Simian Virus 40-Like Sequences from Early and Late Regions in Human Thyroid Tumors of Different Histotypes

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Simian virus 40 (SV40) sequences were investigated in human thyroid tumors of different histotypes, Graves’ disease thyroid specimens, normal thyroid tissues, and peripheral blood mononuclear cells (PBMC) of healthy donors. Specific SV40 large T antigen (Tag) sequences were detected, by PCR and filter hybridization, in human thyroid tumors with a frequency ranging from 66% in papillary thyroid carcinomas (PTC) to 100% in anaplastic thyroid carcinomas (ATC). SV40 was revealed in 60% and 100% of normal thyroid tissues adjacent to PTC and ATC, respectively, but in only 10% of control normal thyroid tissues (NTT) from patients affected by multinodular goiter. Thyroid tissues from patients affected by the Graves’ disease were found to be SV40 positive with a frequency of 20%. In agreement with previous investigations, the presence of SV40 sequences was detected in 25% of PBMC of healthy individuals. SV40 Tag mRNA was detected by RT-PCR, whereas the viral oncprotein was revealed by immunohistochemistry with a specific monoclonal antibody. The high prevalence of SV40 footprints in human thyroid tumors indicates that the oncogenic virus may participate as a cofactor in the onset/progression of specific human thyroid cancers. Detection of SV40 sequences in NTT adjacent to thyroid cancers suggests that the viral infection may spread from transformed cells to normal cells surrounding the tumor. The presence of the SV40 footprint in PBMC implies that blood cells are vectors of the virus in other tissues of the host. (J Clin Endocrinol Metab 88: 892–899, 2003)
and in brain tumor samples (5), thus adding further support to a role for SV40 in human tumorigenesis. The evidence that SV40 Tag sustains immortalization of human neoplastic cells is supported by experiments showing the induction of apoptosis in SV40 Tag-positive mesothelioma cells transfected with antisense Tag sequences (40).

SV40 is a monkey virus that was believed to be transmitted to humans only under exceptional situations in natural infection (32). SV40-contaminated vaccines (32), in particular antipolio vaccines, were administered to hundreds of millions of humans worldwide between 1955 and 1963 (34). However, the presence of this viral agent in humans, before the introduction of SV40-contaminated vaccines, cannot be discarded (41).

Malignant thyroid cancer encompasses a spectrum of different histotypes ranging in aggressiveness from the slow-growing, indolent papillary thyroid cancer (PTC) up to the rapidly fatal anaplastic thyroid cancer (ATC). Moreover, thyroid C cells may give rise to so-called medullary thyroid cancer (MTC), which has a malignant potential between papillary and follicular thyroid cancer (42). Specific oncogenes have been shown to be involved in thyroid carcinogenesis; ras (43) and peroxisome proliferator-activated receptor-γ mutations have been reported in follicular thyroid cancer (44). The most important genetic alteration of PTC is the rearrangement of the RET proto-oncogene, producing several chimeric oncogenes, named RET/PTC. These rearrangements are present in nearly 50% of naturally occurring PTC and in nearly 80% of radiation-induced PTC (45–47). Tyrosine receptor kinase rearrangements are found in PTC at a low frequency (48), whereas the MET oncogene is overexpressed in 50% of the cases (49). ATC, the most aggressive thyroid cancer, is characterized by the presence of p53 inactivating mutations in 22–83% of the cases (50). MTC can be either sporadic or hereditary, and the main genetic alterations of the diseases are somatic or germline RET gene point mutations, respectively (51, 52). Graves’ disease (GD) is an organ-specific autoimmune disorder characterized by the presence of TSH receptor-stimulating antibodies leading to continuous stimulation of the follicular cells and consequent hyperthyroidism (53).

In a previous investigation, in a small proportion of papillary thyroid carcinomas, SV40-like DNA sequences have been found integrated into the genomic tumor DNA (18). These data were obtained by the low sensitive Southern blot hybridization technique, and they were limited to the papillary histotype.

