Human papillomaviruses (HPVs) are small DNA tumor viruses that cause benign and malignant tumors of squamous epithelia. Among more than 100 subtypes that have thus far been identified, a group of high-risk human papillomavirus types including HPV-16 or HPV-18 are the causative agents of cancer of the uterine cervix, a leading cause of cancer-related deaths in women worldwide.1–3 Low-risk mucosotropic HPV types typically cause benign lesions such as genital warts. The development of cervical cancer has been linked to persistent infections with high-risk HPVs and is generally preceded by a lengthy latency period. Moreover, only a minority of women infected with high-risk HPVs eventually develops cancer. These observations strongly suggest that accumulation of host genetic changes and predisposing host factors play central roles in malignant progression of high-risk HPV associated lesions.4,5

High-risk HPVs encode two oncoproteins, E6 and E7, which can each individually extend the life span of primary human cells and, when co-expressed, facilitate their immortalization. High-risk HPV E6 or E7 oncoproteins are not sufficient to directly transform human cells to accumulate additional genomic aberrations and undergo transformation upon extensive passaging, or when exposed to mechanisms that limit viral survival such as clearance of infected virions occurs in the upper differentiated layers of the infected squamous epithelium in cells that would have normally permanently ceased to proliferate. The high-risk HPV E6 and E7 oncoproteins target critical negative growth regulatory signaling circuits to allow viral genome replication in these post-mitotic cells (Fig. 1). The high-risk HPV E7 oncoproteins bind and degrade the retinoblastoma tumor suppressor protein (pRB) and the related pRB family members p107 and p130.16 High-risk HPV E7s also inactivate the cyclin-dependent kinase (CDK) inhibitors p21Waf1 and p27Kip1.17 This profound dysregulation of cellular proliferation control pathways results in aberrant activation of E2F-mediated gene transcription with increased expression of cyclin E and cyclin A and aberrant CDK2 activity.21–24 E2F can activate p14ARF thereby stabilizing p53 through inhibition of MDM2.25 Activation of p53 results in induction of cell cycle arrest or apoptosis. To abrogate this antiproliferative response, the high-risk HPV E6 oncoprotein induces the rapid proteasomal degradation of p53 by interacting with the host cell protein E6-AP, a HECT

Chromosomal instability at a numerical or structural level is a hallmark of malignant tumors and is particularly common in cancers of epithelial origin.12 Chromosomal instability develops at early stages of cervical neoplasia and can be detected even in preneoplastic lesions.13 Genomic instability may contribute importantly to the rapid selection of clonal cell populations that are able to overcome the various environmental challenges that arise during carcinogenic progression.14 In this light, genome instability has been described as an "enabling characteristic" of tumor cells.15

The high-risk HPV-encoded E6 and E7 oncoproteins encode multiple functions that enable the virus to overcome growth regulatory mechanisms of the host cell cycle machinery. Genomic instability induced by high-risk HPVs may be an additional mechanism that by increasing the plasticity of the host cell genome facilitates the evolution of cell populations that support long-term viral persistence.

