

Human Papillomavirus Type 18 DNA Is Integrated at a Single Chromosome Site in Cervical Carcinoma Cell Line SW756

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SW756, a cervical carcinoma cell line, has multiple copies of human papillomavirus type 18 DNA sequences. The integration site of human papillomavirus type 18 DNA was localized by in situ hybridization to chromosome 12 at band q13. This single integration site corresponds to a heritable fragile site, which may have facilitated the integration of the viral DNA.

Human papillomavirus types 16 and 18 (HPV16 and HPV18) have been implicated in the etiology of genital cancer (1, 5, 17). Integrated single or multiple copies of HPV18 DNA have been detected in cervical carcinoma cell lines and biopsies (17). Two cell lines derived from cervical carcinomas, SW756 and HeLa, have abnormal chromosome constitutions with numerical and structural alterations, contain 10 to 50 copies of HPV18 DNA (17), and have similar HPV18 transcription patterns (16). In HeLa cells, HPV18 DNA is integrated at four chromosome sites: on normal chromosomes 8 and 9 and on two abnormal chromosomes derived from 5 and 22, respectively (12a). To determine whether HPV18 DNA in SW756 cells is integrated at the same locations as in HeLa cells, we localized the integration site(s) in SW756 chromosomes by an in situ hybridization technique. The present analysis demonstrates that multiple copies of HPV18 DNA are integrated at a single site in SW756 chromosomes.

HPV18 DNA was molecularly cloned from a cervical carcinoma biopsy (1). The 7.8-kilobase *EcoRI* fragment used cross-hybridizes with other papillomavirus types only under low-stringency conditions, and its genome is aligned colinearly with the genomes of human papillomavirus type 6 (HPV6) and HPV16. This DNA probe was used to assign the HPV18 integration sites in HeLa cells (12a) and SW756 cells (present study). The DNA was labeled with all four ³H-deoxy nucleoside triphosphates to a high specific activity (2.7×10^7 cpm/ μ g) by using a nick translation kit (Amersham Corp., Arlington Heights, Ill.). Chromosomes were obtained from synchronized SW756 cell cultures (3). The in situ hybridization procedure for mapping single-copy genes was carried out under stringent conditions of 50% formamide-2 \times SSC (1 \times SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at 42°C for both hybridization and subsequent washings (7, 12a). Autoradiography and details of G-banding through the emulsion have also been described previously (12). No specific hybridization at any chromosome region was detected by in situ hybridization of HPV18 DNA with human chromosomes derived from normal leukocyte cultures (12a).

SW756 is a near tetraploid cell line; normal chromosomes were present in at least two copies, with the exception of chromosome 13, which was frequently monosomic or nulisomic. Several abnormal marker chromosomes (M) were identified by G-banding (10) (Fig. 1): M1 is an iXq chromosome; M2 is a 2p- chromosome; M3, M4, and M5 have long arms consistent with the banding patterns of chromosomes

13q, 9q, and 7q, respectively; M6 is an iXp chromosome; M7 has two prominent, darkly stained G-bands, one on the long arm and one near the centromere, with the rest of the long arm being stained homogeneously (6); M8 is a deleted 6; and M10 is an i9p chromosome. A small acrocentric chromosome (not present in Fig. 1) was probably derived from chromosome 18p. The interpretation of these abnormal chromosomes is consistent with the initial cytogenetic characterization of this cell line (6).

Fifty metaphases and prometaphases were examined after in situ hybridization with the HPV18 DNA probe, autoradiography, and G-banding for chromosome identification. Of the total 260 silver grains localized, 141 (54%) were on chromosome 12, with the largest accumulation at band q13 (Fig. 2). Thirty-seven metaphases had grains on normal chromosome 12; 51% of these had a single chromosome 12 labeled, 29% had two labeled (Fig. 3), and 20% had three labeled. From a total of 62 chromosome 12's labeled, 23% had one grain, 42% had two grains, 21% had three grains, and 14% had four grains. With the exception of the hybridization site on chromosome 12, no significant accumulation of grains was observed on other normal or abnormal chromosomes. Therefore, HPV18 DNA is integrated at a single site, chromosome band 12q13.

