

Genomic Instability Induced By Human Papillomavirus Oncogenes

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Abstract

Cervical cancer is one of the leading causes of cancer death in women worldwide. Human papillomavirus (HPV) infection is necessary but not sufficient for the development of cervical cancer. Genomic instability caused by HPV allows cells to acquire additional mutations required for malignant transformation. Genomic instability in the form of polyploidy has been implicated in a causal role in cervical carcinogenesis. Polyploidy not only occurs as an early event during cervical carcinogenesis but also predisposes cervical cells to aneuploidy, an important hallmark of human cancers. Cell cycle progression is regulated at several checkpoints whose defects contribute to genomic instability.

The high-risk HPVs encode two oncogenes, E6 and E7, which are essential for cellular transformation in HPV-positive cells. The ability of high-risk HPV E6 and E7 protein to promote the degradation of p53 and pRb, respectively, has been suggested as a mechanism by which HPV oncogenes induce cellular transformation. E6 and E7 abrogate cell cycle checkpoints and induce genomic instability that leads to malignant conversion.

Although the prophylactic HPV vaccine has recently become available, it will not be effective for immunosuppressed individuals or those who are already infected. Therefore, understanding the molecular basis for HPV-associated cancers is still clinically relevant. Studies on genomic instability will shed light on mechanisms by which HPV induces cancer and hold promise for the identification of targets for drug development. [N A J Med Sci. 2010;3(2):43-47.]

Key Words: *genomic instability, human papillomavirus (HPV), oncogene, cell cycle checkpoint*

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HPV and Human Cancers

Papillomaviruses are small DNA viruses that replicate in the stratified layers of skin and mucosa. Human papillomavirus (HPVs) can be classified as either high- or low-risk types depending on their clinical associations. The low-risk HPV types such as HPV-6 and HPV-11 are associated primarily with benign lesions including warts or papillomas. The high-risk HPV types, such as HPV-16, HPV-18, and HPV-31, are commonly associated with lesions that can progress to high-grade cervical intraepithelial neoplasia and cervical carcinoma (for review, see ¹). Cervical cancer is one of the leading causes of cancer death in women, worldwide. In addition, HPV infections are linked to more than 50% of other anogenital cancers and cancers of the esophagus (reviewed in ¹). Although tobacco and alcohol are responsible for most of the head and neck squamous cell carcinomas (HNSCCs), there is evidence for a causal association between HPV and a subset of HNSCCs.² Cervical and anogenital cancers and HNSCCs are frequently found among HIV-infected individuals (reviewed in ³). Specific and efficient therapies for HPV infection or HPV-induced malignancies are not yet available. Although the prophylactic HPV vaccine has recently become available, a preventive HPV vaccine is type-specific and is unlikely to be effective for those who are already infected or are immunosuppressed.

Genomic Instability and Cancer

Genomic instability is a hallmark of cancer progression. Genomic instability in the form of polyploidy, wherein cells have more than two sets of chromosomes, has been implicated as a causal role in tumorigenesis. Polyploidy is a major route to centrosome amplification, which in mitosis could form multipolar spindles and result in mis-segregation of chromosomes and subsequently lead to aneuploidy.⁴ Aneuploidy, a state in which cells have extra or missing chromosomes, is another form of genomic instability commonly seen in human solid tumors⁵⁻⁷ and has recently been demonstrated for a causal role in tumorigenesis.⁸ Induction of aneuploidy by polyploidy has been demonstrated in cultured cells (primary rat embryo fibroblasts)⁹ and is best exemplified in the precancerous state of Barrett's esophagus cells.¹⁰ Polyploidy can lead to both numerical and structural chromosome abnormalities by increasing the rate of DNA breakage and damage,^{11,12} the latter has been shown to be an anti-cancer barrier in early human tumorigenesis.^{13,14} Tetrasomy in basal keratinocytes has been found in low-grade squamous intra-epithelial lesions of the cervix infected with high-risk but not low-risk

HPV types.¹⁵ Significantly, a recent study demonstrated that tetraploidy occurred as an early event during cervical carcinogenesis and predisposed cells to aneuploidy.¹⁶ Two other recent studies demonstrated that tetraploid but not diploid mouse or human cells are competent to induce tumors with both numerical and structural chromosome abnormalities.^{17,18}

Polyploidy can be induced in several ways. First, abrogation of the spindle checkpoint is usually followed by failure of cytokinesis.¹⁹ Second, adaptation of the spindle checkpoint and replication of tetraploid cells after abrogating the postmitotic checkpoint will lead to polyploid cells.²⁰ Third, cell fusion can generate polyploid cells.¹² Fourth, after completion of S phase, cells may undergo re-replication,²¹ which can lead not only to polyploidy but also to gene amplification,²² DNA fragmentation,²³ DNA breaks,²⁴ and cellular DNA damage response (²⁵ and references therein).

