JOURNAL OF VIROLOGY, Mar. 1980, p. 1225-1228
0022-538X/80/03-1225/04$02.00/0

Vol. 33, No. 3

Integration Pattern of Human JC Virus Sequences in Two Clones of a Cell Line Established from a JC Virus-Induced Hamster Brain Tumor

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The physical state of the JC virus (JCV) genome was studied in two clonal cell lines (clones 2 and 7) derived from a tissue culture cell line (HJC-15) established from a hamster brain tumor induced by JCV. Saturation-hybridization and reassociation kinetic analyses, using in vitro ^{32}P-labeled JCV DNA, indicated that clone 7 and 2 cells contain 9 to 10 and 4 to 5 copies per cell, respectively, of all or most of the viral genome. Both cell DNAs were analyzed by using the Southern blotting procedure with three restriction endonucleases: *Xho*I, which does not cleave JCV DNA; *Eco*RI, which cleaves once; and *Hin*dIII, which cleaves three times. With each DNA, a variety of JCV-specific DNA fragments were detected. The following conclusions are possible: (i) JCV DNA is integrated into cell DNA in both clonal lines; (ii) both clonal lines contain multiple copies of the viral genome integrated in a tandem head-to-tail orientation; (iii) neither clonal line contains detectable free-form I, II, or III JCV DNA; (iv) each clonal line contains multiple independent sites of JCV DNA integration; and (v) most or all of the sites of integration on the cellular or the viral genome, or both, are different in clone 7 DNA than in clone 2 DNA. Thus, although both clone 7 and clone 2 cells were established from the HJC-15 tumor cell line, they differ in the copy number and integration pattern of JCV DNA.

JC virus (JCV) is a ubiquitous human papovavirus (12) that was first isolated from the brain of a patient with progressive multifocal leukoencephalopathy (13). Relatively little is known about JCV because the only cell culture systems that support its growth are human primary fetal glial cells (12) and, to a lesser extent, human amnion cells (17). An interesting property of JCV is that it is highly tumorigenic in newborn hamsters (18). As a means to investigate the high tumorigenicity of JCV, and to learn more about the molecular biology of the virus, we have used the Southern blotting procedure (15) to study the physical state of the JCV genome in two clonal cell lines established from the same cultured JCV-induced hamster brain tumor. These studies are described in this report and represent the first analysis of the integration of JCV sequences in transformed cell lines.

JCV (strain Mad-1) was grown in human fetal glial cells, and viral DNA was isolated from infected cells as described earlier (9). The DNA was labeled in vitro with ^{32}P by nick translation (8, 19).

The HJC-15 cell line was established from a hamster brain tumor (a mixed malignant glioma of astrocytic and ependymal derivations) induced by intracerebral inoculation of neonatal hamsters with JCV. Attempts to isolate JCV from HJC-15 cells after 12 passages were unsuccessful, but JCV was rescued after Sendai virus-mediated fusion with fetal glial cells (18). Clonal sublines were derived from HJC-15 at the 50th passage by three successive cloning steps. Clones 2 and 7 are morphologically and culturally distinct. Clone 7 requires a more complex growth medium than clone 2, which grows well in minimal essential medium and 3% fetal calf serum. Uncloned HJC-15 cells and clone 7 are tumorigenic in weanling hamsters whereas clone 2 cells are not; both clones synthesize JCV T-antigen (18; B. L. Padgett and D. L. Walker, unpublished observations).

Figure 1A shows hybridization of ^{32}P-labeled JCV DNA with DNAs from the two JCV tumor cell lines and with 0.2, 1, and 10 copies of JCV DNA per cell. Both tumor cell DNAs drove most of the probe into hybrid, as did excess JCV DNA, demonstrating that both lines contain, at least, approximately 80% of the JCV genome. Reassociation kinetic analyses are shown in Fig. 1B and indicate that clone 7 cells contain 9 to 10 copies of the JCV genome per diploid equivalent of cell DNA and that clone 2 cells contain 4 to 5 copies.