HPVS AND THE CELL CYCLE

HPVs contain a double stranded DNA genome of approximately 8,000 base pairs and up to 10 open reading frames (ORFs). The ORFs within the early region code for proteins involved in the regulation of viral replication and the viral life cycle, whereas the 2 ORFs within the late region encode proteins that form viral capsids. In addition, the viral genome contains a region that does not direct the synthesis of viral proteins but contains regulatory DNA elements that act as binding sites for viral and cellular proteins that regulate viral replication and transcription. HPVs do not express the entire complement of enzymes required for genome replication. Therefore, HPVs re-program the host cell’s DNA replication machinery to replicate their own genomes.16 High copy number amplification of viral DNA and assembly of virions occurs in the upper differentiated layers of the infected squamous epithelium in cells that would have normally permanently ceased to proliferate. The high-risk HPV E6 and E7 oncoproteins target critical negative growth regulatory signaling circuits to allow viral genome replication in these post-mitotic cells (Fig. 1). The high-risk HPV E7 oncoproteins bind and degrade the retinoblastoma tumor suppressor protein (pRB) and the related pRB family members p107 and p130.16 High-risk HPV E7s also inactivate the cyclin-dependent kinase (CDK) inhibitors p21Waf1 and p27Kip1.17 This profound dysregulation of cellular proliferation control pathways results in aberrant activation of E2F-mediated gene transcription with increased expression of cyclin E and cyclin A and aberrant CDK2 activity.21–24 E2F can activate p14ARF thereby stabilizing p53 through inhibition of MDM2.25 Activation of p53 results in induction of cell cycle arrest or apoptosis. To abrogate this antiproliferative response, the high-risk HPV E6 oncoprotein induces the rapid proteasomal degradation of p53 by interacting with the host cell protein E6-AP, a HECT
domain E3 ubiquitin ligase.\textsuperscript{26,27} This function of HPV E6, in combination with its ability to stimulate telomerase activity,\textsuperscript{28} enables HPV-infected cells to sustain proliferative activity. In contrast to high-risk HPV types, E6 and E7 proteins encoded by low-risk HPVs do not interact with these targets of high-risk HPV-encoded oncoproteins or do so less efficiently. The p53 and pRB tumor suppressor pathways that are targeted by the high-risk HPV E6 and E7 proteins, respectively as part of the viral replication strategy are dysfunctional in the majority of non-virus-related human carcinomas.

\section*{GENOMIC INSTABILITY INDUCED BY HIGH-RISK HPVS}

The notion that dysregulation of cell cycle control mechanisms can result in disruption of genomic integrity has first been established in model organisms such as \textit{Saccharomyces cerevisiae}.\textsuperscript{29} Studies in Thea Tlsty’s laboratory have shown that expression of HPV oncoproteins can render normal human cells genomically unstable, thus predisposing them to accumulate chromosomal alterations.\textsuperscript{30} Whereas HPV-16 E6 expressing cells exhibited evidence for structural chromosomal changes,\textsuperscript{30} HPV E7 oncoprotein expressing cells were prone to accumulate numerical chromosomal abnormalities and developed aneuploidy.\textsuperscript{30,31}

\section*{CENTROSOME-MEDIATED MITOTIC DEFECTS}

There are several mechanisms that can account for chromosomal gains and losses including mitotic non-disjunction, spindle checkpoint defects, or disturbances of the polarity of the cell division. The latter mechanism is particularly appealing in the context of HPV-associated carcinogenesis because abnormal multipolar mitoses in suprabasal epithelial layers have long been recognized as a hallmark of high-risk HPV-associated lesions of the uterine cervix.\textsuperscript{32} Such cell division abnormalities typically result from the presence of supernumerary mitotic spindle poles.\textsuperscript{33} Spindle poles are formed by centrosomes, small cytoplasmic organelles that were first recognized by Theodor Boveri to play important roles in orchestrating the cell division process.\textsuperscript{34} Abnormal centrosome numbers are a very frequent finding in cancers and can cause the formation of multipolar mitotic spindles that trigger asymmetric cell divisions with increased risk for chromosome missegregation, aneuploidy and carcinogenic progression. Co-expression of HPV-16 E7 with the E6 oncoprotein, which induces an accelerated proteasomal degradation of p53, further increases the proportion of cells with centrosome-related mitotic defects, most likely by abrogating G2/M checkpoint control (see text for details).

\begin{figure}[h]
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\caption{HPV-16 E6 and E7 oncoproteins abrogate negative growth regulatory signaling pathways of the host cell. The HPV-16 E7 oncoprotein binds and degrades the retinoblastoma tumor suppressor protein (pRB) and the related pRB family members p107 and p130. This leads to aberrant E2F-mediated gene transcription and unscheduled activation of cyclin/CDK2 complexes as well as elevated CDC25A levels. Several lines of evidence suggest that dysregulated CDK2 activity can give rise to abnormal centrosome duplication thus leading to an increased risk for chromosome missegregation, aneuploidy and carcinogenic progression. Co-expression of HPV-16 E7 with the E6 oncoprotein, which induces an accelerated proteasomal degradation of p53, further increases the proportion of cells with centrosome-related mitotic defects, most likely by abrogating G2/M checkpoint control (see text for details).}
\end{figure}
cellular defects. This model is supported by studies that showed that in HPV E7 oncoprotein-expressing cell populations aneuploid cells emerge even before immortalization (Fig. 2). Interestingly, the proportion of multipolar metaphase cells in such populations is much higher than the proportion of ana- or telophase cells with a multipolar arrangement suggesting that there are regulatory mechanisms that inhibit mitotic progression in the presence of abnormal numbers of spindle poles. Nonetheless, expression of HPV oncoproteins allows a significant number of cells to complete mitosis in the presence of an asymmetric spindle pole arrangement.

