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Methylation of Human Papillomavirus 16 L1 Gene in Cervical Intraepithelial Neoplasia and Cancer

Pathologie

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It is estimated that 15-20% of all human cancers are caused by oncogenic viruses, one example of which being cervical squamous cell cancer that is caused by different types of human papillomaviruses.

Most infections by human papillomaviruses get cleared by the immune system. However, in some cases, this does not occur leading to persistent infections that might transform into cancer.

The L1 protein, one of the two capsid proteins, is the most immunogenic protein and therefore used in vaccination. However, the L1 gene is only expressed in the most superficial epithelial cell layers where it cannot be targeted by the immune system. Strict regulation of L1 gene expression is therefore one mechanism of immune evasion by the virus.

The mechanisms regulating L1 gene expression are only beginning to be understood. One potential mechanism might be methylation of the L1 gene.

L1 gene methylation has been found to increase from CIN lesions of increasing grades to cancer. However, methylation analysis has so far only been performed on smears that only contain the most superficial cells, or on biopsies that contain huge volumes of tissues and therefore a mixture of asymptotically infected epithelium, pre-cancerous lesions of different grades and cancer itself. Methylation values obtained in those studies only represent an average.

In this work, I wanted to test the hypothesis that L1 gene methylation depends on host cell differentiation in the way that methylation increases with decreasing degree of differentiation.

In order to test this hypothesis, I analyzed methylation at all 19 CpGs in the L1 gene on 9 cervical squamous cell carcinomas (SCC), 11 cervical intraepithelial neoplasia (CIN) grade 2 lesions and 5 CIN 3 lesions on microdissected tissue of formalin-fixed paraffin-embedded tissue which has never been done before. I analyzed the 11 CIN 2 lesions and the 5 CIN 3 lesions as one group due to the restricted number of samples.

I found that median methylation levels and the proportion of highly methylated samples was higher in the SCC group than in the CIN 2-3 group. Stratification into subgroups showed an

increasing median methylation of CIN 2 over CIN 3 to cancer. My results therefore confirm my hypothesis.

Previous research in which an increase of methylation level from CIN lesions of increasing grades to cancer lesions confirm this finding.

Furthermore, I wanted to examine whether L1 methylation is also correlated to integration of the HPV genome into the host genome. Previously, a positive correlation between high L1 methylation and integration of the HPV genome has been shown on smears and biopsies.

I tested the hypothesis that L1 methylation would be high in both SCC samples with episomal HPV genome status and with integrated HPV genomes as they are both cancer samples, but that methylation would still be higher on integrated genomes.

Therefore, I analyzed methylation at all 19 CpGs of the L1 gene in 23 fresh frozen cancer lesions with known integration status, out of which 11 samples contained episomal HPV genomes and 12 samples contained integrated HPV genomes.

I found higher median methylation levels and higher proportions of highly methylated samples in carcinomas with integrated genomes which confirms my hypothesis.

In conclusion, this study constitutes a further step towards a better understanding of the role of HPV genome methylation as an epigenetic mechanism in the context of oncogenic progression of an HPV infection. Further research on methylation and its function in viral life cycle and tumorigenesis could be based upon these two descriptive studies which might have contributed to development of future diagnostic and therapeutic strategies of HPV-related cancers.