In the present study we analyzed by the more sensitive PCR technique a new large series of human thyroid tumors of different histotypes and other benign thyroid disease samples. We also studied normal thyroid tissues (NTT) from patients affected by multinodular goiter and PBMC from blood donors. Three different SV40 genomic regions, corresponding to the Tag amino (N)-terminal, regulatory, and VP1 structural protein carboxyl (C)-terminal sequences, were analyzed. The SV40 specificity of different regions, amplified by PCR, was investigated by filter hybridization with internal oligoprobes and was further assessed by DNA sequencing. Tag expression was revealed by RT-PCR and immuno-histochemistry with the specific monoclonal antibody (mab) Pab 101.

Materials and Methods

 Patients, clinical specimens, and cell lines

In this study 109 patients, 80 females and 29 males, between 30 and 84 yr of age, were enrolled. Twenty-seven primary PTC, 2 lymph node metastases, and 10 NTT adjacent to the carcinoma were from 29 patients affected by PTC. Eighteen primary MTC and 2 lymph node metastases were obtained from 20 patients affected by MTC. Twenty primary ATC and 10 NTT near the tumor were from 20 ATC patients, whereas 20 GD thyroid tissues were from 20 GD patients. Twenty NTT were from 20 patients affected by multinodular goiter. In addition, 20 PBMC samples from individuals, relatives of patients affected by sporadic MTC, were analyzed. All samples were obtained from patients after informed consent.

Four human thyroid carcinoma cell lines, designated FRO and ARO from anaplastic carcinoma, WRO from follicular carcinoma, and NPA from poor differentiated papillary carcinoma, provided by Dr. J. Fagin (University of Cincinnati, Cincinnati, OH), were grown in RPMI 1640 medium supplemented with 10% fetal calf serum, 100 U/ml penicillin, 0.1 mg/liter streptomycin, 1 mM sodium pyruvate, and nonessential amino acids.

DNA purification

Fresh tissues were immediately frozen and kept at −80°C. Each sample was cut, minced, and digested with sodium dodecyl sulfate (1%) and proteinase K (500 μg/ml), followed by extraction with a mixture of phenol-chloroform-isooamyl alcohol (25:24:1). DNA was precipitated with ethanol/sodium acetate (0.2 M), resuspended in TE buffer (10 mM Tris-HCl, pH 8, and 1 mM EDTA), and stored at −20°C (6). Sections from formalin-fixed, paraffin-embedded tissues were extracted using a commercial kit (Qiagen, Milan, Italy) following the manufacturer’s instructions.

PCR, filter hybridization, and DNA sequence analyses

SV40 DNA from wild-type VA-45-54-I (our laboratory) and 776 (Sigma-Aldrich, Milan, Italy) strains (34) were used as controls in PCR amplification and DNA sequence experiments. Each DNA sample was first tested for suitability for PCR by amplification of p53 gene (exons 7–8) sequences (Table 1). Only positive samples were further investigated for amplification of SV40 sequences. All experiments were carried out in triplicate at the Section of Endocrinology, University of Pisa. Precautions to avoid PCR contamination were carefully taken. In the first step of our analysis, all samples were screened for the highly conserved SV40 Tag sequences of 172 bp, coding for the N-terminal portion of the oncoprotein (1) using the SV40 detection kit (Poisays Research, Trieste, Italy; Table 1). Positive samples were further analyzed for regulatory and VP1 C-terminal sequences (Table 1).

