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Acetylation of intragenic histones on HPV16 correlates with enhanced HPV16 gene expression

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ABSTRACT

We report that many histone modifications are unevenly distributed over the HPV16 genome in cervical cancer cells as well as in HPV16-immortalized keratinocytes. For example, H3K36me3 and H3K9Ac that are common in highly expressed cellular genes and over exons, were more common in the early than in the late region of the HPV16 genome. In contrast, H3K9me3, H4K20me3, H2BK5me1 and H4K16Ac were more frequent in the HPV16 late region. Furthermore, a region encompassing the HPV16 early polyadenylation signal pAE displayed high levels of histone H3 acetylation. Histone deacetylase (HDAC) inhibitors caused a 2- to 8-fold induction of HPV16 early and late mRNAs in cervical cancer cells and in immortalized keratinocytes, while at the same time increasing the levels of acetylated histones in the cells and on the HPV16 genome specifically. We concluded that increased histone acetylation on the HPV16 genome correlates with increased HPV16 gene expression.

Introduction

Human papillomavirus (HPV) is the most common sexually transmitted virus in the human population. It is present in 99.7% of all cervical cancers and is tightly associated with other anogenital cancers as well as head and neck cancers (Walboomers et al., 1999). More than 500,000 cases of cervical cancers are diagnosed each year in the world and cervical cancer is one of the main causes of death of women in the developing world (Walboomers et al., 1999). Although the vast majority of the genital HPV infections are cleared by the immune system within a year after infection, and as such pose little threat to the health of the infected individual, HPVs may in rare cases persist and cause cancer (zur Hausen, 2002). These persistent HPV infections are generally caused by a subset of the sexually transmitted HPVs termed high-risk HPV types. HPV type 16 is the most common high-risk type in cervical cancers as well as in the human population (Bouvard et al., 2009; Bosch et al., 2002). How HPV16 can persist in the presence of a functional immune system is an enigma but is likely a result of the ability of HPV16 to interfere with the immune system of the host, and its ability to restrict expression of the highly immunogenic L1 and L2 capsid proteins to a late stage in the viral life cycle, at the very top of the infected mucosal epithelium (Chow et al., 2010; Bodily and Laimins 2011; Doorbar, 2005). Controlling HPV16 gene expression is therefore of paramount importance for establishment of persistence (Johansson and Schwartz, 2013).

The HPV16 DNA genome can be divided into an early and a late region (Fig. 1A) (Howley and Lowy, 2006). The early region is coding for E1, E2, E4, E5, E6 and E7 and is followed by the early polyA signal pAE, while the late region encodes L1 and L2 and is

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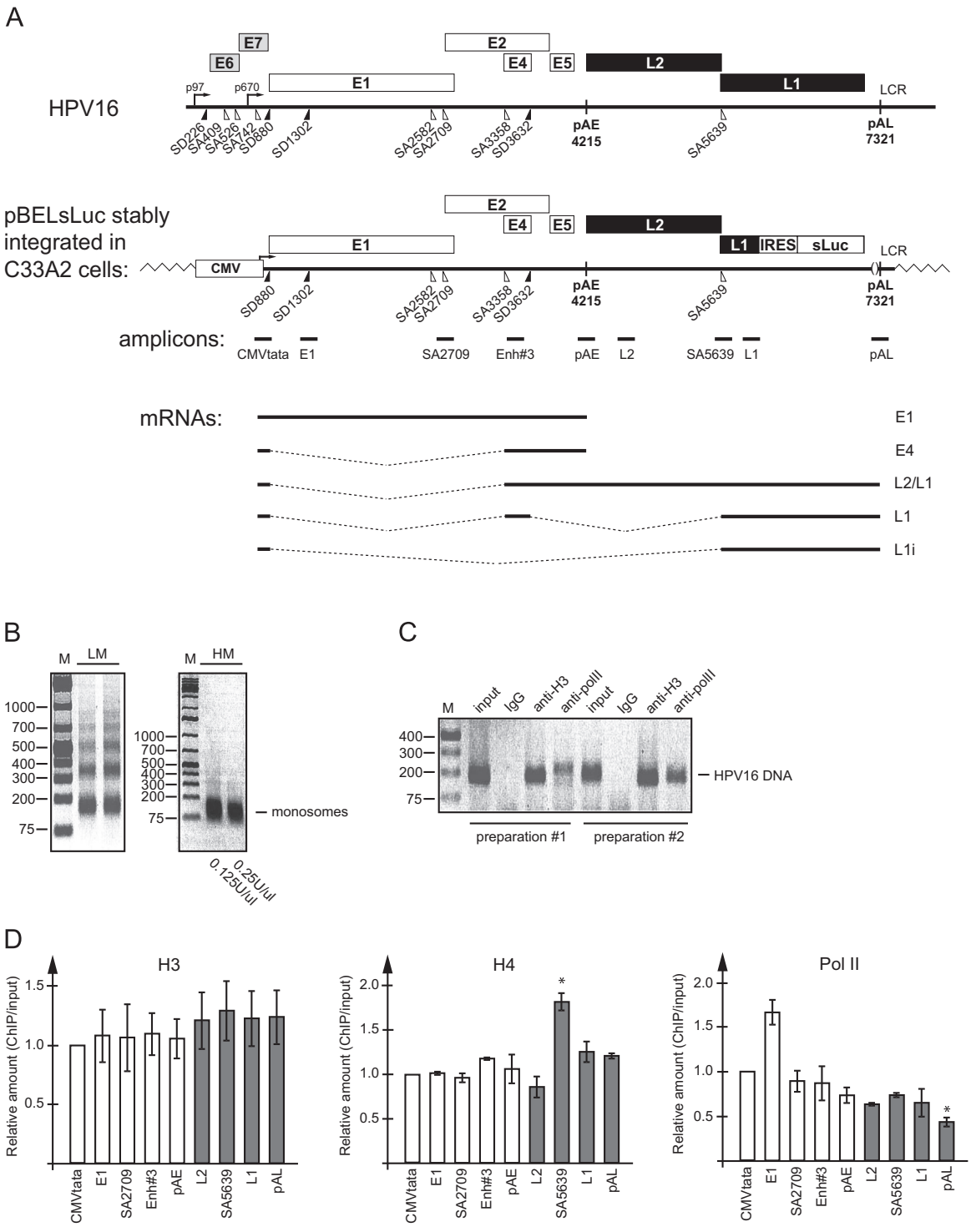

