INTRODUCTION

Failure of the immune system to clear persistent HPV infections can lead to the development of cervical cancer (CC) after several decades. In precancerous lesions, most HPV genotypes persist in an epithelial state whereas, in many high-grade lesions, genomes are found integrated into the host chromosome. Although no apparent hotspots have been identified, HPV integration often occurs in common fragile sites, which are naturally occurring regions of genomic instability. The majority of CC contain one or many copies of HPV, integrated more or less randomly into the host chromosome, with the viral integration sites frequently lying within the regulatory E1 or E2 genes. One-half of HPV 16-positive cancers and most HPV 18-positive malignancies contain integrated HPV genomes, suggesting that integration may, in some cases, contribute to malignant progression.

Recent studies have suggested that an important step in HPV carcinogenesis may be the coexistence of HPV episomes with integrated copies. Expression of the E1 and E2 viral replication proteins from episomes can initiate DNA replication from integrated viral origins, resulting in their amplification and the induction of chromosomal abnormalities. Replication of integrated origins also results in the activation of DNA repair and recombination systems, which increases the likelihood of acquiring cellular mutations, increased genomic instability and, eventually, malignant progression.

The E1 protein possesses DNA helicase and ATPase activities that catalyze the unwinding of DNA and recruits cellular replication machinery to viral origins. E2 is a DNA-binding protein that helps to load E1 onto origins and tethers the viral episomes to the host chromosome during segregation. Increased expression of E1 and E2 occurs upon differentiation and is necessary for genome amplification.

E2 proteins form complexes with E1 to initiate viral replication. E2 also regulates the expression of E6 and E7, and can exert suppressive or activating effects depending on the abundance of E2. Disruption of E2 ORF as a result of integration of viral genome into the host genome allows an uncontrolled overexpression of viral oncoproteins E6 and E7, which is a hallmark in CC. An elevation in the level of E6 and E7 is directly related to the increasing severity of neoplasia, and that the deregulated expression of these genes is directly responsible for the accumulation of genetic errors in the infected cell and the eventual integration of viral episomes into the host cell chromosome, which is seen in many CC.

OBJECTIVE

To investigate the physical state (episomal or integrated) of HPV 16 and HPV 18 genome, using a PCR combining 10/11 primers that covering the E1-E3 region, in samples from patients infected by HPVP16 or 18, harboring cervical lesions in different stages of progression and cancer. In addition to establishing the mapping of rupture of the virus genome within this region investigated.

RESULTS

Toilet & E2 Amplification Strategy

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DISCUSSION & CONCLUSION

Recent investigations suggest diverse and contradictory results related to integration pattern of viral in different stages of neoplastic progression. It has been observed that viral integration mainly from specimens of high-grade lesions, whereas others found that viral integration takes place early during the course of infection detected in significant proportion of low-grade lesions. Additionally, it has been suggested that viral integration is associated with specific genotypes. It is more of a cause of chromosome instability.

Frequently, in the preneoplastic stages of CC, HPV is predominantly present in the episomal form, without changing the nucleotide sequence of DNA that regulates gene expression. Conversely, some degree of integration may be present in LSI, suggesting that, LSI may possibly represent a prenevalence lesion.

Frequencies of HPV integrated genome in CC are variable (from 30% to 100%) in different studies.

Our preliminary HPV16-results showed a reasonable number of patients with mixed forms (partially integrated): 32,2%(30/93). It seems possible, that elimination of episomal forms may not be essential during tumorigenesis transformation, and there could be some selective advantage of these forms in concomitant state for persistent and progressive HPV infection. Considering only integrated form, we have identified 13,5%(12/93), this value is within the average range. Finally, episomal forms were identified in 27,9%(32/63) of the cases. However, in the cases of infection those of SHL, this value drops to 18,6%(4/3). fact that should be further evaluated.

HPV16-positiveness in the Brazilian population was evaluated in previously described studies [4/27/3], followed by mixed 21,6%(37/100), and episomal 13,5%(37/279) in HPVP16 infections, showing a different integration process.

Surprisingly, our data showed that E1 is more frequently broken [91,9%(34/37)) than E2 [79,2%(21/37)] in the integrated forms of HPV16, and also partially broken in mixed forms. E1[100%(30/30)] and E2 [26,0%(8/30)], contrary to previous studies that described E2 as the gene region most commonly disrupted. Conversely, HPV 18 cases is consistent with the literature, with 59,5%(27/24) E2 disrupted and 33,3%(8/24) E1 at integrated forms, and in partially broken forms detected only E2[100%(8/8)].

In the literature, has been demonstrated by sequence analysis that all sites of viral gene disruption occurred from E6 to L1 genes, more frequently in L1 gene (70%), followed by E1 gene (67%). Among some HPV genotypes, there are different integration into the host genomes does not appear to be an entirely random event but occurs preferentially at certain chromosomal locations, while HPV genomes could be disrupted at any gene, and cells with viral disruption at the L1 genes may be selected against during the clonal selection process. And the use of cervical lesions at an early stage of cancer progression, possibly contain different clonal differences.

Integration usually disrupts the E1 or E2 genes, potentially leading to a deregulation of viral gene expression. Among others, multiple-HPV infections, and different HPV variants, are also plausible examples of confounding variable.

Recent analyses have indicated that levels of E2 transcript and E2 protein expression in HPV-infected lesions do not correlate as closely as it has been previously thought, and that disruption of E2 protein expression is not always accompanied by disruption of the corresponding gene.

Anepiducy can be detected in pre-malignant HPV-associated cervical lesions. These activities are limited to high-risk E6 and E7 proteins as none of them are seen in cells expressing their low-risk counterparts. Activation of the three-negative functions in cells bearing both episomal and integrated forms of the viral genome could therefore result in chromosomal alterations and induction of genomic instability, which are likely to be important in the progression to malignancy.

Several molecular studies have suggested that the deregulation of E5/E7 expression, even in the absence of genome integration, is a critical event in determining neoplastic grade. Although it is not clear exactly how gene expression from the viral episome can become deregulated in early CIN. In these instances, deregulated gene expression may be due to changes in cell signalling as can be brought about by hormonal changes, or epigenetic modifications such as viral DNA methylation, which may depend on the nature of the infected epithelial cell.

We herein describe a very specific methodology that can successfully map the HPV 16 and 18 genome fragile areas. Data are being analyzed in order to search for statistical correlation between integration and severity of the lesion but it has been observed that E1-E2 absence were suggestively frequent in HSL and cancer. In a few cases, episomal forms were observed in cancer samples, suggesting additional biomarkers is involved in carcinogenesis.

It has been observed a higher frequency of intact forms for HPV16 than for HPV18 with predominance of disruption in E1/E2 together and E2 exclusive gene, respectively. The specific sites for disruption observed, suggest diversity in fragile areas between HPV types.

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