1-VP3:	VP3: 5′-CTCACAGGAGCTTGAAAGAAGAAC-3′	nt 1089–1112	55	570
SV3V.rev:	5′-AAAAGCACTCCACCTCAGTGAAGC-3′	nt 1658–1635		
VP1:	VPA: 5′-AACTGGAGTAGACAGCTTCACT-3′	nt 1621–1642	55	682
	LA4: 5′-GTCAACAGCAGAAACATACAAGCTG-3′	nt 2302–2281		
VP1:	SV1V.for: 5′-GTTGGGCCCTTGTGCAAAGCT-3′	nt 2255–2275	55	667
	SVTV3: 5′-GATGATGATGATGAAGACAGCCA-3′	nt 2921–2899		
Tag:	T5: 5′-TTATCAGCATTTTCCTGGCTGTCTTC-3′	nt 2884–2909	55	620
	SVT.rev: 5′-TTGCTTGAATTATGTGGGGGGA-3′	nt 3503–3482		
Tag:	REV4: 5′-GTTCAGCCTGTCCAAGGGCAAATT-3′	nt 3444–3467	55	460
	Svcore.rev: 5′-TATTAAAAAAGAACAGCCCAGCCA-3′	nt 3903–3880		
Tag:	SVREV2: 5′-GTACTTATAGTGGCTGGGCTG-3′	nt 3870–3890	55	705
	PYV.for: 5′-TAGATTCCAACCTATGGAACTGA-3′	nt 4574–4552		
Tag-tag:	SV2T.for: 5′-AAAGGCATTCCACCACTGCTCC-3′	nt 4524–4545	55	422
	SV.for2: 5′-CTTTGGAGGCTTCTGGGATGCAACT-3′	nt 4945–4921		

[a]SV40 strain 776, GenBank accession number AF316139.
[b]PCR annealing temperature (°C). Cycling times for each step of the PCR, with each set of primers, after DNA denaturation at 95°C. for 5 min, were denaturation 1 min at 94°C, annealing 1 min at the temperatures indicated in the column T of this table, and extension 1 min at 72°C. The long PCR steps for 10 cycles, after DNA denaturation at 94°C for 10 min, were denaturation 1 min at 94°C, annealing 1 min at 57°C, and extension 1 min at 72°C; then for additional 14 cycles, denaturation 1 min at 94°C, annealing 1 min at 57°C, and extension 1 min at 72°C, increased of 30 s each cycle from cycles 11 to 24.
[c]Size of the amplified products in bp.

but differ in a single nucleotide from the SV40 wt strain 776.

The virus isolated in the second transfection experiment was neutralized, like the SV40 strain rescued in the first experiment, by a specific goat serum to SV40 capsid antigens. Because we were able to repeat the rescue of SV40 from a previously positive human DNA, we transfected again into CV-1 cells six other DNA specimens (extracted from three human PBMC samples and from three human brain tumors) from the first experiment. Sixty days after transfection, no CPE was apparent in the transfected CV-1 monolayers and the rescue experiment was considered negative. Thus, the results of transfection of a considerable number of SV40-positive human DNA samples into SV40-susceptible monkey cells indicate that SV40 rescue from human DNA is a rare event. This seems to be due to the low amount of SV40 DNA present in human tissues, which is at the limits of the sensitivity of the transfection methods, or to the SV40-defective genomes found frequently in human DNA samples (Martini *et al*, 2002). Most likely, only some of the samples containing SV40 DNA over a certain threshold yielded infectious virus upon transfection. On the other hand, the repeated rescue obtained with one sample of cellular DNA demonstrates the reproducibility of the experiment and rules out an accidental contamination with a control SV40 laboratory strain during transfection, inasmuch as in the repeated transfection experiment no SV40 laboratory strains were used as positive controls.

