Human papillomavirus (HPV) and cervical cancer

Tumor virology is the science that researches the role of viruses in human tumor genesis. Main players in tumor virology are human papilloma viruses that play a role in genital, skin and oropharyngeal cancers. At present more than 100 genotypes have been isolated, 77 of which have been identified in humans. A low mutation rate is suggested by the small differences in base sequences of the different genotypes. Cervical cancer is the second leading cause of cancer in women. Although it has been observed that cervical cancer behaves like a sexual transmitted disease, it was not until the 1970s that HPV was shown to be the causative agent. In cervical cancers, HPV has been detected in up to 95% of the cases. The long latency period between infection and the development of cancer, the variety of progression to cervical cancer and the clonal origin of cervical cancer show that HPV infection alone is not sufficient for the development of cervical cancer, but requires a multi-step event. Epidemiological data on the occurrence of cervical cancer in biologically related women also suggests the involvement of a hereditary factor in the etiology of cervical cancer. The multi-step nature of cervical cancer is illustrated by the distinct pre-neoplastic epithelial changes called intraepithelial neoplasia (CIN) which form a spectrum of atypia, graded CIN I to CIN III. The proteins expressed by the HPV genome interfere with the cell cycle regulation process. Regression or progression in cervical preneoplasia thus depends on the genetic instability caused by the interaction of HPV and the cell cycle.

Screening for HPV

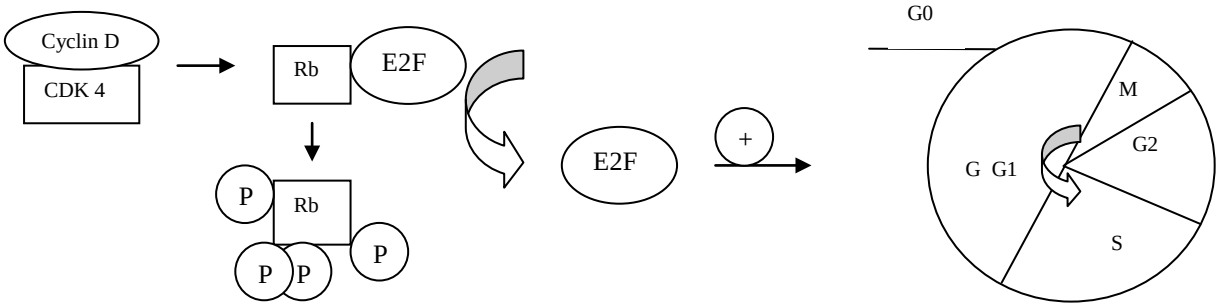
Women with an intact immunological response system will not carry the virus their entire live. Infections by HPV subtypes will generate an immune response that protects against subsequent infections by the same type. The derived immune response will temper some infections with other types and result in transient lesions in many women. The overall prevalence of HPV in the target population is 20%. Since HPV exposure is greatest in young sexually active women, detection of the virus will exceed by several-fold the ability to detect abnormalities. In post-menopausal women, the HPV infection index is much lower, but cytological abnormalities are detected more often. It is clear that the PAP screening procedure is effective and it has been demonstrated that a positive HPV test is a powerful independent risk factor for the development of CIN lesions, as 93-100% of invasive carcinomas are associated with HPV infections. However, the relative value of screening for HPV in a population is shown by combining the high prevalence of HPV with the long time frame between infection and development of cancer. In other words, the incidence of cancer in women under 25-30 years old is very low whereas the incidence of HPV infection is about 40%. High throughput population screening without morphological-based diagnosis will result in high numbers of virus positive patients, most of whom will never suffer any consequences of the infection, making such testing redundant and a financial burden for healthcare. It is therefore more advisable to target HPV screening, i.e. screening those women for HPV who have developed early cytological abnormalities in order to monitor possible

developments towards more serious abnormalities, thus being able to interfere with the disease at an early stage.

The in situ HPV hybridization test enables visual control on the cells and the localization of infection as opposed to testing procedures in liquid formats. The information on histological details in combination with HPV in situ hybridization can therefore exclude false positives and innocent positives (young women). Moreover, in situ hybridization makes it possible to differentiate between episomal or integrated HPV DNA. **Studies have shown that episomal HPV induces genomic changes such as tetrasomies and single trisomies, while HPV integration correlates to aneusomies and polysomies, which are predominantly detected in CIN III and micro-invasive carcinoma, demonstrating that integration of HPV DNA is a pivotal step in the transition of CIN to micro-invasive carcinoma. Integration of the HPV genome together with histological information makes it possible to determine between high risk CIN II/III and low risk CIN I/II and it is here that in situ hybridization provides the tools for improved diagnosis.**
Last but not least:
HPV has also been linked to the etiology of other cancers such as cancer of the vulva, penis, non-melanoma skin cancers (basal and squamous cell carcinoma), cancers of the oral cavity, larynx and esophagus. These observations emphasize the importance of this virus group as proven and suspected human cancer carcinogens.

