Journal of Cellular Biochemistry, Supplement 23:55-60 (1995)

The Molecular Biology of Cervical Cancer

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Abstract Infections with specific high-risk types of human papillomavirus constitute a major risk factor in development of precancerous and cancerous lesions of the uterine cervix. Laboratory studies suggest that the human papillomavirus has a mechanistic role in development of these lesions. The two viral proteins consistently expressed in cervical carcinomas functionally abrogate critical cell cycle regulatory pathways, including those governed by the p53 tumor suppressor protein and the product of the retinoblastoma susceptibility gene, pRB. Subversion of these pathways by viral proteins causes genomic instability, resulting in the accumulation of chromosomal abnormalities followed by clonal expansion of malignant cells. Since continued expression of the papillomavirus proteins is critical for maintenance of the transformed state, they are attractive targets for prevention and therapy of precursor as well as cancerous lesions of the cervix. © 1995 Wiley-Liss, Inc.

Key words: Cervical cancer, genomic instability, human papillomaviruses, p53, retinoblastoma protein

Papillomaviruses are small epitheliotropic DNA viruses which induce mostly benign skin lesions, including squamous warts and papillomas. More than 70 human papillomavirus (HPV) types have been described thus far; about 30 have been associated with lesions of the anogenital tract [reviewed in 1]. These anogenital-associated HPVs are further classified as *low risk* (*e.g.*, HPV-6, HPV-11), associated with benign genital warts, and the *high risk* (*e.g.*, HPV-16, HPV-18), associated with lesions such as squamous intraepithelial neoplasia (SIN) which can progress to anogenital cancer. Approximately 85% of all cervical carcinomas are conservatively estimated to be high-risk HPV-positive. Epidemiological studies have shown that infection with a high-risk HPV is a significant risk factor for developing cervical neoplasia and cancer [reviewed in 2].

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INTEGRATION OF VIRAL DNA INTO THE HOST GENOME DURING CARCINOGENIC PROGRESSION

In benign lesions associated with HPV infection, viral genomes are maintained in an episomal state. In contrast, viral DNA is frequently integrated in the host cellular genome in anogenital carcinomas. The process of integration is an irrevocable step in the life cycle of the virus. The integration is stable, with no mechanism for specific excision of the viral DNA; often viral integration is accompanied by deletions and rearrangements of the HPV DNA. Viral integration has no consistent locus in the host genome, although in some cases HPV integration may occur in the proximity of cellular protooncogenes [3]. There is, however, a striking pattern of integration with respect to the viral genome, which frequently disrupts the E1 and/or the E2 open reading frames (ORFs). This compromises the integrity of the HPV genome and annuls the tight regulation of viral gene expression, the normal function of the regulatory proteins encoded by E1 and E2. The E2 gene encodes a DNA binding protein with transcriptional targets in the

viral regulatory region. The main activity of the high-risk HPV E2 protein is to repress transcription at the viral promoter that directs the expression of the E6 and E7 oncogenes [reviewed in 4]. In agreement with this model, expression of the HPV E2 protein decreases cellular transformation and immortalization in several experimental scenarios. As an additional consequence of viral DNA integration, the HPV E6 and E7 genes are consistently expressed in cervical cancers; the major mRNA species detected in cervical carcinoma cells are polycistronic with the capacity to encode full length or internally spliced versions of E6 (designated E6*), as well as the full length E7 protein. Tissue culture experiments indicate that continued expression of E6 and E7 is required to maintain the transformed phenotype [reviewed in 2].

HIGH RISK AND LOW RISK CLASSIFICATION CORRELATES WITH *IN VITRO* TRANSFORMATION

Over the last several years, a number of tissue culture systems have become available to assay the transforming functions of HPVs. Interestingly, the transforming activity of a given papillomavirus type *in vitro* correlates with the clinical association of low risk and high risk. This is true for focus formation in established rodent fibroblast cell lines and in *ras* cooperation assays in baby rat kidney cells. The finding that high-risk but not low-risk HPVs can immortalize squamous genital epithelial cells are potentially relevant findings in HPV biology. A genetic analysis revealed that both E6 and E7 proteins were required for keratinocyte immortalization, and that both E6 and E7 genes had to be derived from a high-risk HPV type for immortalization to occur [reviewed in 5]. These observations agree with more recent studies which demonstrate that the E6 and E7 genes of high-risk but not low-risk HPVs can efficiently abrogate certain cell cycle checkpoints and induce genomic instability [6].