This assigned site coincides with the location of a heritable fragile site at 12q13.1 (21). In HeLa cells, three integration sites of HPV18 DNA are at or in close proximity to fragile sites (12a). In two simian virus 40 (SV40) transformed human-mouse cell hybrids and four lymphoblastoid Epstein-Barr virus-transformed cell lines, some integration sites also correspond to the location of fragile sites (13, 19). Physical and chemical carcinogens break chromosomes at fragile sites (20), and this vulnerability may be also important for the clastogenic action of viruses. Recently, a postintegration rearrangement of hepatitis B virus DNA in a human hepatocellular carcinoma was directly implicated in the generation of chromosome aberrations resulting in the formation of a 17;18 translocation (8).

The identification of cells with silver grains on three copies of normal chromosome 12 strongly indicates that polyploidization of SW756 cells occurred after HPV18 DNA integration. Multiple copies (10 to 50) of HPV18 DNA are integrated at a single site in SW756 cells and at four sites in HeLa cells (12a). Whether HPV18 DNA sequences were originally integrated in HeLa cells at only one site or independently at multiple sites is not known. Three integration sites in HeLa cells correspond to the locations of *c-myc*, *c-abl*, and *c-sis* proto-oncogenes (12a). The integration site

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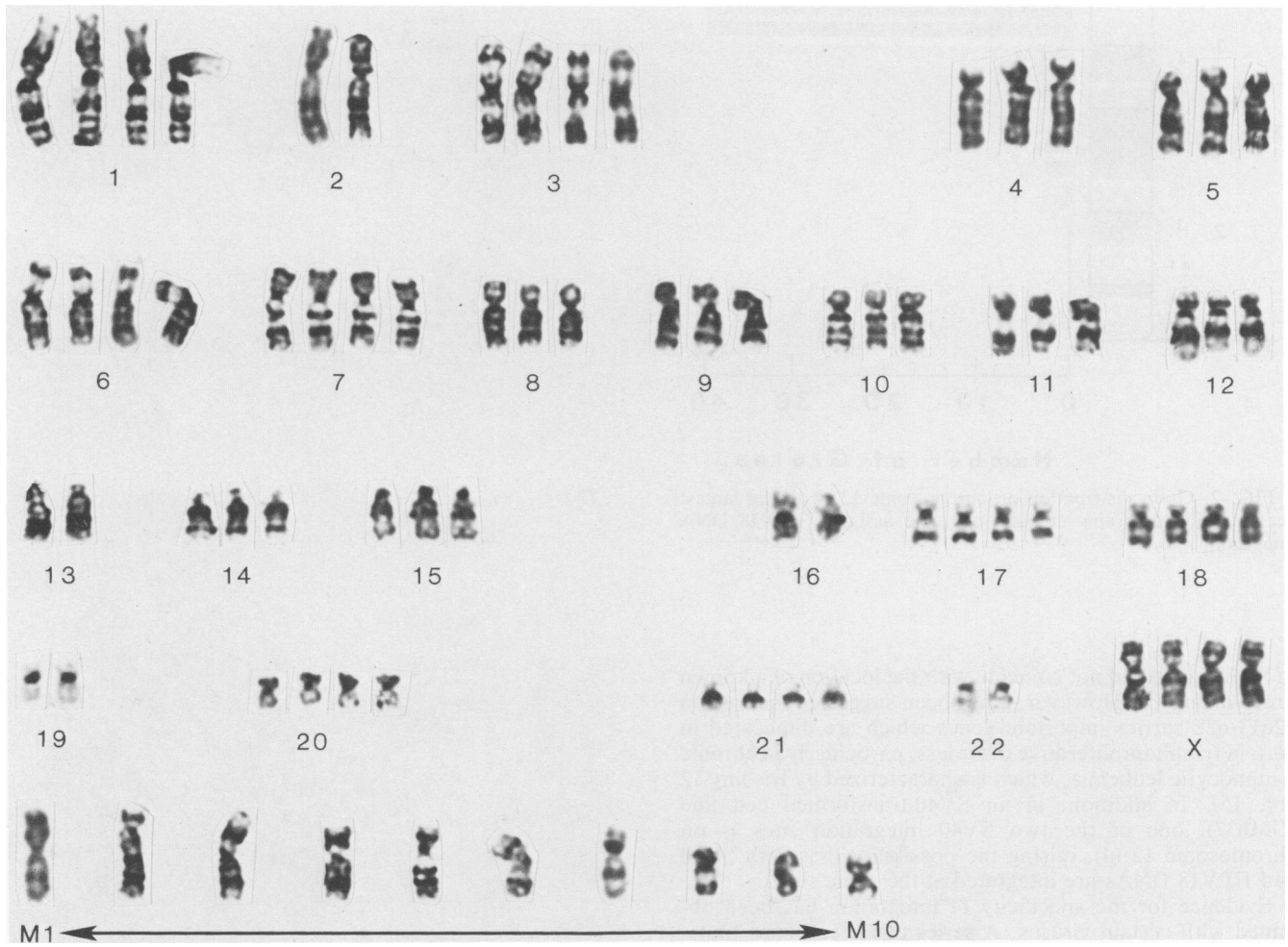