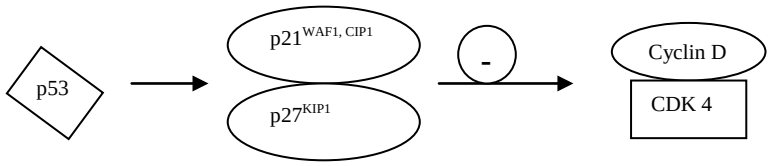
The cell cycle

The cell cycle is regulated by sequential protein kinases. These consist of a cyclin and a cyclin-dependent kinase (CDK). Progression of the cell cycle is largely regulated by phosphorylation of Retinoblastoma protein (Rb). Upon release of E2F transcription factor, DNA synthesis is activated.



Inhibition of the cell cycle progression is supported by cyclin-dependent kinase inhibitors (CDK) such as $p16^{INK4}$, $p21^{WAF1, CIP1}$ and $p27^{KIP1}$ that inactivate cyclin-CDK complexes.

In stress situations that lead to DNA damage, wild type p53 suppressor gene product is overexpressed and leads to an arrest in the cell cycle in the G1 phase, allowing repair of DNA damage. The G1 arrest induced by p53 is mediated by $p21^{WAF1, CIP1}$ protein that binds to and inhibits the activity of cyclin-dependent kinase (CDK) and proliferating cell nucleolar antigen (PCNA). Alternatively, p53 can also induce apoptosis.



Loss of wild type p53 is seen in many types of human tumors and is an important step in the development of almost all cancers.

The cell cycle, HPV and malignant transformation in cervix epithelium

HPV type viruses express proteins that exhibit transforming capacities interfering with cell growth regulation and therefore may contribute to malignant transformation. Proteins playing a role in this process are encoded by the HPV E5, E6 and E7 genome open reading frame (E=early).

E5:
Expression of E5 has a negative effect on the cell-cell communication via gap-junctions, which in turn has an insensitivity effect on cell growth regulators, which further has a positive effect on the expression of E6 and E7 oncogenes.

- → inhibits down regulation of Epidermal Growth Factor Receptor (EGFR)
- → induction of proto-oncogenes c-jun, junB and c-fos
- → suppresses cyclin-dependent kinase inhibitor $p21^{WAF1, CIP1}$

The effect is a boost of mitogenic response of host cells to stimulate viral replication.

E6:
Complexes with p53 tumor suppressor gene product

- → accelerates p53 degradation, consequently preventing G1 arrest and DNA repair
- → inhibits p53 regulatory properties i.e. by cyclines and PCNA
- → prevents p53 mediated apoptosis, thus damaged cells are maintained
- → interacts with calcium-binding proteins and thus may interfere with cell growth and differentiation

The effect is an induction of genetic instability.

E7:
Interacts with the Rb gene product, thus interfering with their control on the G1 / S transition of the cell cycle. After binding E7 to Rb, the E2F transcription factor is released and induces uncontrolled cell proliferation. By binding and inactivation of E7 to CDK inhibitor $p27^{KIP1}$ the G1 arrest is overruled, resulting in Cyclin E / CDK 2 release. The latter are involved in cell cycle progression. E7 is also involved in repression of p53 transcription activity as well as complexes with transcription factors as c-jun, junB and c-fos.

HPV infections are cell differentiation dependent. Transcription and amplification occur only in squamous epithelia undergoing terminal differentiation. HPV replication relies entirely on host cell replication. HPV DNA has been detected in the earliest stages of pre-neoplastic epithelial changes and can be interpreted as a very early event in cervical carcinogenesis. The viral genome in its latent form is present in proliferating cells. Replication and assembly of viral particles as well as the translation and functional activity of viral proteins are restricted to the differentiating layers of the dermis. In pre-malignant lesions, HPV is only present episomally. In cervical carcinomas, however, the viral DNA is integrated in the host cell genome. Upon integration, the viral genome is disrupted, often resulting in the loss of E2 gene which encodes functions that may repress E6 and E7 gene transcription. The E6 and E7 transcription in basal cells is very low (resulting in low levels of mRNA). In high grade lesions, however, a high transcription activity is often noticed throughout the proliferating epithelial cell layers. The triggering of the carcinogenic mechanism by accumulation of chromosomal changes that are required for malignant progression may be provided by changes in the p53 and Rb expression caused by the known interference of E6 and E7.

Viral integration guarantees expression of viral oncogenes and is most consistently found in ~~(??)~~high risk HPV types 16 and 18. Features of CIN II or CIN III are observed in 90% of squamous precursor lesions caused by HPV 16. Many other types (31, 33, 35, 39, 45, 52, 56 etc.) may be associated with invasive cancer. HPV types 31, 33 and 35 have been associated with less aggressive carcinoma because of the observation that there are significant differences in mortality in women caused by cervical cancers that were associated with HPV 16, 18 versus HPV 31, 33, 35.

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