In striking contrast, centrosome abnormalities in response to HPV E6 expression primarily arise in cells that display pronounced nuclear abnormalities including appearance of multiple nuclei as well as micromolecules, or contain large multilobulated nuclei that may have formed as a consequence of a persistent block of cytokinesis. Centrosome abnormalities in HPV-16 E6 expressing cells only become evident after prolonged passaging indicating that they accumulate in parallel with other cellular alterations. When HPV E6 and E7 are co-expressed, however, there is clearly an additive effect on the incidence of numerical centrosome abnormalities and multipolar cell divisions. This may be explained in part by HPV E6-induced relaxation of G2/M checkpoint control thereby allowing an increased proportion of cells to enter mitosis in the presence of multipolar spindles.

The ability of high-risk HPV E7 to rapidly disrupt normal centrosome and centriole duplication is independent from its ability to degrade pRB and the related pRB family members p107 and p130. The integrity of the LXCXE motif, which also mediates the interaction with p21Cip1, is necessary. Because dominant-negative mutants of E2F and CDK2 can each suppress HPV-16 E7 induced centrosome duplication errors, it is conceivable that dysregulation of cyclin/CDK2 complexes importantly contributes to this activity of the E7 oncoprotein. A series of recent reports, however, showed that murine cells deficient for cyclin E or CDK2 proliferate normally and that CDK2 inhibition does not necessarily impede cellular proliferation or centrosome duplication. Even though it is possible that functional redundancy between CDKs may compensate for loss of CDK activity, these findings raise the possibility that cyclin/CDK2 complexes may be dispensable for normal centrosome duplication, but may trigger abnormal centrosome duplication when dysregulated. The contribution of additional mechanisms engaged by the HPV E7 oncoprotein for example, an abrogation of a recently described intrinsic block of centrosomes to re-replicate, awaits further experimental clarification.

Integration of the HPV genome into a host chromosome and subsequent overexpression of HPV E6 and E7 is not a prerequisite for HPV-induced genome destabilization to occur. Studies in oncotypical raft cultures have shown that expression of HPV-16 E6 or E7 from HPV episomes present at low copy numbers, as it is usually found early during infection, is sufficient to induce numerical centrosome anomalies, multipolar mitoses, and aneuploidy.

Modeling cervical carcinogenesis in transgenic mice also recapitulated the appearance of numerical centrosome abnormalities. Transgenic animals with expression of HPV-16 E7 driven by a cytokeratin 14 promoter and treated with low doses of estrogen not only develop numerical centrosome abnormalities in the cervical mucosa but are also prone invasive cervical carcinomas. In contrast, however, HPV-16 E6 expressing transgenic mice, which display a comparable level of numerical centrosome aberrations, develop only low grade cervical lesions that do not progress to malignant tumors. These findings further support the notion that the mere presence of centrosome abnormalities in a tumor or cell culture system is not a generally relevant marker for ongoing chromosomal instability that accurately predicts malignant progression.

A small study has shown that human premalignant cervical lesions and carcinomas display centrosome abnormalities, and that their incidence increases with malignant progression. Similar findings have been reported for several other cancer types. Again, the functional relevance of such abnormalities needs to be considered carefully because even high levels of centrosome abnormalities do not necessarily correlate with ongoing numerical chromosomal instability.

**DISRUPTION OF HOST CELL MITOTIC FIDELITY BY ADDITIONAL MECHANISMS**

In addition to centrosome abnormalities, the HPV-16 E6 and E7 oncoproteins have been reported to independently disrupt the mitotic spindle checkpoint. From these studies it is unclear, however, whether the observed defects represent true checkpoint aberrations or whether they represent abnormalities to properly halt DNA re-replication upon spindle checkpoint activation. Cells that engage mitotic checkpoints and fail to complete mitosis can eventually enter a G1 like state (adaptation) with a tetrablastic chromosome complement. Normal cells remain tetraploid but abnormal cells may be able to undergo additional rounds of DNA replication eventually yielding polyploid cells; a process that is facilitated by impaired p53 function. In such cases, tetra- or polyploid cells with multiple centrosomes arise, which can be a source for the emergence of aneuploid progeny, particularly when p53 function is impaired. In addition to polyploidization, HPV-16 E6 or E7 expressing cells also frequently show additional cell division abnormalities, including lagging chromosomes that may in part be caused by premature chromosome segregation.