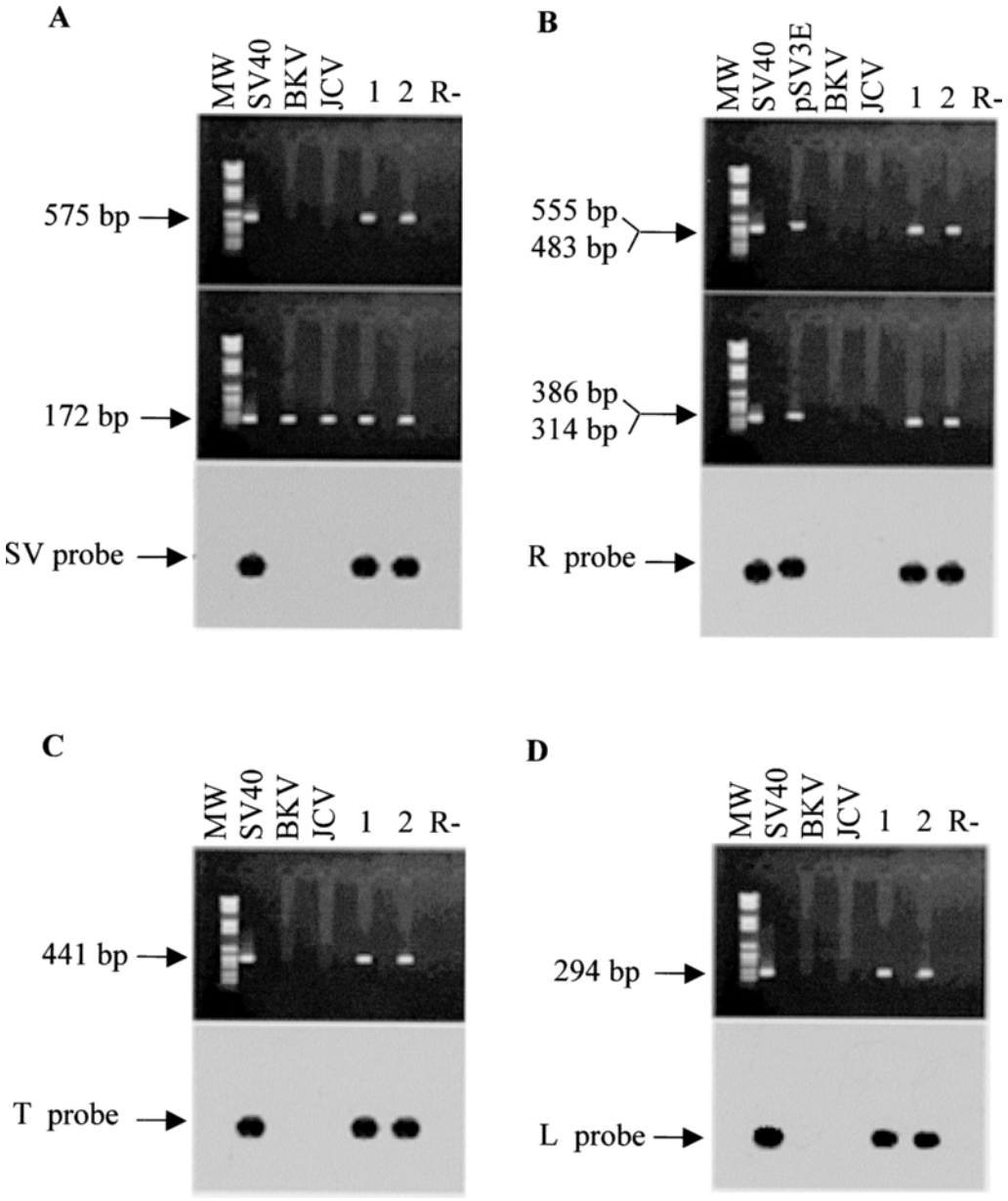

Figure 2 Agarose gel stained with ethidium bromide and film images of DNAs from the two reactivated SV40 strains analyzed by PCR and filter hybridization. DNA (0.5 μg) was PCR amplified, for 35 cycles, in a total volume of 50 μl containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 0.01% gelatine, 150 μM of each dNTP and 25 μM of each primer, 1 unit of Taq-DNA polymerase (Roche, Milan), together with 1 unit of Platinum Taq antibody as indicated by the supplier (Life Technologies, Milan). By adding the Taq antibody, the Taq polymerase activity was blocked up to 94°C, thus avoiding the generation of nonspecific amplification products at room and ramping temperatures (Martini *et al*, 2002). MW, molecular weight markers (marker VI, Roche Diagnostics, Milan, Italy). SV40, BK virus (BKV), and JC virus (JCV) are the PCR positive controls from recombinant plasmids. 1 and 2 are the rescued samples, from PBMCs of a blood donor and from a vulvar tissue, respectively. R− is the negative control of the PCR reaction without DNA template. (**A**) *Top and middle panels*: PCR analysis for the SV40 Tag N-terminal sequences. Arrows indicate the product size obtained by PCR (575 bp) and nested (n)PCR (172 bp). *Bottom panel*: film images of PCR amplified products of the middle panel hybridized to the SV40 specific internal SV probe (Table 2). (**B**) *Top and middle panels*: PCR analysis for the SV40 regulatory region. pSV3E is the control recombinant plasmid carrying three 72-bp repeats (Lednicky and Butel, 1997; Martini *et al*, 2002). The arrows indicate the 483 bp and 314 bp of SV40 776 as well as the 555 bp and 386 bp of the pSV3E product from PCR and nPCR, respectively. *Bottom panel*: film image of PCR amplified DNAs of the middle panel hybridized to the SV40 specific internal R probe (Table 2). (**C**) *Top panel*: PCR analysis for the SV40 Tag C-terminal region. The arrow indicates the 441 bp PCR product size. *Bottom panel*: film image of PCR amplified DNAs of the top panel hybridized to the SV40 specific internal T probe (Table 2). (**D**) *Top panel*: PCR analysis for the SV40 VP1 region. The arrow indicates the 294-bp PCR product. *Bottom panel*: film image of PCR amplified DNAs of the top panel hybridized to the SV40-specific internal L probe (Table 2).

