

Persistent transforming HPV infection may correlate with persistent histologically defined CIN II+

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Introduction

Latent infection has been described as infection without viral DNA replication and without production of viral particles. Latent infection will therefore probably not be detected with HPV mRNA based methods and should most probably not be defined as an infection but merely as a presence of HPV. A clinical infection is usually defined by active replication of the viral genome. The active lytic phase of HPV may be defined as a transient infection causing cell changes and proliferation but not malignant cell abnormalities. This infection may be discovered by cytology as koilocytosis without nuclear abnormalities, and by histology as CIN I or metaplasia (Figure 2). This clinical infection may be discovered by in situ hybridization as episomal HPV (diffuse staining) or by immunohistochemistry using proliferation markers (Figure 1). These assumptions are strengthened by the discovery of HPV types without oncogene expression in cytologically and histologically negative cervical samples (Molden et al., CEBP 2005, Sotlar et al., JVM 2004, Cushierie et al., JVM., 2004).

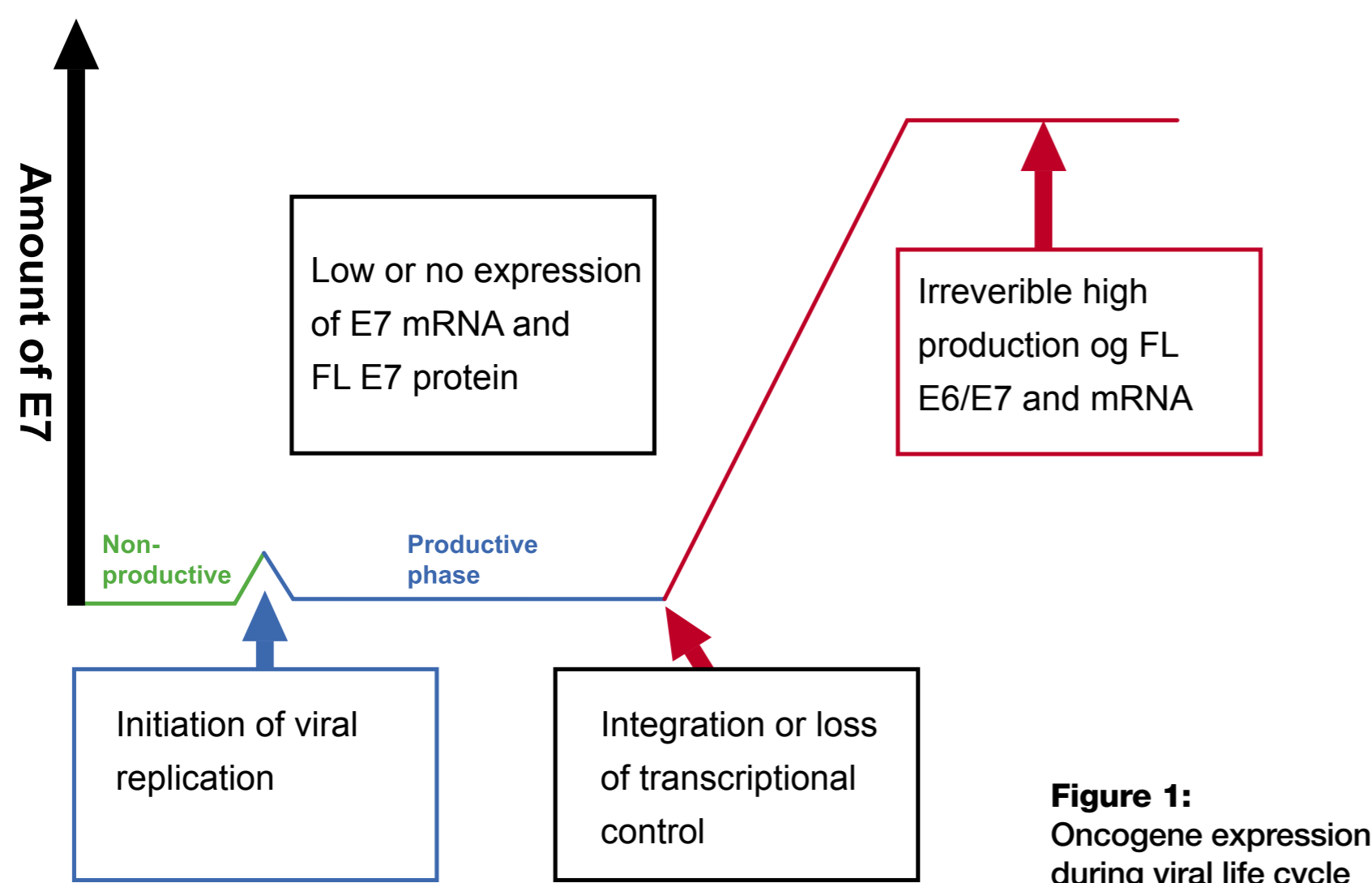


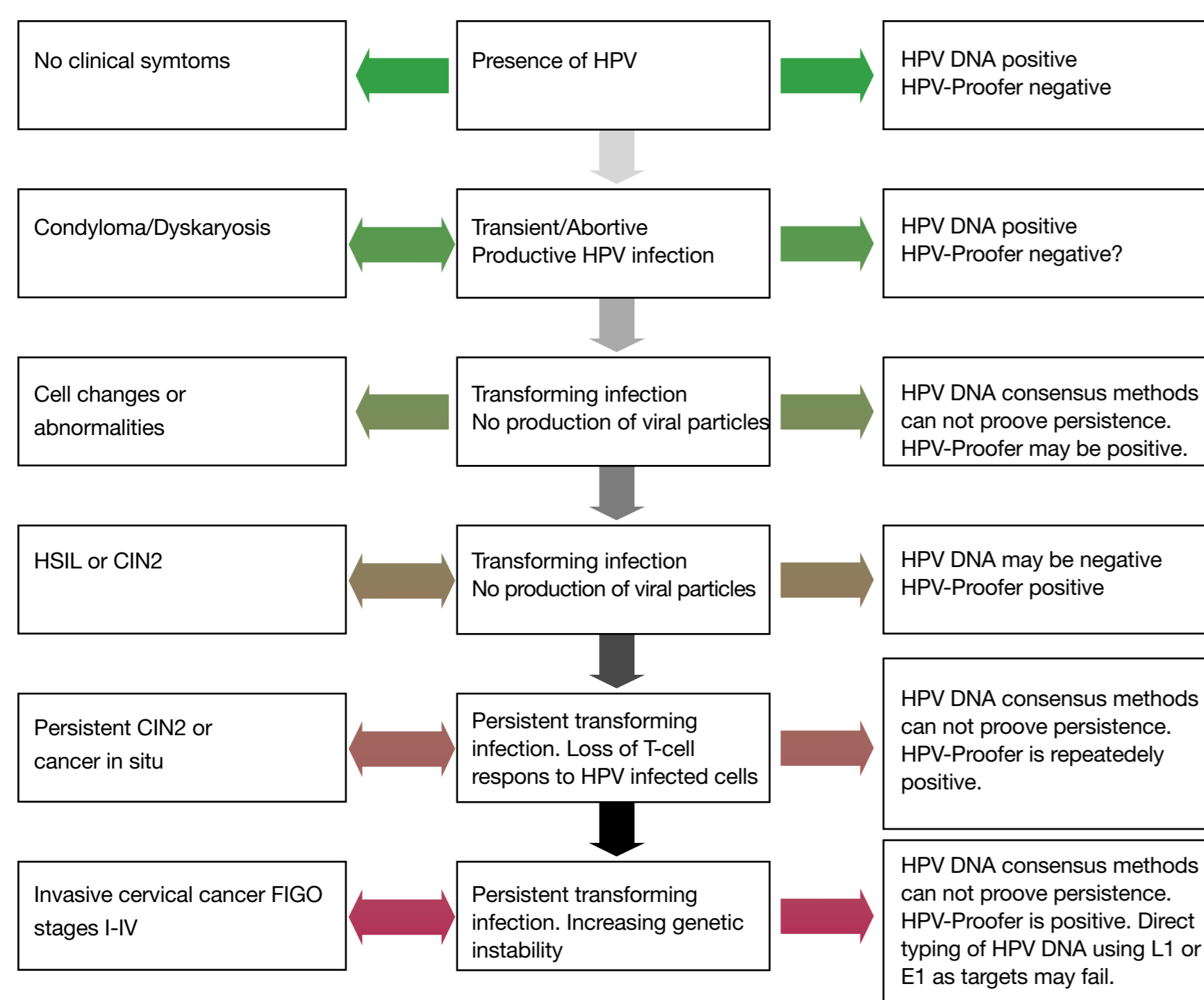
Figure 1: Oncogene expression during viral life cycle

Prior to entering the cell and in the initial non-productive phase, viral genes are not transcribed. This phase spans a relatively short time interval. When entering the basal cell layer, for example through microlesions, production of E7 mRNA is initiated. The expression of E7 mRNA and proteins are at first limited to the area immediately above the basal cell layer and will subsequently be down regulated not to induce cell apoptosis or loss of viral production. However, in some cases the regulation of viral transcription and/or mRNA splicing is altered. HPV DNA integration and and/or stability of E6/E7 mRNA will cause high production of E6 and E7 proteins. Whether the E7 3D protein structure change after alterations in the transcriptional regulation remains to be elucidated.

