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Transcription of Human Papillomavirus Type-18 DNA in Human Cervical Carcinoma Cell Lines *

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A. Introduction

DNA of human papillomavirus (HPV) type 16 or 18 has been identified in about 70% of all human cervical carcinomas analyzed so far [1] and in cell lines established from cervical carcinomas [2, 3]. In the malignant tumors and all cell lines, DNA of HPV 16 or 18 is integrated into the host cell genome. Integration consistently results in disruption of the viral genome within the open reading frame (ORF) E1, E2 region. With regard to the host cell genome, however, HPV 16/18 DNA integration seems to occur at different sites in different tumors and cell lines. Since tissue culture systems susceptible to transformation by HPVs were not available, we used the three HPV 18-positive cervical carcinoma cell lines HeLa, C4-1, and SW756 for a comparative analysis of HPV 18 transcription.

B. Results

HPV 18 transcription was studied by cDNA cloning and sequence analysis of HPV 18-positive cDNA clones isolated from the HeLa, C4-1, and SW756 cDNA library.

The HPV 18-positive cDNA clones of all three cell lines are derived from virus-cell fusion transcripts. They are composed of 3'-terminal host cell sequences spliced to 5'-proximal viral sequences. Splicing involves a viral splice donor site located a few nucleotides downstream from the start codon of HPV 18 ORF E1 (Fig. 1). The 3'-terminal cellular sequences are different in cDNA clones from the three cell lines and also differ – due to variable use of cellular splice acceptor sites – within the same cell line. They do not harbor long ORFs. Furthermore, no viral-cellular fusion ORFs of considerable protein-encoding capacity have been detected in the cDNA sequences. These data indicate that the chimeric viral-cellular transcripts do not contain the information for specific cellular proteins.

The 5' viral sequences are derived from the 5' part of the early region of HPV 18. Three types of cDNA clones can be distinguished due to the splicing patterns observed in the 5' HPV 18 sequences (Fig. 1). As potential protein-coding regions they contain ORFs E6 or E6*, followed in 3' by E7 and E1 (only in HeLa). Northern blot hybridizations with E6/E6* exon- and E1 exon-specific oligonucleotides revealed that mRNAs types 1 and 2 are present in C4-1 and SW756 cells, whereas types 2 and 3 (and type 1 in very small amounts) are present in HeLa cells. Primer extension experiments were performed to determine the 5' ends of the mRNAs. Several transcriptional initiation sites were identified, which are located directly at the A residue of the E6/E6* start codon or up to eight nucleotides upstream from it. These data indicate that a TATA box sequence located 35 nucleotides upstream from the E6/E6* ATG initiates transcription in all three cell lines. The very close proximity of the 5' ends of the mRNAs to the E6/E6* start codon may play a role in

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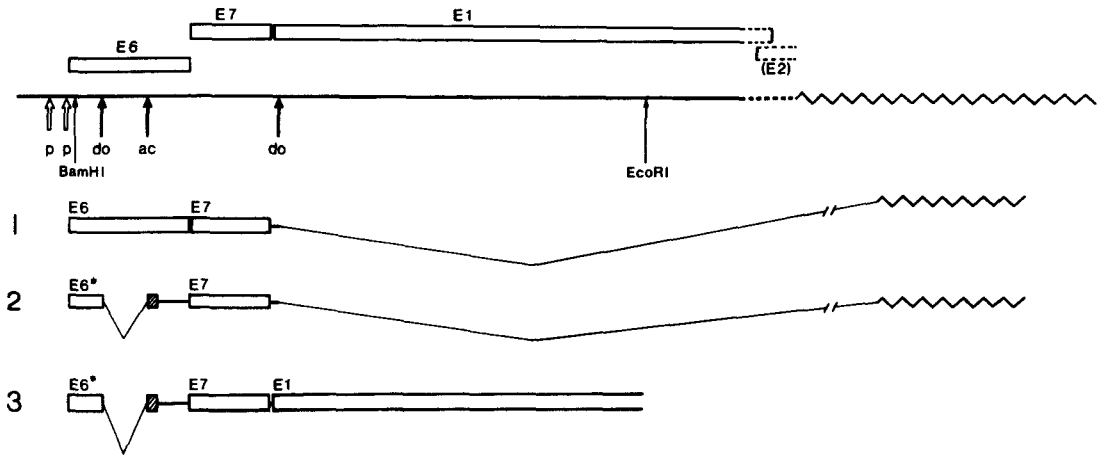

Fig. 1. Splicing patterns of HPV 18-positive cDNA clones from HeLa, C4-1, and SW756 cells. *Upper part:* The E6-E7-E1 region of the integrated HPV 18 DNA is shown together with the 3′ flanking cellular sequences (indicated by *zig-zag lines*). ORFs are indicated by *open boxes*, the viral-cellular junctions by *dotted lines*. The positions of TATA box sequences (*p*), and splice donor (*do*) and splice acceptor (*ac*) sites are indicated. *Lower part:* Three types of cDNA clones can be distinguished according to the splice patterns of the 5′-terminal HPV 18 sequences. As possible protein-coding regions they contain ORFs E6 or E6*, followed in 3′ by ORF E7 and E1 (type 3 is present only in HeLa cells). Intron sequences excised by splicing are represented as *thin, slanted lines*

regulating the synthesis of the E6/E6* and E7 proteins by shifting translation from the 5′-proximal cistron (E6 or E6*) to the second cistron (E7).

ORF E6* is generated by splicing (see Fig. 1) and encodes a putative protein of only 57 amino acids (aa) that shares the N-terminal 43 aa residues with the ORF E6 gene product. Generation of an E6* ORF due to splicing in E6 was not described for other papillomaviruses. Nucleotide sequence comparisons with other genital HPV DNAs revealed that only HPV 16, 18, and 33 contain possible splice sites at the corresponding positions, but HPV 6 and 11 do not, due to single nucleotide exchanges. Thus, it is tempting to speculate that mRNAs with an E6* splice pattern possibly encoding a E6* protein are specific for HPV types associated with genital carcinomas. Sequence comparisons revealed a certain homology between the putative E6* gene product and epidermal growth factors.

The similar transcription patterns of HPV 18 DNA in the three human cervical carcinoma cell lines indicate that expression of specific HPV 18 early genes (E6, E6*, E7) may have a functional role in the maintenance of the malignant phenotype of these cells.

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