In the present study, about 65% of the SV40-positive specimens was obtained from individuals who received anti-poliovirus vaccination after 1965, in a period when polio vaccines were not contaminated by SV40 (Sangar *et al*, 1999), suggesting that SV40 is circulating in the human population. This epidemiological condition may explain the lack of difference in cancer incidence between individuals vaccinated with SV40-contaminated and SV40-free poliovaccines (Strickler *et al*, 1998). In this connection, the presence of SV40 in humans, before the introduction of SV40-contaminated vaccines, cannot be discounted (Geissler *et al*, 1985). Reactivation of wt SV40 from PBMCs of a blood donor and from a non-neoplastic vulvar tissue, together with the detection of SV40 DNA in cells from buffy coats, PBMCs, B and T lymphocytes, lymphoblastoid cell lines, and sperm fluids of normal individuals (Martini *et al*, 1995, 1996, 1998, 2002; Dolcetti *et al*, 2003), point to blood and sexual contacts as common routes of SV40 spread in human tissues and in the human population. Besides, the presence of SV40 in human kidneys and urine (Li *et al*, 2002b) points to the kidney as a potential site of virus latency in humans, as it occurs in monkeys (Barbanti-Brodano *et al*, 1998; Butel and Lednicky, 1999). Excretion of SV40 in urine may contribute a means of interhuman virus transmission through the contaminated environment.

In this study, as well as in previous investigations carried out in Italy (Barbanti-Brodano *et al*, 1998; Pacini *et al*, 1998), the SV40 sequences of the viral DNA detected in clinical samples were not distinguishable from the nucleotide sequence of the SV40 wt strain 776, which has two 72-bp repeats in the enhancer domain of the regulatory region. For many years, SV40 wt strain 776 was considered a laboratory strain that duplicated, during serial passages in cell cultures, the 72-bp sequence present in the enhancer domain of the natural strain. The two 72-bp repeats give a growth advantage to SV40 in CV-1 and TC-7 cells (Butel and Lednicky 1999). However, a recent investigation showed that different SV40 strains replicate in BSC-1 cells with a distinct growth kinetics independently from the enhancer copy number (Minor *et al*, 2001). The SV40 strains that contaminated poliovaccines show a regulatory region that contains either one or two 72-bp repeats (Rizzo *et al*, 1999). Thus, SV40 strains with two 72-bp repeats detected in human tissues may represent viruses circulating in the human population after poliovirus vaccination. SV40 strain variability, found in the United States (Butel and Lednicky, 1999), could be due to the heterogeneous human population of this country, although SV40 strain 776 was the main representative among the different SV40 strains detected in kidney, urine, and blood samples of an American group consisting of normal persons and patients affected by nephropathies (Li *et al*, 2002a, 2002b). On the other hand, SV40 wt strain 776, the only strain so far detected by different groups in Italian patients (Barbanti-Brodano *et al*, 1998, 2004; Pacini *et al*, 1998), may reflect the more homogeneous population present in Italy. In this context, it should be noted that JC virus (JCV), which is closely related to SV40, has a geographical strain distribution (Barbanti-Brodano *et al*, 1998). Due to this characteristic, JCV fingerprinting was employed to study migrations of human populations (Agostini *et al*, 1997).

This study provides the first evidence of reactivation of infectious SV40 from normal human tissues, suggesting that infection of humans by SV40 may cause virus transmission and circulation in the human population. This assumption is in agreement with the detection of SV40 sequences in human tissues (Barbanti-Brodano *et al*, 1998, 2004; Butel and Lednicky, 1999; Jasani *et al*, 2001) and of SV40 virions in urine (Li *et al*, 2002b). These results and recent data obtained by other investigators indicate that SV40 may behave as a new human polyomavirus, because SV40 infection in humans seems now to occur independently from administration of contaminated vaccines.

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