FIG. 1. G-band karyotype of an SW756 cell with 83 chromosomes, 10 of which are abnormal and arranged at the bottom (M1 to M10). See the text for the derivation of the abnormal chromosomes.

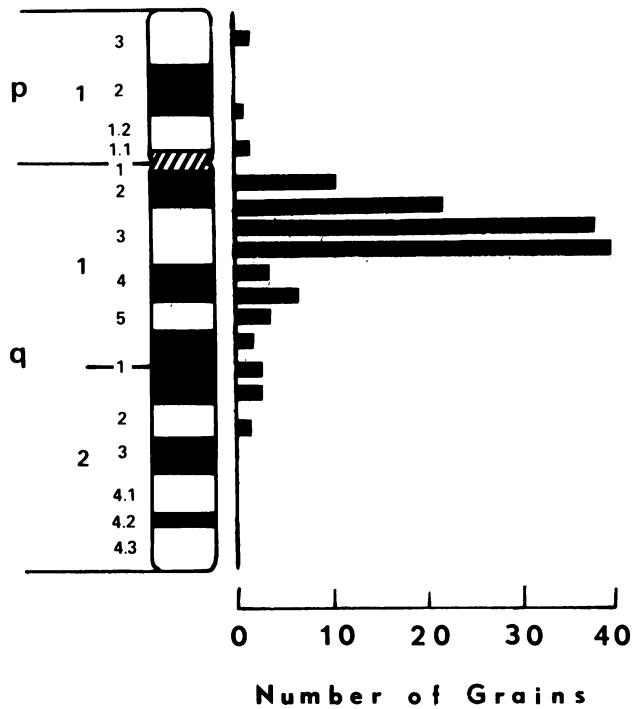


FIG. 2. Grain distribution on chromosome 12, with the largest accumulation of grains at band q13, the assigned HPV18 DNA integration site.

in SW756 cells did not coincide with the location of a known proto-oncogene. However, it has been suggested that region 12q13-q22 carries important genes which are duplicated in certain lymphoproliferative disorders, particularly in chronic lymphocytic leukemia, which is characterized by trisomy 12 (14, 15). In addition, in an SV40-transformed cell line (GM637), one of the two SV40 integration sites is on chromosome 12 (4), raising the possibility that both SV40 and HPV18 DNAs are integrated at the same site.

Evidence for the specificity of integration has been obtained with certain viruses. A series of rat cell lines transformed by SV40 showed identical blot hybridization patterns of the integrated viral sequences (11). Moloney leukemia virus DNA sequences were found preferentially or exclusively at sites with a transcriptionally active conformation (2). In a human hepatoma cell line, hepatitis B virus DNA was detected at the centromeric heterochromatin of chromosomes 1 and 16 as well as the heterochromatic region of the Y chromosome (18). Intracisternal A particles, which are retroviruslike entities, were detected by in situ hybridization at specific regions in the heterochromatin of Syrian hamster chromosomes (9). Recently, another group mapped the cellular sequences flanking integrated papillomavirus DNA in SW756 cells to chromosome 12 by using somatic cell hybrids (4a). Although HPV18 DNA may integrate at different chromosome sites in cervical cells, the results with these cervical carcinoma cell lines show that regions which are prone to breakage and which carry genes important in cell growth regulation and tumorigenesis are nonrandomly affected. Additional studies are required to demonstrate whether such regions are privileged integration sites for HPV18 DNA.

