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Meeting abstract

Analysis of the methylation status of HPV DNA in human cell lines

Rocio S Méndez*[1], Cristian P Miguez[2], Miriam C Guido[3] and Alejandro García-Carrancá[1,3]

Address: [1]Division of Basic Research, Instituto Nacional de Cancerología, Mexico City, Mexico, [2]Facultad de Química, UNAM, Mexico City, Mexico and [3]Department of Molecular Biology and Biotechnology, Instituto de Investigaciones Biomédicas, UNAM, Mexico City, Mexico

Email: Rocio S Méndez* - rocmenmar@yahoo.com

* Corresponding author

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Background

DNA methylation is an important epigenetic process that regulates gene expression mainly through its effect on chromatin conformation. It involves the stable maintenance of methylated cytosines at CpG dinucleotides (meCpGs) which in turn can regulate gene expression by two mechanisms: by direct interference with transcription factors binding to their target sequences, or by binding MeCP2 proteins, which recognize methylated DNA and recruit histone deacetilases, resulting in a highly compact chromatin structure. Human Papillomavirus (HPV) E2 protein controls various viral processes, including gene transcription and DNA replication. These activities rely upon its ability to bind as an homodimer to its target sequences (5'-ACCGN_4CGGT-3') in the viral DNA. Since these sequences contain CpG dinucleotides, they are potential targets for DNA methylation, and, in fact, it has been demonstrated that E2's ability to bind to its cognate sequence is inhibited by methylation. Different studies have shown that the methylation patterns of HPV DNA, in the long control region (*LCR*) and the 3' region of the *L1* gene, are heterogeneous in human cell lines and clinical samples. The objective of this study was to determine the methylation status of HPV type 18 and 16 DNA in different human-derived cell lines.

Materials and methods

DNA from HeLa, ViPa and CaLo cell lines (with integrated HPV type 18 DNA) was extracted and modified with sodium bisulfite. The 5' region of the *L1* gene, the complete *LCR* (divided in three regions) and the 5' region of the *E6* gene, were amplified using specific primers designed for modified DNA. These PCR fragments were cloned in TOPO A vector and 10 clones from each cell line were sequenced for analysis.

Results

DNA methylation patterns observed in three different cell lines containing HPV type 18 DNA showed great similarity. No methylation of CpG dinucleotides was observed in the complete *LCR* and the 5' region of the *E6* gene in CaLo cell line. Similarly, the enhancer and promoter regions within the *LCR*, and the 5' region of *E6* were unmethylated in HeLa and ViPa cell lines. However, the 5' region of the *L1* gene, although mostly hypermethylated in HeLa and CaLo, exhibited an heterogeneous pattern in ViPa cells. Interestingly, the CpG at position 7318 (within the 5' region of the *LCR*) was consistently found methylated in two cell lines (HeLa and ViPa), as opposed to CaLo where it was found unmethylated.

Conclusion

The methylation patterns of HPV 18 DNA in these three cell lines are very similar, with the *LCR* generally unmethylated. The heterogeneity of the methylation patterns in the 5' region of the *L1* gene reported in previous studies was confirmed in the present work. Further studies are needed in order to determine if these patterns are consistent in tumor samples, and how they correlate with various stages of the carcinogenic process and the viral life cycle.

Further analysis using methylation-specificand Headloop suppression PCR will be useful to establish the CpG methylation patterns of the viral genome in different types of lesions (low and high grade) and during different stages of the viral life cycle.