Ana Ligia Gutierrez Solis
Dr.sc.hum.

Methylation Analyses of Human Papillomavirus L1 and Its Implication for the Treatment of HPV-Associated Cancer Cells

Fach/Einrichtung: Pathologie
Doktorvater: Prof. Dr. med. Magnus von Knebel Doeberitz

Persistent infections with high-risk human papillomavirus (HR-HPV) are the main cause for the development of cervical cancer. These infections are highly prevalent worldwide. Transformation of HPV-infected cells is a rather rare event and linked to a shift of HPV gene expression patterns that is mediated by shifts of the methylation pattern of the viral genome. Details of the methylation status of HPV genomes have not yet been characterized in detail and the question remains, how methylation regulates the viral life cycle.

This study aimed to find out whether methylation of distinct CpG sites within the L1 gene region of the HPV 16 genome changes during epithelial differentiation and transformation. Therefore, the methylation pattern of all 19 CpG sites within the L1 gene was accessed in 45 microdissected samples at different stages of cervical intraepithelial neoplasia (CIN 1, 2, 3) and at the level of squamous cell carcinoma (SCC). It was found that the L1 gene methylation levels were substantially higher in CIN 3 and SCC samples ($p16^{INK4A}$-positive) compared to CIN 2 samples ($p16^{INK4A}$-positive). The methylation levels of the majority of the CIN 2 samples were low, for CIN 3 samples they were heterogeneous and the SCC samples were highly methylated throughout all 19 CpG sites.

To assess whether the L1 methylation is associated with the integration of the HPV genome into the host DNA, twelve carcinoma samples with integrated HPV 16 genomes were evaluated and significantly higher methylation levels in the 3'-region of L1, particularly at the CpG sites between 6365 and 6794 of the L1 genome, were observed. A correlation between the L1 gene methylation and the

chromosomal integration status could be established. These results substantiate previous findings suggesting increasing methylation of integrated HPV genomes.

The use of demethylating agents like 5-aza-2'-deoxycytidine (DAC) could provide additional advantages for eliminating HPV-associated cells. In order to explore the potential of the demethylating agents, a total of nine HPV-associated cell lines were treated. DAC treatment lead to the re-expression of the HPV L1 gene. Furthermore, DAC did suppress the expression of E6 andE7 oncogenes. In addition, DAC treated cells revealed increased expression of the CTAs, NY-ESO 1 and MAGE-A3, in HPV-associated cancer cell lines. Moreover, the expression of PD-L1 was evaluated and showed a cell line-dependent expression profile.

Altogether, the data presented in this thesis suggest that the HPV life cycle is linked to the “epigenetic milieu” of the host cell. It further shows that treatment of HPV transformed cells with demethylating agents like DAC results in suppression of the viral oncogene, activation of the HPV capsid antigen L1 and cellular cancer testis antigens like NY-ESO 1 and MAGE-A3.