DNA was amplified for 35 cycles in a total volume of 100 μl containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 200 μM deoxy-NTP, 50 pmol of each primer, and 1 U of Tag DNA polymerase (Amersham Pharmacia Biotech, Milan, Italy). Primers, oligoprobes, annealing temperatures, and PCR product size are reported in Table 1. Ten microliters of each PCR reaction were loaded on 2% agarose gel and electrophorised in 1× TAE (40 mM Tris acetate and 1 mM EDTA, pH 8), stained by ethidium bromide, and photographed. DNA was transferred to nylon membranes and cross-linked to filter by UV irradiation (3, 6). All filters were hybridized to SV40-specific internal oligoprobes (Table 1) at 42°C in 5× SSC (0.5 M NaCl and 0.5 M sodium citrate), 0.1% sodium dodecyl sulfate, block solution, and 0.5% dextran sulfate (Amersham Pharmacia Biotech). Oligoprobes were 3′-end labeled employing the enhanced chemiluminescence labeling kit and revealed by a chemiluminescent reagent (Amersham Pharmacia Biotech) (6). The stringency of the final wash was adjusted according to the melting temperature. Filters were exposed to x-ray films (Kodak, Rochester, NY) for 15–60 min. PCR-amplified products were DNA-sequenced by Sanger’s technique with the USB Sequenase kit (Amersham Pharmacia Biotech) or auto-
TABLE 1. Oligonucleotides used as primers in PCR and as probes in filter hybridization

<table>
<thead>
<tr>
<th>SV40 DNA regions</th>
<th>Oligonucleotidesa</th>
<th>Reference positionb</th>
<th>Tc</th>
<th>Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tag NH2</td>
<td>PYV for: 5'−TAGTGGCCAAACTTGGAAAGAG−3'</td>
<td>nt 4574−4552</td>
<td>54</td>
<td>172</td>
</tr>
<tr>
<td></td>
<td>PYV rev: 5'−GGAGTCTTTAGGGTCTTACCT−3'</td>
<td>nt 4040−4425</td>
<td>4452−4473</td>
<td>55</td>
</tr>
<tr>
<td>SV probe: 5'−ATTGGAGATCTAGCATAGCC−3'</td>
<td>nt 5195−5218</td>
<td>29−50</td>
<td>67</td>
<td>294</td>
</tr>
<tr>
<td>RA1: 5'−ATATCTGTCATCGAGTTAA−3'</td>
<td>nt 5195−5218</td>
<td>29−50</td>
<td>67</td>
<td>294</td>
</tr>
<tr>
<td>RA2: 5'−TCCAAAAACCGCTCTCCTACT−3'</td>
<td>nt 5195−5218</td>
<td>29−50</td>
<td>67</td>
<td>294</td>
</tr>
<tr>
<td>R probe: 5'−TCTAGCAGCCTAGGGGAGGAG−3'</td>
<td>nt 5195−5218</td>
<td>29−50</td>
<td>67</td>
<td>294</td>
</tr>
<tr>
<td>VP1 COOH</td>
<td>LA1: 5'−GGGTGTTGGCCCCTTTGCTCAAGAC−3'</td>
<td>nt 2251−2277</td>
<td>67</td>
<td>294</td>
</tr>
<tr>
<td></td>
<td>LA2: 5'−CATGTCTGGATCCCCAGGAAGC−3'</td>
<td>nt 2545−2522</td>
<td>67</td>
<td>294</td>
</tr>
<tr>
<td></td>
<td>L probe: 5'−TAAACAGAGGACACAGAGGTTGGATGG−3'</td>
<td>nt 2432−2460</td>
<td>67</td>
<td>294</td>
</tr>
<tr>
<td></td>
<td>Rev: 5'−TACCTGCTTAGTGGCTCTCT−3'</td>
<td>nt 14591−14572</td>
<td>55</td>
<td>151</td>
</tr>
</tbody>
</table>

a Oligonucleotides used as primers in PCR and as probes in filter hybridizations.
b nt, Reference nucleotide positions in SV40 strain 776 (71) and human p53 gene (European Molecular Biology Laboratory/GenBank accession no. X54156).
c Tc, PCR annealing temperature (C).

