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Cervical Cancer: Molecular Mechanisms of HPV-induced Carcinogenesis

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The causal role of persistent human papillomavirus (HPV) infections in the development of cervical cancer and its precursors has been proved beyond reasonable doubt. In high-grade lesions an abortive infection is established in which the viral gene expression becomes deregulated, and the normal life cycle of the virus cannot be completed. HPV contributes to neoplastic progression predominantly through the action of E6 and E7 viral oncoproteins. These proteins bind to and inactivate the tumor suppressor proteins p53 and pRb, respectively, causing deregulation of the cell cycle.The essential steps in HPV-induced carcinogenesis are: integration of HPV DNA into the host genome, overexpression of E6 and E7 oncoproteins, genetic instability and accumulation of the cellular genetic damage.

Key words: HPV, cervical cancer, carcinogenesis, abortive HPV infection, E6 and E7 oncoproteins, HPV integration

Human papillomaviruses (HPV) are small DNA viruses that demonstrate significant genetic variation, with more than 100 types identified to date. About 40 of them can infect the genital tract and fall into two categories: high risk (HR) and low risk (LR). The HR HPV types are associated with the development of anogenital cancers including cervical cancer.

HPV life cycle and cervical cancer

Structure of the HPV genome

The HPV genome is a double-stranded, circular DNA molecule that contains approximately 8000 bp. The genome is functionally divided into three regions [9]: the early (E) region encodes non-structural viral proteins E1–E7, the late (L) region encodes the L1 and L2 proteins and a control region called the Long Control Region (LCR) or Upper Regulatory Region (URR) without coding potential. E1 and E2 encode proteins that are essential for viral replication and control of gene transcription. E2 also plays

an important role in the regulation of the levels of E6 and E7 oncoproteins and in the viral genome encapsidation. In addition, HR E2 proteins could induce abnormal mitotic phenotypes and overexpression of Skp2, a main regulator of the G0-G1/S transition, indicating a potential role of E2 proteins in HPV-induced carcinogenesis [2]. The E4 encoded protein is expressed and acts late in the viral life cycle as regulator of late gene expression, virus assembly, maturation and release. E5 gene encodes for the protein that interacts with various transmembrane proteins like the receptors of the epidermal growth factor and induces mitogenic signalling and transformation of cells via this receptor. The E6 and E7 regions encode for oncoproteins. The late region encodes the capsid proteins L1 and L2. The noncoding region of HPV genome regulates DNA replication by controlling the transcription.

Normal productive HPV infection

The productive life cycle of HPV is linked to the differentiation of the infected epithelial cells and is initiated by the infection of basal epithelial cells [9]. Viral proteins are expressed sequentially with differentiation, and mature virions are produced only in the most superficial layers of the epithelium. HPV DNA replicates as an episome (circular DNA molecule which is separate from the host cell DNA) in the para-basal and squamous cell layers. In the basal layer, the early proteins E6 and E7 facilitate replication and maintenance of the viral genome and cause cellular proliferation as well. Expression of the late proteins (L1 and L2) occurs in the upper layer of the epithelium. This is followed by packaging of the DNA into the capsid and release of infectious virions from the normally desquamated epithelial cell.

Abortive HPV infection as precursor to cancer

In contrast to the productive infection where new virus particles are produced, in high-grade squamous intraepithelial neoplasia (HSIL) and cancer production of HPV proteins and viral DNA is quite different [9]. In this case an abortive infection is established in which the viral gene expression becomes deregulated, and the normal life cycle of the virus cannot be completed. There is little viral DNA replication and only a subset of viral proteins is produced. The late viral proteins, L1 and L2, are only weakly expressed or not expressed at all. Therefore, there is minimal, if any, viral particle assembly and release at the epithelial surface. At the same time viral E6 and E7 oncogenes are highly expressed.