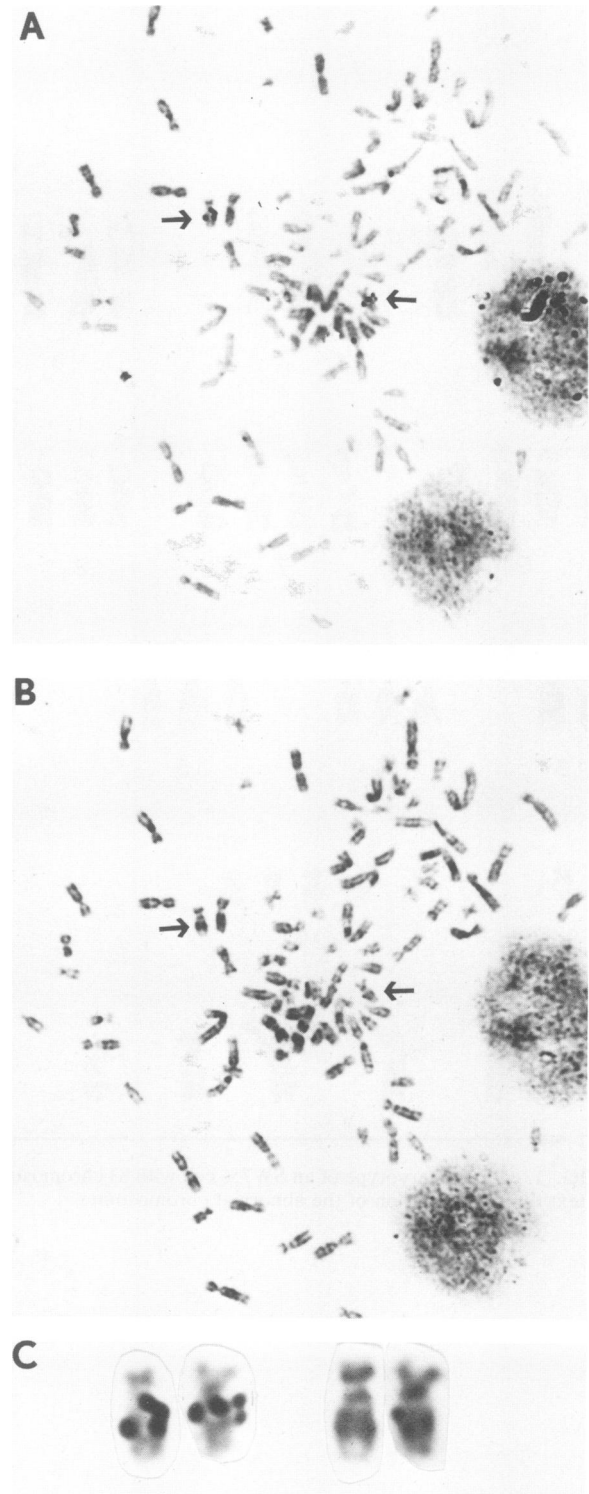


FIG. 3. (A) Representative metaphase after in situ hybridization with an HPV18 DNA probe exhibiting several grains on two chromosomes (arrows). (B) The same spread as in panel A after G-banding induced by trypsin-EDTA treatment showing that the labeled chromosomes are 12's (arrows). (C) Enlargement of the chromosome 12's indicated by the arrows in panels A and B.