RT-PCR and immunohistochemistry

RNA was extracted from frozen tissue samples with RNAzol according to the manufacturer's protocol (Tel-Test, Friendswood, TX), whereas from formalin-fixed paraffin-embedded sections that were deparaffinized with xylol and digested with proteinase K (Roche, Milan, Italy), the RNA was purified with a mixture of phenol/chloroform/isoamyl alcohol (25:24:1). The concentration of RNA was determined by spectrophotometry. Five micrograms of cytoplasmic RNA were treated with 10 U deoxyribonuclease I (Amersham Pharmacia Biotech) for 20 min at 37°C, purified with phenol/chloroform/isoamyl alcohol, precipitated with ethanol/sodium acetate, RNA was resuspended in diethylpyrocarbonate-treated water and retrotranscribed with 500 ng random examers, 20 U AMV retrotranscriptase (Promega Corp., Milan, Italy), 80 U RNasin (Promega Corp.), and 100 µM deoxy-NTP, buffer 1× [50 mM Tris-HCl (pH 8.3), 50 mM KCl, 10 mM MgCl₂, 10 mM dithiothreitol, and 0.5 mM spermidine] in 100 µL. cDNA was then PCR amplified with primers specific for the Tag N-terminal region using an annealing temperature of 54°C and 45 cycles (3, 6) (Table 1).

Immunohistochemistry was carried out in samples previously found to be SV40⁺ to investigate the expression of SV40 Tag oncoprotein. SV40 Tag was analyzed with the mab Pab 101 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), which recognizes a specific C-terminal epitope of the viral oncoprotein (3). All tissues were fixed in 10% formalin, dehydrated, and paraffin-embedded at the Section of Pathology, University of Pisa. Thin sections (5 µm) were deparaffinized with xylol and rehydrated with ethanol solutions. All slides were subjected to antigen retrieval using 10% citrate buffer in a microwave oven, as indicated by the supplier (Milestone, Bergamo, Italy). Washes were performed with PBS for 5 min. Endogenous peroxide activity was blocked with 5% H₂O₂ for 15 min. Tissue sections were incubated with the purified Tag mab, diluted 1:100, at room temperature for 1 h, and subjected to avidin and biotin block for 20 min each, to streptavidin-peroxidase for 10 min, and to diaminoenzidine chromogen substrate for 5 min. The sections were then counterstained with hematoxylin.

RESULTS

PCR analysis of SV40 sequences in human thyroid tumors, NTT, and PBMC from donors

In this investigation human thyroid tumors of different histotypes, NTT from patients affected and not affected by thyroid carcinomas, GD thyroid tissues, and PBMC from blood donors were analyzed by PCR for sequences of three different SV40 genomic regions (Table 1). In the first step, DNA samples were analyzed by PCR for the conserved SV40 Tag N-terminal coding sequences by the PYV set of primers, which efficiently amplify these viral sequences (1, 3) (Table 1). Tumor samples were found SV40-positive with high prevalence, ranging from 66% of PTC to 100% of ATC (Fig. 1 and Table 2). A similar prevalence, ranging from 60–100%, was detected in the corresponding NTT surrounding the thyroid tumors, whereas in the thyroid tissue from patients affected by multinodular goiter and GD thyroid specimens, the frequency of SV40 sequences was of 10% and 20%, respectively (Fig. 1 and Table 2). ARO from anaplastic carcinoma, WRO from follicular carcinoma, and NPA from poor differentiated papillary carcinoma, were all SV40 negative (Table 2). The different frequencies of SV40 Tag N-terminal coding sequences detected in PTC, MTC, and ATC compared with NTT are statistically significant (Table 2).

Statistical analysis

Data were analyzed by the univariate statistics test for difference between two independent groups. The prevalence of SV40 Tag N-terminal coding sequences in each thyroid tumor type was compared with the control represented by NTT from patients affected by multinodular goiter. P value less than 0.05 was considered statistically significant.
sequenced for the three regions investigated, i.e. Tag N-terminal, regulatory, and VP1 C-terminal sequences. DNA sequencing showed a complete identity with the SV40 wild-type strain 776. Only a single DNA sample from a GD tissue showed a silent point mutation, aCt oT transition, at nucleotide 2482 of VP1 C-terminal coding sequences (Fig. 2).

**Tag expression by RT-PCR and immunohistochemistry**

Tag expression was investigated at the mRNA level by RT-PCR analysis, followed by filter hybridization with the specific internal oligoprobe SV (Table 1). The Tag transcript was searched in 24 thyroid cancer specimens previously found to be SV40-positive by PCR, whereas 30 samples found to be SV40 negative were used as negative controls (Table 3). Nine of 13 PTC and 8 of 11 ATC samples showed the expression of the mRNA specific of SV40 Tag, whereas none of the negative controls was positive for the Tag transcript (Fig. 3 and Table 3).