Fig. 1. (A) Schematic representation of the HPV16 genome depicting open reading frames as rectangles, the early and late promoters p97 and p670 respectively, as arrows and splice sites as triangles. The long control region (LCR) and the early and late polyA signals pAE and pAL are indicated. Schematic drawing of the HPV16 pBELsLuc reporter plasmid integrated in the cellular genome of the C33A-derived reporter cell line C33A2 (Li et al., 2013). A subset of early and late mRNAs that may be produced from the pBELsLuc is indicated. The locations of amplicons produced in ChIP assays with primers listed in Table S1 are indicated. (B) DNA extracted from MNase treated chromatin prepared from C33A2 cells were separated on agarose gels and stained with GelRed nucleic acid staining. LM and HM indicated low and high levels of MNase, respectively. The higher levels yielded primarily monosomes and were used for all preparations. (C) PCR of HPV16 DNA with primers L2F and L2R (L2 amplicon) on chromatin immunoprecipitations performed with IgG, anti-histone H3- or anti-RNA polymerase-II-antibodies on two independently prepared nucleosome preparations. PCR with the same primers was also performed on each nucleosome preparation that was used for ChIP (input). (D–F) ChIP analyses on C33A2 cells using antibodies to proteins indicated in each histogram and qPCR of the indicated amplicons. Primers and antibodies are listed in Tables S1 and S2 respectively. Mean values with standard deviations of the amount of immunoprecipitated DNA compared to input DNA are displayed. For antibodies to H3, H4 and RNA polymerase II, the values for the CMVtata PCR was set to 1 to correct for differences between different ChIP extracts. White bars represent HPV16 early genes and gray bars represent late genes. *p*-Values were calculated for each ChIP result in relation to the CMVtata amplicon (D) or the E1 amplicon (E, F and H). *p*-Values less than 0.05 (*) or 0.01 (**) are indicated. All samples were analyzed in two independent ChIP assays and all qPCR reactions were performed in triplicates. (G) Secreted luciferase activity in the cell culture medium of C33A2 cells incubated with 5 μM of the indicated demethylase inhibitors for 24 h. DMSO was used as a negative control and TPA as a positive control. Graph shows mean values and standard deviations of fold induction of sLuc activity of each drug compared to DMSO. Results are from three independent experiments performed in triplicates. *p*-Values were calculated with sLuc results from each drug compared to sLuc results from C33A2 cells incubated with DMSO. All sLuc measurements were obtained from at least three independent cell treatments and were analyzed in triplicates. *p*-Values less than 0.05 (*) or 0.01 (**) are indicated. (H) Chromosomal DNA prepared from C33A2 cells subjected to methyl-cytosine DNA immunoprecipitation followed by qPCR of the indicated amplicons. *p*-Values were calculated for each primer pair in relation to the E1 amplicon. *p*-Values less than 0.05 (*) or 0.01 (**) are indicated. All samples were analyzed in two independent assays and all qPCR reactions were performed in triplicates. (I) sLuc activity in the cell culture medium of C33A2 cells incubated with 5 μM of the indicated DNA methyltransferase inhibitors azacitidine or RG108. *p*-Values were calculated with sLuc results from each drug compared to sLuc results from C33A2 cells incubated with DMSO. All sLuc measurements were obtained from at least three independent cell treatments and were analyzed in triplicates. None of the *p*-values were less than 0.05.