ONCOLOGY

Human Spontaneous Laryngeal Carcinoma HEp-2 Cells Are Chronically Infected with SRV-1 Virus, a Variant of Simian Type D Retrovirus

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A type D retrovirus chronically persisting in HEp-2 cells from human laryngeal carcinoma was analyzed by PCR and sequenced. This virus is most similar to SRV-1 and probably represents one of its subtypes.

Key Words: *type D retroviruses; SRV-1; HEp-2 cells*

Exogenous type D retroviruses (RV/D) are lymphotropic viruses. They were isolated from some monkey species kept in primate research centers in the USA. These classic retroviruses contain no oncogens and belong to so-called «simple» viruses. The group of simian RV/D includes Mason—Pfizer monkey viruses (MPMV or SRV-3), SRV-1, and SRV-2, as well as less frequent SRV-4 and SRV-5. MPMV, the first isolated virus of this group, was obtained from a female macaque with breast cancer [3]. In addition, MPMV and similar SRV/D isolates were identified as causative agents of certain infectious immunodeficient diseases in macaques clinically similar to AIDS in humans [6]. The nucleotide sequences of SRV-1 and SRV-2 are very similar to that of MPMV; these viruses can cause AIDS in monkeys [8,9].

Anti-RV/D antibodies were found in healthy inhabitants of West Africa [7] and patients with lymphadenopathies, including AIDS [2]. SRV/D was also isolated from a patient with B-cell lymphoma and AIDS [2]. Nucleotide sequences of RV/D were recently found in lymphocytes from children with Burkitt lymphoma [5].

HEp-2 cell culture maintained for several years at the Laboratory of Immunology of Oncogenic Viruses (N. N. Blokhin Russian Oncology Research Center) was isolated from spontaneous laryngeal carcinoma of a deceased female patient. This line chronically pro-

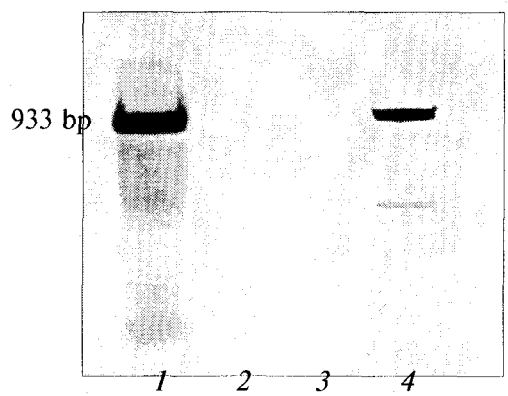

Fig. 1. Electrophoresis of PCR products of the *pro* region of a Mason—Pfizer virus (MPMV, *1*), cell lines Raji (*2*) and Jurkett (*3*). and SRV subtype from HEp-2 cells (*4*) in 5% PAAG.

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                    ---------G--T----------------      -2884   MPMV
                    ---------C--T--------------GA      -2479   SRV-2
CCTCAGTTCCACCTCCCACACAGTATTAACACCGGAAATGGGGCCCCAAG     -2500   SRV-1
                    ||||||||||||||||||||||||||||||
                    AGTATTAACACCGGAAATGGGGCCCCAAG      -29     HEp-2
----------------------A--A--G----------------------2934 MPMV
-C---GCT------G-------A--T--G--C--T--T--G-----G---2529 SRV-2
CGTTAAGCACTGGAATATATGGGCCCCTACCTCCCAACACTTTTGGATTA          -2550 SRV-1
|| |||||||||||||||||||| |||||||||||||||||||||||||| ||
CGTTAAGCACTGGAATATATGGGCCCCTACCTCCCAACACTTTTGGATTA  -79     HEp-2
---C-----C-------------------G--------------------2984 MPMV
--AC--------G-C--T-CC--AG--------T------A----C-----       -2579   SRV-2
ATCTTAGGCAGAAGTAGCATTACTATAAAAGGTCTACAAGTTTATCCAGG -2600   SRV-1
|||||||||||||||||||||||| ||||||||||||||||||||||||
ATCTTAGGCAGAAGTAGCATTACTATGAAAGGTCTACAAGTTTATCCAGG  -129    HEp-2
--------------------C--------------T--------A----3034 MPMV
---T-----------T-----A------T-C-----------C-G----A-2629 SRV-2
AGTAATTGATAATGACTATACTGGGGAAATTAAAATAATGGCAAAGGCTG -2650   SRV-1
|||||||||||||||||||| ||||||||||||||||||||||||
AGTAATTGATAATGACTATACTGGAGAAATTAAAATAATGGCAAAGGCTG          -179  HEp-2
-T--                                                        MPMV
--TC                                                       SRV-2
TCAACAATATTGTTACTGTTCCTCAAGGCAACAGGATAGCTCAATTAATC  -2700   SRV-1
||||
TCAA                                               -183    HEp-2
.....................................................................................
                                   ----------C---------3434 MPMV
                                   -C--C-----C-----G---3029 SRV-2
GAAAATAATTCTGGTCTCATTAAACCGTTTGTTATTCCTAATTTACCTGT  -3050   SRV-1
                                   ||||||||||||||||||
                                   TTATTCCTAATTTACCTGT      HEp-2
---T---------C-----T-A--------------------------- -3484   MPMV
T--T---------C-----T-A-----C----------C----------  -3079   SRV-2
CAACCTTTGGGGCAGAGATCTCCTTTCTCAAATGAAAATTATGATGTGTA -3100   SRV-1
|||||||||||| |||||||| ||||||||||||||||||||||||||
CAACCTTTGGGGCAGAGATCTNNTTTCTCAAATGAAAATTATGATGTGTA      HEp-2