The SV40 Tag oncoprotein was analyzed by immunohistochemistry in 9 PTC and 8 ATC specimens found positive by RT-PCR for the specific Tag mRNA, whereas the negative controls were 30 SV40-negative thyroid tissues (Table 3). Immunoreactive samples, 3 PTC and 8 ATC specimens (Table 3), showed weak (5 specimens), medium (2 specimens), and strong (1 specimen) cytoplasmic staining (Fig. 4D). None of the negative controls immunoreacted for SV40 Tag oncoprotein (Table 3).

**Discussion**

In our study, carried out by PCR, filter hybridization, and DNA sequence analyses, SV40 sequences from different viral

![Agarose gel electrophoresis of PCR-amplified SV40 regions](image)

**FIG. 1.** Agarose gel electrophoresis of PCR-amplified SV40 regions stained by ethidium bromide and hybridization with specific internal oligoprobes. MW, Molecular weight markers (marker IV, Roche). Lane C, Positive control represented by SV40 DNA, strain 776. Lanes 1–3, PTC samples. Lanes 4–6, MTC samples. Lanes 7–10, ATC samples. Lanes 11 and 12, GD samples. Lanes 13 and 14, NTT from PTC samples. Lanes 15 and 16, NTT from ATC specimens. Lanes 17 and 18, NTT from multinodular goiter samples. Lanes 19 and 20, PBMC (Table 2). Lane R, negative control of the PCR reaction without DNA template. A, Tag N-terminal region amplified with primers PYV.for-PYV.rev (top) and hybridized with the internal SV oligoprobe (bottom). The arrow indicates the product size obtained by PCR (172 bp). B, Regulatory region amplified with primers RA1-RA2 (top) and hybridized with the internal R oligoprobe (bottom). The arrow indicates the product size obtained by PCR (314 bp). C, VP1 region amplified with primers LA1–LA2 (top) and hybridized with the internal L oligoprobe (bottom). The arrow indicates the product size obtained by PCR (294 bp; see Table 1).

**TABLE 2.** SV40-like sequences in human thyroid tumors, tumor cell lines, normal tissues, and PBMC

<table>
<thead>
<tr>
<th>Tissues and cell lines</th>
<th>Tag N-terminal sequences</th>
<th>Regulatory sequences</th>
<th>VP1 sequences</th>
<th>Statistical analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary thyroid carcinoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Papillary</td>
<td>19/29 (66)</td>
<td>13/19 (68)</td>
<td>12/19 (63)</td>
<td>$P = 0.02$</td>
</tr>
<tr>
<td>Medullary</td>
<td>18/20 (90)</td>
<td>15/18 (83)</td>
<td>16/18 (88)</td>
<td>$P = 0.01$</td>
</tr>
<tr>
<td>Anaplastic</td>
<td>20/20 (100)</td>
<td>15/20 (75)</td>
<td>15/20 (75)</td>
<td>$P = 0.01$</td>
</tr>
<tr>
<td>Thyroid tumor cell lines</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GD tissue</td>
<td>0/4</td>
<td>0/4</td>
<td>4/4 (100)</td>
<td></td>
</tr>
<tr>
<td>Normal thyroid tissues</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjacent to PTC</td>
<td>6/10 (60)</td>
<td>6/6 (100)</td>
<td>6/6 (100)</td>
<td></td>
</tr>
<tr>
<td>Adjacent to ATC</td>
<td>10/10 (100)</td>
<td>10/10 (100)</td>
<td>7/10 (70)</td>
<td></td>
</tr>
<tr>
<td>Patients with multinodular goiter</td>
<td>2/20 (10)</td>
<td>0/2</td>
<td>0/2</td>
<td></td>
</tr>
<tr>
<td>PBMC</td>
<td>5/20 (25)</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

ND, Not determined.

