Abstract. Earlier we found that SiHa cervical squamous carcinoma cells that harbor HPV type 16 respond to ATRA treatment in a dose-dependent manner: high-dose (10^{-5} to 10^{-4} M) but not low-dose (10^{-7} to 10^{-6} M) ATRA induced growth arrest. Growth of HPV-infected cells is highly dependent on the expression of the viral E6/E7 proteins. Thus, targeting expression of the viral E6/E7 genes might influence growth properties of HPV-infected cells. Here, we demonstrated that high-dose ATRA inhibited expression of HPV16 E7 through suppression of the HPV16 promoter (p97) activity. Gelshift assay (EMSA) revealed that binding of the AP-1 transcription factor to an oligonucleotide originated from the HPV type 16 promoter was diminished after high-dose, but not low-dose ATRA treatment. This suggests that high-dose ATRA suppresses HPV 16 promoter activity, at least in part, via a decreased AP-1 binding. Our data might be useful in treatment of cervical dysplasias and/or carcinomas.

Retinoids, such as ATRA, are widely used in the treatment of cervical dysplasias and cervical cancers (1, 2). ATRA inhibits the growth of cervical cells and also causes apoptosis in vitro (3). Our earlier studies demonstrated that ATRA-induced growth arrest was dose-dependent in SiHa cervical carcinoma cells (4-6), which harbor an integrated copy of HPV 16 (7). The growth properties of HPV-infected cells are highly influenced by the expression of viral early genes such as E6 or E7 (8). Studies by other groups showed that ATRA inhibits transcription of HPV E6/E7 (9, 10) indicating its potential antiviral activity.

Accordingly, we wanted to determine if ATRA treatment does affect transcription of HPV type 16 in SiHa cells and whether this effect is dose-dependent.

Materials and Methods

Cells. The HPV16-positive cervical squamous carcinoma cell line (SiHa) was purchased from ATCC and maintained at 37 °C in DMEM supplemented with 10% FCS in a 5% CO2 atmosphere.

RNA isolation and real-time RT-PCR. Cells were washed with PBS and directly lysed in TriReagent-LS (Molecular Research Center, Inc., Cincinnati, OH, USA) and precipitated according to the manufacturer’s recommendation. mRNA levels of HPV16 E7 were determined by a one-step real-time RT-PCR procedure as described elsewhere (11).

Electrophoretic mobility shift assay (EMSA). SiHa cells were treated with high (10^{-4} M) or low (10^{-6} M) doses of ATRA for 30 minutes and nuclear extracts were prepared (12). Six μg of nuclear extract
was incubated in the presence of a labeled AP-1-binding oligonucleotide from the proximal promoter site of HPV16 (CTACTGAATCACTATG). The DNA-protein complexes were separated from free probe by electrophoresis on a 6% polyacrylamide gel. The gel was dried and subjected to autoradiography.

Results and Discussion

The early genes (E6 or E7) of oncogenic HPVs interact with antioncogenes, cell cycle regulatory proteins or various transcription factors causing impairment in cellular proliferation (13) or immune responses (14) of the infected cells. Therefore, various antiviral/immunomodulatory therapies against HPV infections target the expression of those early genes (15). Studies showed that retinoids, such as ATRA, inhibit HPV E6/E7 transcription (9, 10). Using a one-step real-time RT-PCR method, we determined the mRNA levels of the E7 gene of HPV16 at different time points after high- or low-dose ATRA treatment (Figure 1). Our data clearly demonstrated that only high-dose ATRA treatment was able to reduce the steady-state mRNA level of HPV16 E7 significantly.

HPV transcription is controlled by elements present in the URR (16). The early promoter (p97) is located in the 3’end of the URR and from this promoter transcription of both the E6 and E7 genes is initiated. Using a SEAP-reporter plasmid that contains the full length URR of HPV16, we found that high-dose ATRA treatment significantly suppressed transcription from the p97 (Figure 2A). This suggests that the observed decrease in E7 mRNA levels after high-dose ATRA treatment (Figure 1) occurs at the level of transcription.

Binding sites for a number of cellular transcription factors that influence HPV transcription have been identified in proximity to the early promoter (17). The AP-1 transcription factor is critical for activation of the p97 promoter and, thus, the expression of E6/E7 genes of
HPV16 (18). It has also been reported that retinoids are capable of transrepressing AP-1-mediated gene activation (19). Accordingly, we examined the binding of AP-1 to an oligonucleotide that originated from the promoter proximal region of HPV16 after ATRA treatment. As is shown in Figure 2B, high-dose but not low-dose ATRA treatment significantly reduced binding of AP-1 proteins to that oligonucleotide (Figure 2B). This suggests that the observed repression of transcription was due, at least in part, to the diminished AP-1 binding.

Our data indicate that repression of E7 transcription by ATRA might be part of the ATRA-induced growth arrest. However, the molecular basis for the dose-dependent repression has yet to be unfolded.

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References


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