**HIGH-RISK HPV-INDUCED STRUCTURAL CHROMOSOME INSTABILITY**

Cervical carcinomas and HPV-immortalized cell lines frequently acquire structural chromosomal changes together with numerical chromosome imbalances. Such abnormalities can include chromosomal translocations, deletions, amplifications of certain chromosomal regions, or other changes. HPV-16 E6 and E7 can independently induce structural chromosome instability in vitro. This is reflected by the induction of anaphase bridges in high-risk HPV oncoprotein expressing cells. Anaphase bridges frequently occur when unprotected chromosome ends are generated for example, after telomere attrition or DNA breakage. Mechanistically, chromosome breaks in HPV oncoprotein expressing cells are likely to involve increased susceptibility to DNA damage or defective DNA damage repair. In support of this model, HPV oncoprotein expressing cells are impaired in their ability to respond to DNA damage presumably as a consequence of loss of p53 or pRB function. Primary human keratinocytes expressing the HPV-16 E7 oncoprotein display an increased number of phospho-H2AX foci in the nucleus when compared to HPV-16 E6 expressing cells. Histone H2AX becomes rapidly phosphorylated in the vicinity of DNA damage, which constitutes
a critical step for assembling other DNA repair factors at the site of damaged DNA.69 In contrast, telomere shortening was not found to be a major source of chromosomal breakage in HPV-expressing pre-immortalized cell populations.45 Increased DNA breakage or impaired DNA repair may also account for the enhanced ability to integrate foreign DNA70 and may thus ultimately promote integration of viral DNA into the host cell genome.

Microsatellite instability (MIN) is observed in many human tumors and arises as a consequence of impaired DNA repair processes.71 MIN has been observed in some HPV-associated tumors but seems to be confined to advanced carcinomas and thus it is unlikely that it is directly caused by HPV oncogene expression.72

In summary, high-risk HPV oncoproteins rapidly disrupt the chromosomal integrity of the host cell on a numerical and structural level. It is conceivable that the majority of events causing genomic instability are not associated with the production of viable daughter cells, but trigger programmed cell death46 or other abortive cellular responses such as senescence.41 Persistent infection with high-risk HPVs and the integration of the viral genome into a host cell chromosome followed by enhanced expression of HPV E6 and E7, however, increase the risk for the accumulation of genetic changes that may ultimately reach a critical threshold necessary for malignant conversion.

**DOES GENOMIC INSTABILITY PROMOTE PERSISTENT HPV INFECTION?**

Productive viral infection depends on replication of the viral genome in differentiated epithelial cells. However, differentiating epithelial cells have normally undergone irreversible growth arrest, which would not support viral genome synthesis. The presence of abnormal, DNA synthesis competent cells in differentiated strata of a squamous epithelium may therefore trigger various antiproliferative responses. If they are not abrogated by viral functions, these cellular defense responses would lead to the elimination of HPV infected cells and thus limit viral propagation.11 Cellular defense mechanisms may include upregulation of CDK inhibitors such as p16INK4a, which is commonly detected in cervical lesions,13 but also clearance of abnormally replicating host cells by apoptotic cell death.74 Host immune surveillance,75 or a permanent proliferative block by cellular senescence.76–78 The ability of high-risk HPV E6 and E7 to subvert growth-suppressive signaling pathways of the host contributes importantly to the ability to overcome such barriers and allows for establishment of persistent high-risk HPV infection, which has been identified as a major risk factor for malignant progression.11 Moreover, the increased genomic plasticity in cells expressing HPV oncoproteins may provide an additional selection advantage allowing for the emergence of tumor cell clones that have acquired host genetic changes that thwart the above-mentioned cellular defense mechanisms.14