HIGH-RISK HPV E6 PROTEINS TARGET THE p53 TUMOR SUPPRESSOR PROTEIN FOR DEGRADATION

A biochemical mechanism for the high-risk HPV E6 protein's biological activities was provided by the discovery that they can specifically interact with the p53 tumor suppressor protein (Fig. 1) [7]. In agreement with the low levels of wild type p53 detected in cervical carcinoma cells or in HPV immortalized cell lines [7,8], interaction between p53 and high-risk HPV E6 protein results in the rapid degradation of p53 through the ubiquitin-mediated proteolysis pathway [9]. As a consequence, the metabolic half-life of p53 in HPV E6-expressing cells is markedly decreased [10]. Biochemical studies showed that the interaction between E6 and p53 is mediated by a 100 kD host cellular protein designated E6-AP [11]. The complex of E6 and E6-AP is directly involved in the ubiquitination reaction of p53 and acts as a ubiquitin ligase (E3 activity) [12]. The E6/E6-AP-mediated ubiquitination and functional inactivation of p53 is mediated by a ubiquitin cascade with the E6/E6-AP complex directly conjugating a ubiquitin moiety on p53 [13].

Part of the function of p53 as "the guardian of the human genome" [14] is to "sense" cellular DNA damage and prevent division of compromised cells. Inactivation or mutation of the p53 checkpoint is predicted to allow the continued replication of cells with damaged DNA and is manifested by increased genomic instability (Fig. 1). Indeed, primary human cells which express the high-risk HPV E6 oncoprotein do not arrest at the G_1/S boundary after DNA damage and show enhanced genomic instability [15–17]. This directly and causally implicates the high-risk HPV E6 oncoproteins in establishing genomic instability, a hallmark of tumorigenic progression of cervical lesions.

HPV E7 PROTEINS FUNCTIONALLY INACTIVATE THE RETINOBLASTOMA TUMOR SUPPRESSOR PROTEIN

The HPV E7 genes encode small zinc-binding nuclear phosphoproteins. The high-risk HPV E7 genes function as oncogenes in a number of transformation assays, including focus formation in NIH/3T3 cells, and they can cooperate with *ras* to induce transformation of primary baby rat kidney cells. HPV E7 proteins function as transcriptional activators of several viral and cellular promoters [review in 5]. Insight into the biochemical basis for some of these biological activities came from observing that the amino terminal half of E7 contains a binding site for pocket pro-