Southern blotting analyses of DNAs from the

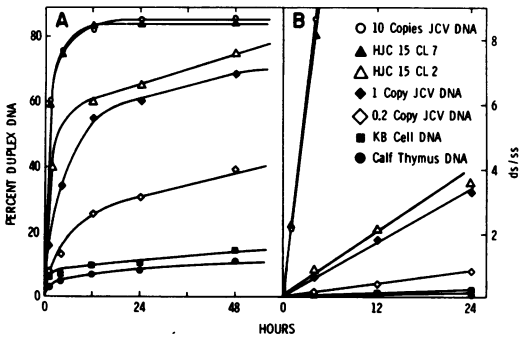

FIG. 1. *Hybridization of in vitro ^{32}P-labeled JCV DNA with 0 to 10 copies of JCV DNA or HJC-15 hamster tumor cell line DNA. ^{32}P-labeled JCV DNA (1.2×10^8 cpm/μg, 500 cpm/25 μl) was annealed with 6 mg of HJC-15 clone 7 (CL 7) or clone 2 (CL 2) tumor cell line DNA per ml, 6 mg of KB cell DNA per ml, or 6 mg of calf thymus DNA per ml mixed with 0 to 10 copies of JCV DNA. Samples were taken at times indicated, and duplex DNA formation was determined by batchwise hydroxylapatite chromatography. The procedures for hybridization and extraction of tumor cell DNA have been described (19). ds/ss, Double stranded/single stranded.*

two cell lines are shown in Fig. 2. Three restriction endonucleases were used: *Xho*I, which does not cleave JCV DNA; *Eco*RI, which cleaves once; and *Hin*dIII, which cleaves three times to yield fragments that are 88, 8, and 4% of the length of simian virus 40 DNA (9, 10). *Xho*I digestion of both DNAs yielded a diffuse series of fragments that migrated more slowly than form II JCV DNA and that were not resolved in our gel system (Fig. 2, lanes A and B). No form I, II, or III JCV DNAs were detected, even after long exposures to X-ray film. (Note that despite being a no-cut enzyme, in control digestions, i.e., lanes C and D, *Xho*I generated form II and even a little form III DNA, probably because of nonspecific nuclease present in the enzyme preparation.) We conclude that these cells do not contain detectable free genome-length JCV DNA and that all JCV sequences are present in high-molecular-weight fragments which probably represent viral-cell DNA junction fragments. However, we cannot exclude the unlikely possibility that the JCV sequences are not integrated into cellular DNA, but rather are in the form of an unusual free heterogeneous collection of oligomers of JCV genomes.

*Eco*RI digestion of both cell DNAs yielded a prominent JCV-specific fragment that comigrated with form III DNA, as well as additional fragments, most of which were larger than form III DNA (Fig. 2, lanes F and G). This result is strong evidence that some of the JCV sequences in both cell lines are in a tandem head-to-tail orientation. Also consistent with this conclusion is that *Hin*dIII digestion of both cell DNAs yielded a fragment that comigrated with the *Hin*dIII-A fragment of JCV DNA (the small *Hin*dIII-B and -C fragments have run off the gel) (Fig. 2, lanes K and L). A variety of other JCV-specific *Hin*dIII fragments, both larger and smaller than the *Hin*dIII-A fragment, were also observed.

The portions of the JCV genome in the *Eco*RI fragments other than form III (Fig 2, lanes F and G), and the *Hin*dIII fragments other than *Hin*dIII-A (Fig. 2, lanes K and L), cannot be determined without further blotting analyses with subgenomic fragments of the JCV genome as probe. However, certain deductions are possible. If all JCV sequences integrated into cell DNA were in a tandem orientation, then only two virus-cell junction fragments would be present. Since many fragments are observed, it is likely that each cell line contains JCV sequences integrated at multiple, independent sites. This possibility is consistent with the *Xho*I results (Fig. 2, lanes A and B), which suggest a variety of JCV-specific, high-molecular weight fragments.