This is underlined by the finding that cervical lesions, in contrast to most non-virus related tumors, initially consist of polyclonal precursors that are replaced by clonal populations at more advanced stages of malignant progression.79,80 In this context, integration of viral DNA results in an additional growth stimulus.81

Structural and numerical chromosomal changes may each contribute to the outgrowth of rapidly proliferating clones of HPV infected cells (Fig. 3). Previous experiments have shown that the introduction of entire chromosomes, for example chromosome 6, into HPV-immortalized cells can induce growth arrest and decreased telomerase activity.83 Similar results have also been obtained when additional copies of chromosome 1 or chromosome 11 were transferred into HPV-18-positive HeLa cells.83 These results imply that losses of certain chromosomes may endow HPV infected cells with a survival advantage by allowing replication beyond the normal constraints of host cells. Chromosomal losses may also affect other characteristics of malignant growth such as tumor angiogenesis.84

Increased DNA damage or insufficient repair in HPV-oncoprotein expressing cells may promote the development of structural chromosome abnormalities. Aberrations of chromosome 3 are particularly frequent in cervical carcinomas and gains of the long arm of chromosome 3 (3q) have been reported to be associated with malignant progression.85 This region of chromosome 3 harbors sequences for the RNA component of the human telomerase gene (TERC) on 3q26 and may thus contribute to a growth advantage of HPV-infected cells.86

Loss of the FHIT (fragile histidine triad) gene on the short arm of chromosome 3 (3p) is frequently found in premalignant and cancerous cervical lesions as well as in various other human malignancies.87,88 The FHIT gene locus encompasses the FRA3B common fragile site, a highly unstable region of the human genome. There is a correlation between infection with oncogenic HPVs and loss of heterozygosity at the FHIT locus and high-risk HPVs can integrate into this site.89 Even though the biological activity of the FHIT protein is not fully understood, re-expression of FHIT in lung cancer cells results in apoptosis and G1 cell cycle arrest.90 Loss of FHIT may therefore support cellular survival and proliferation.91 Increased DNA breakage or impaired DNA damage repair in HPV-oncoprotein expressing cells may promote breakage at the FRA3B locus leading to FHIT gene inactivation and may thus provide an additional route leading to a growth advantage of HPV-infected host cells.

![Figure 3](https://example.com/figure3.png)

**FIGURE 3** – HPV oncoprotein-induced chromosomal instability as a co-factor for viral infection. The high-risk HPV E6 and E7 oncoproteins have evolved to target negative growth-regulatory signaling pathways of the host cell to promote long-term viral replication and survival. High-risk HPV E6 and E7 also rapidly induce numerical and structural chromosome instability. Such abnormalities may contribute to persistent HPV infection. For example, transfer of entire chromosomes into HPV-immortalized cells can suppress telomerase activity and result in cellular senescence suggesting that loss of certain chromosomes can promote immortalization. In addition, inactivation of the FHIT (fragile histidine triad) gene locus on chromosome 3p by chromosome breakage can interfere with apoptosis and cell proliferation thereby facilitating the clonal expansion of HPV-infected cells.
OUTLOOK

The high-risk HPV-encoded oncoproteins E6 and E7 have not only evolved to target critical tumor suppressor pathways of the host cell, they also cooperate to rapidly induce enhanced genomic plasticity involving mitotic defects and DNA breakage. These abnormalities do not require physical integration of the HPV DNA into the host genome. Whereas the induction of chromosomal instability will certainly give rise to cells that are not viable and undergo programmed cell death or senescence, there is also an enhanced window of opportunity for accumulation of genetic aberrations that promote the outgrowth of host cells carrying HPV epimorphs. Such cell populations may contribute importantly to the persistence of viral infection and thus transmission to other hosts.

The induction of chromosomal instability is an emerging theme in viral tumorigenesis in humans and not only associated with high-risk HPV types, but also with Hepatitis B virus, Kaposi’s sarcoma herpesvirus (KSHV), and human T cell leukemia virus type 1 (HTLV-1). The mechanisms by which oncopogenic viruses disrupt genomic integrity of the host cell are far from being completely understood. Given that many viral oncoproteins subvert important host cell tumor suppressor pathways, future studies hold the promise to contribute importantly to our understanding of mechanisms that govern tumor development and progression in general.

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REFERENCES