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LITERATURE CITED

1. Boshart, M., L. Gissmann, M. Ikenberg, A. Kleinheinz, W. Scheurlen, and H. zur Hausen. 1984. A new type of papillomavirus DNA, its presence in genital cancer biopsies and in cell lines derived from cervical cancer. *EMBO J.* **3**:1151-1157.
2. Breindl, M., L. Bachelier, H. Fan, and R. Jaenisch. 1980. Chromatin conformation of integrated Moloney leukemia virus DNA sequences in tissues of BALB/Mo mice and in virus-infected cell lines. *J. Virol.* **34**:373-382.
3. Chandler, M. E., and J. J. Yunis. 1978. A high resolution *in situ* hybridization technique for the direct visualization of labeled G-banded early metaphase and prophase chromosomes. *Cytogenet. Cell Genet.* **22**:352-356.
4. Croce, C. M. 1981. Integration of oncogenic viruses in mammalian cells. *Int. Rev. Cytol.* **71**:1-16.
- 4a. Durst, M., C. M. Croce, L. Gissmann, E. Schwarz, and K. Huebner. 1987. Papillomavirus sequences integrate near cellular oncogenes in some cervical carcinomas. *Proc. Natl. Acad. Sci. USA* **84**:1070-1074.
5. Durst, M., L. Gissmann, H. Ikenberg, and H. zur Hausen. 1983. A papillomavirus DNA from a cervical carcinoma and its prevalence in cancer biopsy samples from different geographic regions. *Proc. Natl. Acad. Sci. USA* **80**:3812-3819.
6. Freedman, R. S., J. M. Bowen, A. Leibovitz, S. Pathak, M. Siciliano, H. S. Gallager, and B. C. Giovannella. 1982. Characterization of a cell line (SW756) derived from a human squamous carcinoma of the uterine cervix. *In Vitro (Rockville)* **18**:719-726.
7. Harper, M. E., and G. F. Saunders. 1981. Localization of single copy DNA sequences on G-banded human chromosomes by *in situ* hybridization. *Chromosoma (Berlin)* **83**:431-439.
8. Hino, O., T. B. Shows, and C. E. Rogler. 1986. Hepatitis B virus integration site in hepatocellular carcinoma at chromosome 17;18 translocation. *Proc. Natl. Acad. Sci. USA* **83**:8338-8342.
9. Kuff, E. L., J. E. Fewell, K. K. Lueders, J. A. DiPaolo, S. C. Amsbaugh, and N. C. Popescu. 1986. Chromosome distribution of intracisternal A-particle sequences in the Syrian hamster and mouse. *Chromosoma (Berlin)* **93**:213-219.
10. March of Dimes Birth Defects Foundation. 1981. ISCN: an international system for human cytogenetic nomenclature—high-resolution banding. *Birth Defects Orig. Artic. Ser.* **17**:5.
11. Mougneau, E., F. Birg, M. Rassoulzadegan, and F. Cuzin. 1980. Integration sites and sequence arrangement of SV40 DNA in a homogeneous series of transformed rat fibroblast lines. *Cell* **22**:917-927.
12. Popescu, N. C., S. C. Amsbaugh, D. C. Swan, and J. A. DiPaolo. 1985. Induction of chromosome banding by trypsin/EDTA for gene mapping by *in situ* hybridization. *Cytogenet. Cell Genet.* **39**:73-74.
- 12a. Popescu, N. C., J. A. DiPaolo, and S. C. Amsbaugh. 1987. Integration sites of human papillomavirus 18 DNA sequences on HeLa cell chromosomes. *Cytogenet. Cell Genet.* **44**:58-62.
13. Rabin, M., O. C. Uhlenbeck, D. M. Steffensen, and W. F. Mangel. 1984. Chromosomal sites of integration of simian virus 40 DNA sequences mapped by *in situ* hybridization in two transformed hybrid cell lines. *J. Virol.* **49**:445-451.
14. Robert, K. H., G. Gahrton, K. Friberg, L. Zech, and B. Nilsson. 1982. Extra chromosome 12 and prognosis in chronic lymphocytic leukemia. *Scand. J. Haematol.* **28**:163-168.
15. Sandberg, A. A. 1985. Chromosome changes in lymphoma and solid tumors, p. 185-209. *In* R. S. K. Chaganti and J. German (ed.), *Genetics in clinical oncology*. Oxford University Press, Inc., New York.
16. Schneider-Gadicke, A., and E. Schwarz. 1986. Different human cervical carcinoma cell lines show similar transcription patterns of human papillomavirus type 18 early genes. *EMBO J.* **5**:2285-2292.
17. Schwarz, E., U. K. Freese, L. Gissmann, W. Mayer, B. Roggenbuck, A. Stremlau, and H. zur Hausen. 1985. Structure and transcription of human papillomavirus sequences in cervical carcinoma cells. *Nature (London)* **314**:111-114.
18. Shaul, Y., P. D. Garcia, S. Schonberg, and W. J. Rutter. 1986. Integration of hepatitis B virus DNA in chromosome-specific satellite sequences. *J. Virol.* **59**:731-734.
19. Shiraishi, Y., T. Taguchi, Y. Ohta, and K. Hirai. 1985. Chromosomal localization of the Epstein-Barr virus (EBV) genome in Bloom's syndrome B-lymphoblastoid cell lines transformed with EBV. *Chromosoma (Berlin)* **93**:157-164.
20. Yunis, J. J. 1986. Chromosomal rearrangements, genes, and fragile sites in cancer: clinical and biologic implications, p. 93-128. *In* V. T. DeVita, Jr., S. Hellman, and S. A. Rosenberg (ed.), *Important advances in oncology 1986*. J. B. Lippincott Co., Philadelphia.
21. Yunis, J. J., and A. L. Soreng. 1984. Constitutive fragile sites and cancer. *Science* **226**:1199-1204.