Cell Cycle Checkpoints and Genomic Instability

Cell cycle progression is regulated at several checkpoints whose defects contribute to genomic instability.²⁶ The checkpoints in eukaryotic cells include the G1 checkpoint, the G2/M checkpoint, the spindle assembly checkpoint, and the postmitotic checkpoint.²⁰

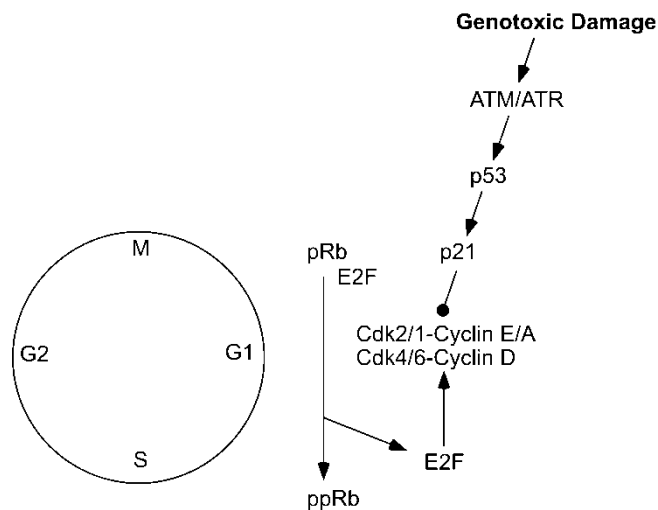


Figure 1. The G1 cell cycle checkpoint. Upon genotoxic damage, p53 is activated via ATM/ATR. p53 activates p21, which inhibits cyclins/Cdks. Phosphorylation of pRb by cyclins/Cdks results in its dissociation from E2F, which mediates transcription of genes required for cell cycle progression. Arrows indicate positive regulation while a knob represents a negative regulation.

The G1 checkpoint is mainly regulated through phosphorylation of the retinoblastoma protein (pRb) by cyclin D/Cdk4-Cdk6 in early G1 and followed by cyclin E1-cyclin A/Cdk2 complexes²⁷ (Figure 1). Cdk1 (Cdc2) also functions in the G1/S phase transition and can substitute for Cdk2.²⁸ Hyperphosphorylation of pRb results in its

dissociation from members of the E2F family of transcription factors. Free E2F mediates transcription of genes required for DNA synthesis and promotes cells to enter S phase.²⁹ Upon exposure to genotoxic agents, p53 is activated via multiple mechanisms including phosphorylation by ATM/ATR and stabilization by ARF (p14/p19).³⁰ p53 activates the transcription of the cdk inhibitor p21, which binds to and inactivates cyclin E1/Cdk2 and cyclin A2/Cdk2 complexes, resulting in pRb hypophosphorylation and cell cycle arrest at the G1-S transition.^{31,32} Other G1 Cdk inhibitors include p16 and p27.²⁷

The mitotic spindle assembly checkpoint monitors chromosome attachment to microtubules and delays chromosome segregation until all chromosomes are correctly aligned on the spindle.³³ Although it was originally thought that p53 might play a role in the spindle checkpoint,³⁴ subsequent studies demonstrated that p53 was not necessary for this process.^{20,35} A recent study demonstrated that down-regulation of Rb could lead to increased expression of the spindle checkpoint protein Mad2, which may result in altered checkpoint function.³⁶

The cells with an intact spindle checkpoint activity that have been arrested in metaphase for prolonged periods of time will eventually adapt to this checkpoint and progress to a G1-like state with tetraploid genomes.^{20,37} The replication of DNA in these cells is usually blocked by p53- and pRb-dependent cell cycle arrest, referred to as the postmitotic checkpoint²⁰ or “tetraploid” checkpoint.³⁷ It appears that the structural integrity and dynamics of the microtubules, rather than tetraploidy per se, is the key to induce cell cycle arrest at this checkpoint.^{35,38-40} The postmitotic checkpoint shares many features with the G1 checkpoint: in all cases, cell-cycle arrest coincides with high concentrations of p21 and hypophosphorylated pRb^{7,41}). p53 appears to play a key role in mediating the postmitotic checkpoint^{9,20,42} and p21 is responsible for at least part of this p53-mediated postmitotic arrest response.^{20,35,43} Activation of the postmitotic checkpoint can eliminate polyploid cells by apoptosis. Tetraploid cells arising due to mitotic slippage are prone to undergo Bax-dependent mitochondrial membrane permeabilization and subsequent apoptosis that is partially dependent on p53.^{44,45}

HPV E6 and E7 Oncogenes and Genomic Instability

The transforming properties of high-risk HPVs primarily reside in the E6 and E7 oncogenes, and the sustained expression of these genes appears to be essential for the maintenance of the transformed state of HPV-positive cells (⁴⁶ and references therein). E6 and E7 encode small proteins that play essential roles in the HPV life cycle.⁴⁷⁻⁵⁰ The oncogenic activities of E6 and E7 have been reflected in multiple biological assays, including immortalization of primary cells, transformation of established mouse fibroblasts, resistance to terminal differentiation of human keratinocytes, tumorigenesis in animals, modulation of

apoptosis, and abrogation of cell cycle check points (reviewed in ⁵¹). The ability of high-risk HPV E6 and E7 protein to promote the degradation of p53 and pRb, respectively, has been suggested as a mechanism by which HPV oncogenes induce cellular transformation.^{52,53} E6 and E7 also have functions independent of inactivating p53 and pRb (reviewed in ^{51,54}). These functions include association with additional cellular proteins, activation of telomerase, and immortalization of primary human keratinocytes. Although E6 and E7 or HPV genome efficiently immortalize primary human epithelial cells, they are not sufficient to directly induce transformation of human cells.⁵⁵ It is believed that the genomic instability caused by E6 and E7 enable cells to accumulate additional genomic aberrations necessary to undergo malignant conversion.