-T--C-A                                                    MPMV
------A                                                    SRV-2
GTCCTAGTGACATAGTCACTGCCCAAATGTTAGCCCAAGGCTACAGCCCC -3150   SRV-1
|||||||
GTCCTAG                                            -3150   HEp-2
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Fig. 2. Nucleotide sequence of the terminal regions of amplified DNA fragment from SRV subtype found in HEp-2 cells. Vertical lines show coincident nucleotides in sequences of SRV-1 and SRV subtype found in HEp-2 cells; letters: noncoincident nucleotides in sequences of SRV-1, SRV-2, and Mason—Pfizer virus. Nucleotide positions for SRV-1 are given according to GB ACCESSION M11841 sequence database.

duces infective virus of RV/D family [4]. Published data suggest that simian RV/D can infect humans and posed the problem of accurate identification of the virus infecting *in vitro* HEp-2 cell. We performed a PCR analysis and sequenced this virus.

MATERIALS AND METHODS

HEp-2 cells were cultured in RPMI medium containing 10% fetal calf serum. DNA was isolated from HEp-2 cells by phenol extraction.

PCR was performed with two oligonucleotide primers *aggggccagccccaggcccc* and *tggggtgcaagtatgtcaatggccc*.

The PCR program consisted of 30 cycles with the following parameters: 30 sec at 96°C, 1 min at 55°C, and 2 min at 72°C. Polyacrylamide gel electrophoresis of DNA fragments was performed as described elsewhere [1]. The specificity of amplified DNA fragments was determined by hybridization with full-length MPMV sequence (plasmid containing MPMV genome insert was kindly provided by Dr. E. Hunter, USA). The probe was prepared and hybridization was performed in accordance with DIG DNA Labeling and Detection Kit protocol (Boehringer).

Sequencing of amplified DNA fragments was performed as described previously [1]; before the sequencing, DNA fragments were purified by PAGE.

RESULTS

The initial characterization of the virus produced by HEp-2 cell line was performed with antiserum against *gag*-coded MPMV proteins [4]. However, homology of SRV genomes suggested that SRV-1 and SRV-2 proteins can also interact with this serum. In light of this, PCR primers used in this work were oligonucleotides corresponding by their primary structures to conserved region of *pro* gene common for all three SRV and, hence, detecting all these viruses.

PCR showed that a SRV-type DNA was actually present in HEp-2 cells (Fig. 1). For MPMV, the predicted length of this fragment is 933 bp, which is consistent with the result of electrophoretic analysis of PCR products. The full-length amplified fragment was mapped to the region 2336-3245 of the SRV-1 genome. This region contains genes *p*14 (2081-2368), *pro* (2296-32-40), and *pol* (3216-5819). Terminal regions of the amplified fragment were sequenced using oligonucleotide primers mapped to 2434-2454 and 3167-3147. It was demonstrated that the studied SRV-type virus is most close to SRV-1 (Fig. 2) and probably represents a subtype of this virus. However, we cannot exclude the possibility that other regions of the SRV genome isolated from HEp-2 cells and SRV-1 are not homogeneous. Therefore, the definite assignment of this virus to a particular SRV type requires its more complete characterization.

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