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ADENOCARCINOMA OF THE CERVIX UTERI. METHODS TO IMPROVE DIAGNOSTICS:BIOLOGICAL MARKERS AND HPV TESTING

Cervical adenocarcinoma (ADC) represents about 20% of invasive cervical cancers. Implementation of screening programs that have led to a decrease in squamous cell carcinoma (SCC) has only had a limited preventive effect on adenocarcinoma as the incidence of ADC, especially among younger women, has steadily increased in recent decades. Human papillomavirus (HPV) is considered the most important single factor for development of SCC. Adenocarcinomas of the cervix are also related to HPV of high risk types (HR-HPV), but the correlation is less pronounced. HPV infection alone is insufficient and other factors are required for oncogenic transformation to ADC.

The aim of this thesis is to investigate molecular markers such as tumor suppressor $p16^{INK4a}$, proliferation marker Ki-67, possible tumor suppressor proteins in the LRIG family, the TP53 target WIG-1, and E6/E7 mRNA in cervical adenocarcinoma in order to provide a deeper understanding of the etiology of the disease and to develop more efficient screening strategies for adenocarcinoma of the cervix.

Formalin-fixed/paraffin-embedded tumor tissue samples of cervical ADC were investigated by immunohistochemistry (IHC) for the occurrence of $p16^{INK4a}$ using the CINtec® kit, of Ki-67 with the MIB-1 antibody, and of LRIG1, LRIG2 and LRIG3 using polyclonal rabbit antibodies. In 38 samples of cervical cancer (ADC or SCC), Wig-1 was examined by IHC using a monoclonal antibody. In cervical cancer cell lines the WIG-1 gene locus was investigated by SKY, CGH, Southern and FISH; mRNA expression by Northern and RT-PCR; and protein expression by Western analysis. Testing for E6/E7 mRNA was carried out using the PreTect HPV Proofer; testing for HR-HPV DNA was done by consensus PCR and subsequent typing by SSCP, direct sequencing and Linear Array®. The examined markers were evaluated regarding prognostic impact and association with HPV infection.

We found that poorly differentiated tumors stain with less intensity and in a lower fraction than do well differentiated tumors. All HPV-positive tumors showed $p16^{INK4a}$ staining, but 60% of HPV-

negative tumors also stained for $p16^{INK4a}$. We found a significant correlation between staining for Ki-67 and histological grade (p=0.031) as well as worse outcome (p=0.004).

High staining intensity for LRIG1 and a high fraction of LRIG3-positive cells were significantly associated with improved patient survival (p=0.03 and p=0.04). LRIG1 and LRIG3 expression correlated with HPV infection, since higher staining intensity was observed in HR-HPV-positive cases.

mRNA extraction from paraffin-embedded tissue samples was successful, as shown by positive results in the GAPDH mRNA integrity control in all cases. HR-HPV infection was detected by mRNA in 64% of the tumors, compared with detection by DNA in 62% of the tumors. There was an 87% agreement in results between the two methods regarding HPV positivity and 84% agreement regarding HPV type.

WIG-1 is not the primary target for genomic alteration on chromosome 3, even though analysis revealed chromosome 3 gains in all lines. WIG-1 mRNA expression was higher in the two HPV-negative cervical cell lines (C33-A, HT-3) than in the HPV-positive lines. Wig-1 expression in tumor tissue, as assessed by IHC, showed significantly higher nuclear Wig-1 levels in ADC than in SCC ($p<0.0001$). We observed higher nuclear Wig-1 expression in the HPV-negative tumors than in HPV-positive tumors ($p = 0.002$). Patients with tumors that demonstrated moderate nuclear and positive cytoplasmic Wig-1 expression had a better prognosis ($p = 0.042$) than those with high nuclear and negative cytoplasmic Wig-1 expression.

According to our findings the tested markers appear to be potential diagnostic supplements for cervical adenocarcinoma as expression of Wig-1 as well as LRIG1 and LRIG3 could serve as prognostic markers, $p16^{INK4a}$ and Ki-67 might be helpful markers for grading endocervical malignancies and mRNA testing has been shown to be as sensitive as DNA testing. The combination of improved detection of precursors through integration of molecular markers into screening programs with vaccination against HPV 16 and 18 will help cervical cancer including adenocarcinoma to become one of the most preventable cancers.