Most of the putative virus-cell junction fragments in the two lines were different in size, indicating that JCV sequences can integrate at several sites in hamster cell DNA. Thus, JCV apparently resembles other papovaviruses in that the cellular DNA itnegration site is not specific (1, 3, 4, 7). However, specificity at the nucleotide level is not excluded.

Our results represent the third papovavirus for which a tandem head-to-tail orientation in transformed cells has been demonstrated; the other viruses are simian virus 40 (3, 4) and polyomavirus (1). These tandem sequences could arise by integration of a multigenomic oligomer, such as has been detected in simian virus 40-infected cells (14), or by amplification (e.g., by chromosome recombination) of a single viral genome after integration. Tandem integration has been hypothesized to explain spontaneous induction of polyomavirus DNA in transformed cells (1); our results do not support the generality of this hypothesis because we did not detect free genomic JCV DNA molecules.

Other published restriction endonuclease digestions of JCV DNA have revealed a variety of submolar fragments that are thought to arise from defective JCV genomes (6, 9–11, 17). We did not usually observe such fragments, except at trace levels in some experiments (not shown).

A striking observation is that although both clonal lines were established from the same hamster brain tumor cell line after 50 passages in tissue culture (18), the concentration and the

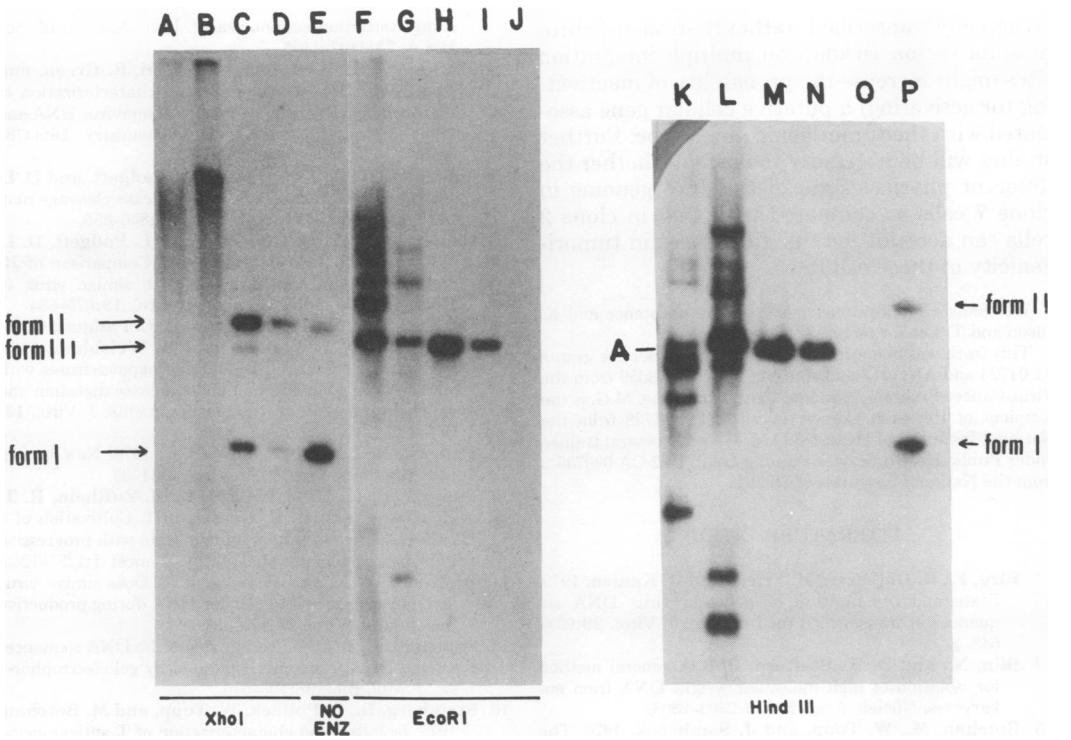

FIG. 2. *Detection of DNA fragments that contain JCV-specific sequences after digestion of two JCV hamster tumor cell line DNAs with XhoI, EcoRI, or HindIII. The products of enzyme digestion were fractionated by agarose gel electrophoresis, transferred to nitrocellulose, annealed with in vitro ^{32}P-labeled JCV DNA, and autoradiographed. The procedures used are as described earlier (19), except that 0.5 M sodium acetate (pH 5.0) was used to neutralize the agarose gel after alkalai denaturation of DNA and for transfer of DNA from the gel to nitrocellulose. High-molecular-weight tumor cell DNA for blotting was extracted by a combination of procedures described by Blin and Stafford (2) and by Graham (5). (A) XhoI-digested clone 2 DNA; (B) XhoI-digested clone 7 DNA; (C) XhoI-digested JCV DNA, five copies per cell; (D) XhoI-digested JCV DNA, one copy per cell; (E) JCV DNA, no restriction endonuclease; (F) EcoRI-digested clone 7 DNA; (G) EcoRI-digested clone 2 DNA; (H) EcoRI-digested JCV DNA, five copies per cell; (I) EcoRI-digested JCV DNA, one copy per cell; (J) EcoRI-digested KB cell DNA (KB cells are a human cell line not expected to contain JCV sequences); (K) HindIII-digested clone 2 DNA; (L) HindIII-digested clone 7 DNA; (M) HindIII-digested JCV DNA, five copies per cell; (N) HindIII-digested JCV DNA, one copy per cell; (O) HindIII-digested KB cell DNA; (P) JCV DNA, no restriction endonuclease.*