Method; PreTect HPV-Proofer

The PreTect HPV-Proofer assay (NorChip AS, Klokkarstua, Norway) is a poly A independent assay that utilizes real-time multiplex NASBA (nucleic acid sequence based amplification). The technique amplifies mRNA in a DNA background with real-time detection of the products by molecular beacon probes. The PreTect HPV-Proofer assay identify individual HPV types 16, 18, 31, 33 and 45.

Figure 2: Clinical manifestations related to viral events and possible outcome of HPV tests



Summary of results

Main conclusion	Basic research	Reference
The E6/E7 transcripts detected by HPV-Proofer show better clinical relevance for detection of high-grade lesions than alternatively spliced E6 transcripts	Basic research	Presence of different HPV 16 RNA transcripts in normal cells compared to CIN2, CIN3 and SCC biopsies (Molden, in prep and poster 22th international papillomavirus conference, 2005)
Indications of differences in the oncogenic potential of individual HPV types. Integration correlate with the carcinogenic process.		Integration frequency of viral genomes differs among cervical cancers infected by different high risk HPV types. (Vinokurova et al., poster 22th international conference)
HPV-Proofer show 97% coverage rate in cervical cancer. All HPV DNA positive cases were found to express E6/E7 mRNA of the corresponding type	Prevalence and coverage rate of mRNA expression from 5 HPV types	E6/E7 expression in 204 squamous carcinoma samples (Kraus & Molden, in prep.)
HPV mRNA was found in 97% of CINIII and was absent in histological normal and low-grade representative LEEP biopsies		HPV expression in the dysplastic portio (190 biopsies) (Kraus, British J Cancer 90:1407-1413, 2004)
In representative normal LEEP biopsies HPV-Proofer was negative.		Follow-up of ASCUS/CIN I cases in Sweden (Bjerre, 2004, Poster # 134, 21th IPC, 2004)
HPV-Proofer show significant better clinical performance compared to hc2; CIN2+ was detected in 83% of the women with normal cytology and positive HPV-Proofer test, but in only 62% of the women with normal cytology and positive hc2 test. HPV E6/E7 mRNA was discovered in 100% of cervical smears collected from women with histological evident cervical carcinoma	Clinical performance compared to DNA-based HPV tests and traditional diagnostic methods	Validation of PreTect HPV-Proofer against hc2 (Lie et al., in press Gyn Oncology, 2005)
HPV-Proofer has the same sensitivity and significantly higher specificity than HPV-DNA based methods. The prevalence of HPV mRNA detected by HPV-Proofer is only 3% in a well screened population		Comparison of human papillomavirus mRNA and DNA detection: A cross-sectional study of 4136 women older than 30 years of age with a two year follow-up of HSIL (Molden, CEBP 14 (2): 367-372, 2005)
HPV-Proofer have two times higher sensitivity than cytology and same specificity for detection of CIN II+. PPV based on only representative biopsies is 5 times higher for HPV-Proofer than for SPF10/LIPA consensus PCR		A high risk population study (Hovland, poster 22th international papillomavirus conference, 2005)
HPV-Proofer has the same sensitivity and significantly higher specificity than HPV-DNA based methods. HPV-Proofer is useful for detection of persistent transforming infection		Human Papillomavirus Type Specific DNA and RNA Persistence—Implications for Cervical Disease. Progression and monitoring (Cuscheri, J Med Virol. 2004 May;73(1):65-70)
A woman having an ASCUS/LSIL Pap smear and a positive PreTect HPV-Proofer result was 69.8 times more likely to be diagnosed with CIN2+ than if a woman was negative for the HPV E6/E7 transcripts. For consensus PCR, the odds ratio was 5.7.		Predicting CIN2+ when detecting HPV mRNA and DNA by PreTect HPV-Proofer and consensus PCR: A two-year follow-up of women with ASCUS or LSIL Pap smear (Molden, 2004, Int J Cancer. 2005 Jan 11)

Discussion

- Depending on the assay, HPV DNA methods seem to detect the most dominating infection. This means that a HPV DNA positive result in cancer samples may detect an additional but transient infection in contrast to an underlying transforming infection. This is based on the fact that HPV DNA methods are able to detect non-productive presence of intact HPV DNA or productive transient infection without production of full-length E6 proteins. Furthermore, it could be difficult to prove detection of persistent HPV only by using consensus HPV DNA methods.
- Persistent transforming HPV infection as defined by persistent presence of type specific E6/E7 HPV mRNA, may directly correlate with persistent CIN II+ and could have a potential role as a prognostic marker in cervical cancer screening.
- Persistent transforming infection is most probably a very serious diagnosis if untreated.