Expression of the high-risk HPV E6 and E7 oncogenes in human keratinocytes leads to polyploidy, which is enhanced by DNA damage and by activation of the spindle checkpoint through microtubule disruption.⁵⁶⁻⁵⁹ These observations are biologically relevant as both E6 and E7 expression can induce DNA damage,^{60,61} and E6-expressing cells contain a high percentage of metaphase lagging (misaligned) chromosomes that could potentially trigger the spindle checkpoint.^{60,62} Previously, it was thought but not directly shown that high-risk E6 and E7 induce polyploidy in response to microtubule disruption by abrogating the spindle checkpoint and that degradation of the tumor suppressor p53 by E6 is a mechanism by which E6 induces polyploidy (⁵⁸ and references therein). The mechanism by which E6 and E7 induces polyploidy upon DNA damage was proposed but not directly demonstrated to be through cytokinesis failure as a result of p53 inactivation and poly-like kinase 1 (Plk1) up-regulation.⁵⁹

Some recent studies demonstrate that HPV-16 E6 and E7 do not have a major effect on the mitotic spindle checkpoint in primary human keratinocytes,^{63,64} a result consistent with what was observed in human fibroblasts and cervical cancer cells.^{35,65} The spindle assembly checkpoint was still active in the presence of E6 or E7 and that expression of E7 did not significantly affect the overall length of mitosis from nuclear envelope breakdown to its reformation in the daughter cells, although a slight prolongation of prometaphase was detected in a separate study.⁶⁶ Instead, HPV-16 E6 and E7 abrogate the postmitotic checkpoint to induce polyploidy after microtubule disruption.^{63,64} The activity of postmitotic checkpoint abrogation is shared by E7 from HPV-58, which is the third most common HPV type in cervical cancer from Eastern Asia.⁶⁷ Interestingly, E6 mutant proteins defective in inducing p53 degradation also induce polyploidy,⁶³ indicating a p53-independent activity of E6 in abrogating the postmitotic checkpoint. However, the p53-independent mechanism by which E6 abrogates the postmitotic checkpoint is not currently known.

In addition to numerical chromosomal changes, expression of HPV-16 E6 and E7 is associated with structural

chromosomal alterations,⁶⁸⁻⁷⁰ which are frequently found in HPV-immortalized cell lines.⁷¹⁻⁷³ Anaphase bridges, which are believed to be a result of chromosomal breaks and structure changes, were observed in E6- and E7-expressing cells.^{60,74} While one study suggests that DNA damage induced by E6 and E7 instead of telomere attrition contributes to anaphase bridges,⁶⁰ another study provided some evidence suggesting that anaphase bridge formation is a result of telomere erosion.⁷⁴

Normal human keratinocytes expressing HPV-16 E6 or E7 display centrosome abnormalities.⁷⁵ In these cells, E7 rapidly induces abnormal centrosome duplication, whereas E6 has no immediate effect on centrosome numbers.⁷⁵ Subsequent studies demonstrated that HPV-16 E7 rapidly stimulated an overduplication of centrioles when transiently expressed in human osteosarcoma U2OS cells, which likely occurred within a single cell division cycle.⁷⁶ In addition to promoting centriole duplication, polyploidy contributed at least in part to centrosome amplification in E7 expressing cells, as more cells with abnormal centrosomes were found in polyploid cells than in diploid cells.⁷⁶

Cells with extra centrosomes in mitosis may form multipolar spindles (also called multipolar mitosis or multipolar metaphase). Extra centrosomes do not always lead to multipolarity. Primary human keratinocytes expressing HPV E6 were shown to have a higher rate of spontaneous multipolar spindle formation but the mechanism is not known.⁶² A correlation of the spontaneous occurrence of multipolar metaphases along with a decrease in the levels of p53 was observed in HPV E6 mutant-expressing cells.⁶² The ability of E7 in promoting multipolar spindle formation is not very clear. While some early studies showed an increase in multipolar spindle formation in E7 expressing primary human keratinocytes,^{60,62,75} a recent study did not observe a significant increase.⁷⁷ The combined effect of E6 and E7 on multipolar spindle formation has not been well established. One study found synergistic effect,⁷⁵ but the other one only detected additive effect,⁶⁰ and still other study did not observe a significant increase in multipolar spindle formation when both E6 and E7 were expressed in primary human keratinocytes as compared with either E6 or E7 alone.⁶²

In summary, induction of genomic instability, polyploidy in particular, is an important step in cervical carcinogenesis. Studies on E6- and E7-induced polyploidy will shed light on mechanisms by which HPV induces tumors and hold promise for the identification of targets for drug development.

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