integration pattern of JCV sequences in clone 7 cells are different from those in clone 2 cells. This suggests that either the arrangement of JCV sequences in the original tumor cell was not stable or the tumor was not monocellular in origin. Our data underscore the importance of cloning cells for studies on virus integration.

An interesting aspect of these studies is that clone 7 cells are tumorigenic in weanling hamsters, whereas clone 2 cells are not (Padgett and Walker, unpublished observation). Of possible significance in this regard is that clone 7 cells contained more copies of the JCV genome, more copies of the genome arranged in tandem, and more apparent sites of JCV DNA integration. More copies of JCV sequences might result in higher cellular concentrations of a JCV-coded tumor antigen(s) which might render the cell tumorigenic. The tandem arrangement of the JCV sequences might also result in increased levels of T-antigens, if a series of early gene promoters in proximity are more likely to be transcribed than are independently integrated early gene promoters. Tandem integration of one or more complete JCV genomes also ensures that at least one uninterrupted version of the early region is present. However, there are simian virus 40-transformed rat cells that harbor only one integrated genome yet express a fully transformed phenotype, including tumorigenicity (16); this does not support (nor does it refute) the gene dosage argument for cell tumorigenicity presented above. Integration could affect tumorigenicity in other ways. For example, integration at multiple sites in cellular DNA might increase the probability of the viral sequences residing in

an actively transcribed (rather than silent) chromosome region. In addition, multiple integration sites might increase the probability of inactivating (or activating) a putative cellular gene associated with the tumorigenic phenotype. Further studies will be necessary to resolve whether the different physical state of the JCV genome in clone 7 cells as compared with that in clone 2 cells can account for the differences in tumorigenicity of these cell lines.

We thank H. Thornton for cell culture assistance and K. Olson and T. Lee for technical assistance.

This work was supported by Public Health Service grants AI-01725 and AI-11217 and contract NO1 CP 43359 from the Virus Cancer Program, National Cancer Institute. M.G. is the recipient of Research Career Award 5K5-AI-4739 from the National Institutes of Health. J.D.M. is a postdoctoral trainee under Public Health Service training grant T 32 CA 090735-2 from the National Institutes of Health.

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