a Total specimens: 129 biopsies, 4 cell lines, and 20 PBMC.

b SV40 DNA sequences from different genomic regions were detected by PCR amplification (Table 1). Positive samples/samples analyzed, and percentage (%) are indicated.

c Prevalence of SV40 Tag N-terminal coding sequences, in different thyroid tumor type, vs. control represented by NTT from patients affected by multinodular goiter.
regions were detected in human thyroid tumors of different histotypes, in nonneoplastic and normal thyroid tissues, as well as in PBMC from blood donors. The use of this sensitive technique disclosed a much higher prevalence of SV40 sequences in human thyroid tumors compared with a previous study carried out in PTC by Southern blot hybridization (18). In the present investigation, SV40 sequences have been amplified by PCR, belong to SV40 wild-type 776 strain and sequence analysis indicated that the different SV40 regions, sequences in the SV40 wild-type strain 776 and in the GD sample were identical, except for the presence of a C to T transition at nucleotide 2482 (arrow), which is a silent point mutation.

### TABLE 3. SV40 Tag mRNA expression and SV40 Tag oncoprotein in human thyroid tumors

<table>
<thead>
<tr>
<th>Thyroid tissue</th>
<th>Tag mRNA*</th>
<th>Tag oncoprotein*</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTC</td>
<td>9/13 (69)</td>
<td>3/9 (33)</td>
</tr>
<tr>
<td>ATC</td>
<td>8/11 (73)</td>
<td>8/8 (100)</td>
</tr>
<tr>
<td>Negative controlf</td>
<td>0/10</td>
<td>0/10</td>
</tr>
<tr>
<td>PTC</td>
<td>0/10</td>
<td></td>
</tr>
<tr>
<td>GD</td>
<td>0/10</td>
<td></td>
</tr>
<tr>
<td>NTT</td>
<td>0/10</td>
<td></td>
</tr>
</tbody>
</table>

* SV40 Tag mRNA was investigated by RT-PCR in specimens previously found SV40Tag by PCR (Tables 1 and 2). Positive samples/samples analyzed, and percentage (%) are indicated.

f SV40 Tag oncoprotein was investigated by immunohistochemistry with the mab Pab 101.

Negative controls are SV40Tag specimens, i.e. 10 PTC, 10 GD, and 10 NTT from patients affected by multinodular goiter.

DNA molecules that may occur in human cells even at low multiplicity of infection. It is also possible that some SV40 regions are not present in our samples (6, 12, 15, 19, 54, 55).

Tag expression was not revealed in all samples previously found to be SV40 positive by PCR. This result could be due to our conditions of RT-PCR, which did not allow detection of the SV40 Tag mRNA present in small amounts. None of the negative controls was positive for the SV40 Tag transcript. Immunohistochemistry analysis for the Tag oncoprotein carried out in thyroid cancers found positive for the Tag viral transcript shows a perfect identity in ATC specimens, whereas only 33% of PTC samples positive by RT-PCR stained positively for the Tag oncoprotein, probably because of the low amount of the viral product. In the 80% of the SV40 Tag-positive PTC and ATC samples, immunostaining was detected in only a fraction of the cells. This result is in agreement with the data from previous investigations, which detected a low SV40 load in human samples. SV40 Tag staining was mainly detected in the cytoplasm of both PTC- and ATC-positive samples. Other studies reported SV40 Tag staining in the cytoplasm (56). In the field, there is a general agreement for considering the cytoplasmic staining of a nuclear protein, such as SV40 Tag, a false positive result. However, a recent investigation reported the characterization of a cytoplasmic cell protein, named p193, belonging to the Bcl-2 family, which interacts specifically with SV40 Tag. The p193/SV40 Tag complex has been found in the cell cytoplasm of SV40-positive cells. Interestingly, it turned out that p193 is an apoptosis-promoting protein, and SV40 Tag bound to it inhibits p193 apoptotic activity (56). It is possible that in our samples the p193/Tag complex occurred, thus explaining the presence in the cytoplasm of the Tag viral oncoprotein. In this connection it should be pointed out that the apoptotic activity in thyroid tumor cells is very low (57).