Papillomavirus E6 and E7 oncoproteins

The E6 and E7 are small proteins of approximately 150 and 100 amino acids with molecular weights of 16–18 kD and 10 kD, respectively. E6/E7 proteins are important for the viral life cycle, for the cell cycle control, and for the carcinogenic processes. Relevant biochemical properties of the HPV-encoded oncoproteins E6 and E7 include inactivation of tumour suppressors, modulation of cell-cycle regulatory, DNA repair and apoptotic processes, deregulation of gene expression and the activation of signal transduction pathways. E6 and E7 proteins function though a number of direct and indirect interactions with cellular proteins, a number of which are well known cellular tumor suppressors.

E6/E7 interactions with p53 and pRb tumor suppressors

The major transforming activities of HR HPV E6 and E7 proteins have been linked to inactivation of the p53 and retinoblastoma (pRb) tumor suppressors, respectively. The HR E6 protein binds to and promotes degradation of p53 through an ubiquitin/ proteasome-dependent mechanism. As a consequence, the normal activities of p53 which govern G1 arrest, apoptosis, and DNA repair are abrogated. To inhibit p53, E6 requires a cellular protein called E6-associated protein (E6AP). In non-infected cells, the ubiquitin-mediated degradation of p53 is triggered by the mdm-2 protein, while in HR HPV-infected cells the E6-E6AP complex replaces mdm-2 in the control of cellular p53 levels [12]. This shift dramatically shortens the p53 half-life, decreases biological function, and reduces p53 protein level in cervical carcinoma cells to less than half the level found in normal epithelial cells [30]. HR HPV E6 proteins also lead to a down-regulation of p53-dependent transcription, independently of E6AP-dependent degradation of the p53 protein.

E7 acts by binding cellular proteins of the pRb tumour suppressor family, which, by interacting with the E2F-family of transcription factors, control cell replication [3]. Association of E7 with pRb causes its degradation, and leads to the loss of pRb control over E2F transcription factors. Binding of E7 to the active form of pRb leads to the release of E2F transcription factors, which then stimulate entry into the S-phase of the cell cycle and lead to cell replication. As a result the cell cycle regulation is disrupted. In addition to binding pRb, E7 can bind to p107 and p130, two other members of the family of pocket proteins. The E6 and E7 proteins of the HR HPV types act as viral oncoproteins, but no such functions are associated with these proteins from the LR types.

E6/E7 interactions with cellular proteins other than p53 and pRb

Besides tumor suppressor proteins p53 and pRb, HR HPV E6 and E7 oncoproteins have a number of additional cellular targets that contribute to their oncogenic activities [20, 21, 23]: transcriptional factors (p300, myc, interferon regulatory factor 3- IRF3, autocrine motility factor 1 -AMF-1/Gps2, p150/Sal2, HDACs, E2F1, CKIs), factors that determine adhesion, cytoskeleton and polarity (paxillin, PDZ domain containing proteins, MAGI proteins, MUPP1), apoptosis factors (Bak, TNFR-1, FaDD, Caspase-8), DNA repair and chromosome stability factors (MCM7, XRCC1, MGMT) and other proteins such as E6 target protein 1(E6TP1). E6 can activate telomerase activity by inducing the expression of human telomerase reverse transcriptase (hTERT) [13].

Key events of HPV-induced cervical carcinogenesis

Integration of HPV DNA into the host genome

Viral DNA integration is a critical event in cervical carcinogenesis. Integrated HPV DNA is found in almost 90% of cervical carcinomas and in a subset of high-grade lesions [11, 16]. Integration is also found in some of low-grade lesions indicating that it may be an early event in cancer progression [24]. The frequency of integrated HR-HPV genomes is different for individual HR-HPV types with HPV16, 18, and 45 found substantially more often in the integrated state compared with HPV types 31 and 33 [28]. Integration of the HPV genome into the host cell chromosome is usually accompanied by the loss or disruption of E1, E2 and E4 viral sequences.The loss of E2 can lead to the deregulation and increased E6/E7 expression, which is critical for the enhanced growth characteristics of cervical cancer cells. Futhermore, as hTERT expression is inhibited

by E2 and activated by E6, HR-HPV integration is an efficient way to activate telomerase, and through cooperative effects with E7, to immortalize epithelial cells.