It has been reported that the SV40 late promoter is regulated by the thyroid hormone receptor-α1 (TRα1) in combination with the retinoid X receptor-α (RXRα). The inhibition is relieved by the thyroid hormone T3 (58, 59). The hypothesis is that TRα1 and RXRα regulators block transcription of the late genes until the onset of viral replication (58, 59). The viral early genes are poorly transcribed when late genes are overexpressed, thus reducing the amount of Tag molecules, viral DNA copies, and virions (60). This mechanism seems cell type specific (60). TRα1 and RXRα are present and active in follicular thyroid cells (61) and perhaps in parafollicular cells,
which are of neural origin. Recently, it has been reported that TRα1 is present in the inactivated form, because of gene mutations, in approximately 60% of PTC (62). It is possible that the low levels of SV40 DNA replication, Tag expression, and virion production in thyroid tumor cells occur because the viral late gene promoter is not inhibited by TRα1 and RXRα, either for the presence of thyroid hormone T3, which relieves the TRα1, or for the inactive TRα1 detected in 60% of PTC.

In this study, which was mainly carried out by PCR, it was found that both neoplastic and normal thyroid tissues from patients affected by thyroid cancer are SV40 positive with high prevalence, suggesting that the SV40 infection may spread from neoplastic cells to the adjacent normal thyroid tissue.

The detection of SV40 DNA in PBMC of healthy individuals confirms the presence of these viral sequences in blood cells (2, 3, 6, 15, 19, 21). The detection of SV40 sequences in PBMC indicates that blood cells may transfer SV40 DNA/virus to different tissues of the host. SV40 after PBMC infection, like JC and BK human polyomaviruses (32), could persist or remain latent for a long period in these cells.

The putative role of SV40 in human tumors is still a matter for investigation and discussion (32, 34). SV40 sequences were detected with different frequencies by several investigators in six different human tumors (brain and bone tumors, mesotheliomas, lymphomas, pituitary adenomas, and thyroid carcinomas) (32, 34, 54), whereas Tag oncogene expression was revealed in human tumor samples by different techniques (32, 34). These data suggest that SV40 is not a simple passenger virus in human tumor cells. Our data indicate that different SV40 DNA regions may be present with different prevalences. As human cells are considered semipermissive, and SV40 generates in these cells defective DNA molecules at a high rate (6, 28), it is possible that SV40 regions absent in a fraction of our samples reflect the presence of incomplete genomes. Moreover, the presence of SV40 sequences in thyroid specimens is not always coupled to the

![A](image1.png)
![B](image2.png)
![C](image3.png)
![D](image4.png)

**FIG. 4.** Immunohistochemical analysis of SV40 Tag in thyroid carcinomas. A, COS-7 cells employed as a SV40 Tag-positive control. The Tag oncoprotein is revealed in the cell nucleus. B, PTC sample T335 with Tag cytoplasmic staining in approximately 30% of tumor cells. C, PTC sample T405 with Tag cytoplasmic staining in the majority of tumor cells. D, ATC sample A7 with a strong cytoplasmic staining revealed in cancer cells.
Tag detection, as observed before in other human tumors (34).

SV40 has a number of characteristics indicating that it may cooperate as a cofactor for the development or progression of human tumors (32, 34). SV40 cooperating with the c-ras-activated oncogene and the catalytic subunit of the telomerase transforms in vitro human fibroblasts (63) and astrocytes (64), and it induces malignant tumors in rodents and transgenic mice (32, 34). Moreover, SV40 Tag activates vascular endothelial growth factor expression (65, 66), and hepatocyte growth factor generating hepatocyte growth factor/scatter factor/c-met autocrine and paracrine loops which drive cell proliferation and invasiveness of both Tag-positive and Tag-negative cells (67). In this context it is worth reminding that hepatocyte growth factor-scatter factor/c-met and vascular endothelial growth factor are found overexpressed in PTC (68–70). Taken together our data and the results of other investigations suggest that the different transforming activities of SV40 may operate in human thyroid tissues during a persistent infection.

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