E7 may induce double-strand DNA breaks or interfere with break repair and this may facilitate viral genome integration. Viral DNA integration is reported to occur randomly throughout the human genome. At the same time several studies have indicated a preference for integration at common fragile sites and transcriptionally active regions [25, 27]. Cases have been reported in which HR-HPV integration has occurred within or adjacent to known oncogenes, most frequently in the region of the MYC gene at chromosomal band 8q24 [10, 29].

In cervical cancer alterations in cellular microRNA (miRNA) patterns have been reported indicating that miRNA biomarkers have a number of promising features [1, 6, 19, 31]. In some cases these alterations were associated with viral DNA integration.

Overexpression of E6 and E7 oncoproteins

High levels of E6 and E7 proteins are a hallmark of HPV-positive cancers. They are consistently found in tumor tissue and in tumor-derived cell lines indicating their role in cancer development. At the same time, there is relatively little E6 or E7 oncoprotein gene expression in normal epithelial cells and low-grade lesions [4]. As the grade increases, however, there is an increase in the levels of E6 and E7 in basal cells and throughout the undifferentiated epithelium. Thus, there is a correlation between the levels of E6 and E7 and the severity of the neoplastic phenotype and therefore the deregulated expression of the viral oncogenes is considered a predisposing factor in the development of HPV-associated cancers. E6 and E7 are transcribed from a promoter, regulated by cellular factors and the viral E2 product. As the viral protein E2 is an important modulator of E6-E7 promoter activity, changes in the levels of E6 and E7 expression occur following E2 disruption caused by the integration of HPV DNA into the cellular genome. The requirement of the E6/E7 genes for the maintenance of the cancer phenotype is shown by studies that have aimed to inhibit viral oncogene activity in cervical cancer cells. Cell lines such as HeLa and SiHa will undergo apoptotic cell death in the presence of molecules that inhibit E6/E7 function or following the reintroduction of E2 [5, 14, 15, 22].

Genetic instability and accumulation of the cellular genetic damage

Although overexpression of HR E6/E7 proteins is considered a predisposing factor, their expression alone is not sufficient for the development of cervical cancer. It is generally accepted that HPV-mediated oncogenesis requires the accumulation of additional genetic changes that occur over time following initial infection. HR HPV E6 and E7 expressing cells have a decreased ability to maintain genomic integrity [18]. Deregulated expression of the viral oncogenes is an important factor in the accumulation of secondary changes in the host cell chromosome that eventually lead to cancer. It was shown that HR HPV E7 oncoprotein acts as a mitotic mutator and induces multiple forms of mitotic abnormalities, including anaphase bridges, unaligned or lagging chromosomes, and multipolar mitoses, the histopathological hallmark of HPV associated cervical lesions and cancer [7, 8]. Structural changes are more commonly detected in chromosomes 1, 3 and 5 and less frequently in chromosomes 7, 8, 10, 12, 13, 16 and 22. Some of these allelic losses have been associated with particular genes that could be involved in malignant conversion and/or progression. Among these, losses in 3p and 10p have been associated with telomerase activation, which is a crucial step for cell immortalization mediated by HR HPVs [17, 26]. In addition, both E6 and E7 can abrogate

normal DNA damage responses which can contribute to the accumulation of genetic alterations in HPV-positive cells.

Conclusion

Papillomaviruses, like many other DNA tumour viruses, cause cancers when their regulated pattern of gene expression is disturbed. HPV infection induces changes in expression of host cell-cycle regulatory proteins. Such differentially expressed host proteins and nucleic acids may have a role as 'biomarkers' of dysplastic cells. At the same time there are a lot of questions to be answered. Many of the cellular targets of the HR E6 and E7 proteins have been identified, but it is not known if each of these interactions represents a physiologically significant association. Little is known about the consequence of different cellular environments on viral gene expression. The factors that regulate viral persistence and the events that lead to latency are other areas that are only poorly understood. All this information would be helpful in identifying novel therapeutic targets and strategies to inhibit the growth of HPV – associated cancers.

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