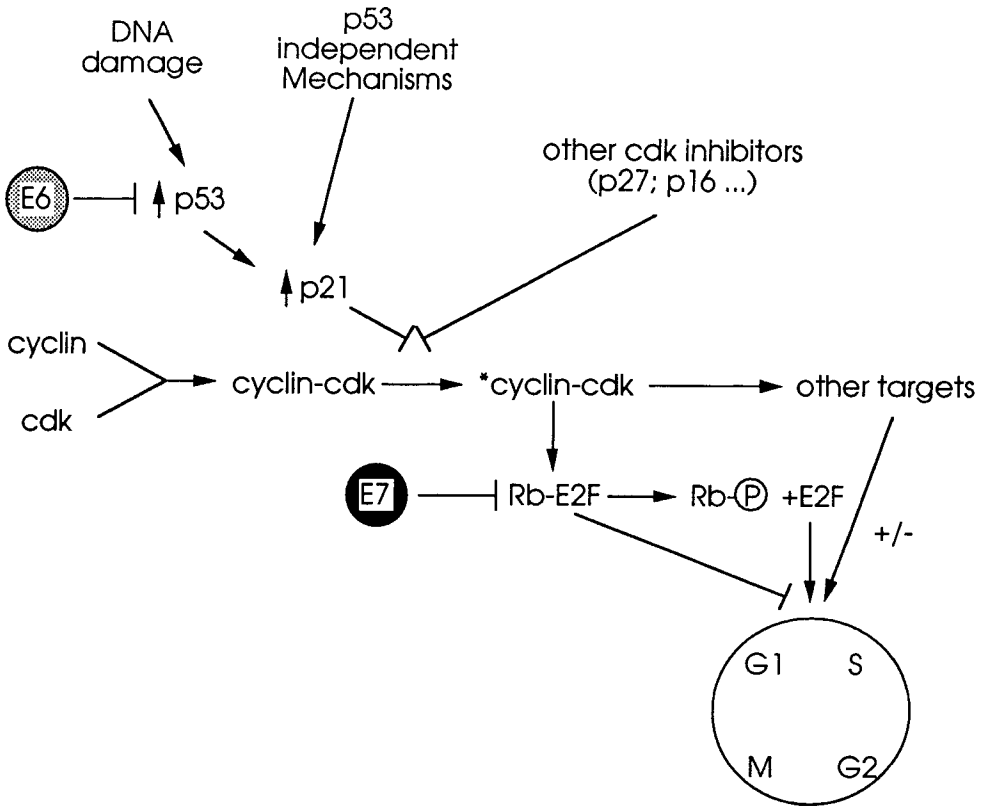

Fig. 1. A schematic representation of some critical regulatory steps of cell cycle progression in mammalian cells targeted by high-risk human papillomaviruses. High-risk HPV E6 proteins interact with the p53 tumor suppressor protein and induce its degradation by the ubiquitin-dependent proteolysis system. HPV E7 proteins form complexes with the retinoblastoma protein pRB (and pocket proteins p107 and p130). By disrupting complexes of pRB with members of the E2F family of transcription factors, they interfere with regulation of the transcriptional activity of E2F, which plays an important role in the temporal regulation of G_1/S cell cycle progression.

teins, a family of structurally related cellular proteins which includes the retinoblastoma tumor suppressor protein pRB, p107 and p130 (Fig. 1).

Members of the E2F family of transcription factors are downstream modulators of pocket protein function. When bound to the G_0/G_1-specific hypophosphorylated form of pRB, E2F acts as a transcriptional repressor. Upon G_1/S-specific phosphorylation of pRB, the E2F/pRB complex is disrupted and E2F has a transcriptional activation function. The delicate balance and tight regulation of E2F's transcriptional activity by the pocket proteins is thought to be a key step for ordered G_1/S transition (Fig. 1) [reviewed in 18]. Disruption of this regulatory network by HPV E7 oncoproteins is regarded as a critical step in carcinogenic progression. In agreement with this model, overexpression of E2F has led to cell cycle progression and morphologic changes characteristic of cellular transformation [19–21]. In cells with an intact p53 pathway, overexpression of E2F induces apoptosis [22,23]. In HPV-positive lesions, the p53 pathway is not functional due to high-risk HPV E6-mediated p53 degradation. The functional abrogation of this pathway by E6 is conditional; targeting the E6 oncoprotein should make it possible to reestablish p53 function, which in turn may lead to reconstitution of the apoptotic pathway triggered by E2F.

ADDITIONAL BIOLOGICAL FUNCTIONS AND PHYSIOLOGICAL TARGETS OF HPV E6 AND E7 ONCOPROTEINS

The majority of studies on high-risk HPV E6 and E7 protein have focused on interactions with p53 tumor suppressor protein and pocket proteins, in

particular pRB, respectively. Many laboratories are defining additional cellular targets of high-risk HPV E6 and E7 oncoproteins using various biochemical and genetic screening methods. Studies with the HPV E6 proteins have been hampered by the absence of definitive structure-function studies. Cellular proteins associated with E6 have been identified, but with the exception of the E6-AP protein [11], their identity and biological importance is unknown [24].

In contrast, the HPV E7 oncoprotein has a modular structure which allows exhaustive mutagenic analyses in an effort to define which domains are important for the multiple biological and biochemical properties of E7. An intact, high-affinity pRB binding site was found to be critical for many of the biological and biochemical properties of E7 [reviewed in 5]. Recent biological and biochemical studies, however, have provided clear evidence for the presence of additional regions which, independent of or in addition to pRB binding, are important for E7's functions in cellular immortalization and transformation. These findings include: pRB binding-deficient E7 mutants, in cooperation with E6, are functional for cellular immortalization of primary human genital epithelial cells, the normal host cells of the high-risk HPVs [25]; pRB binding-deficient E7 mutants of the cotton tail rabbit papillomavirus are still able to contribute to the induction of warts in rabbits [26], a process dependent on E7 expression [27]; mutations in the CR1 homology domain of E7 decrease its transformation potential independent of pRB binding [28]; and the carboxyl terminal domain of E7 is required for the disruption of pRB/E2F-1 complexes [29,30]. The definition of additional cellular targets of E6 and E7 may provide new pharmacological targets for prevention and/or therapy of HPV-associated disease and cancer.

ADDITIONAL GENETIC ABNORMALITIES IN CERVICAL CANCERS

Only a small percentage of women infected with high-risk HPVs eventually develop clinical symptoms, including cervical carcinoma. This implies that infection with a high-risk HPV constitutes only one step in cervical carcinogenesis; additional cellular events are likely to be necessary to the develop of cervical carcinoma. As pointed out above, the deregulated expression of high-risk HPV oncogenes as a consequence of viral integration may be mechanistically related to chromosomal abnormalities observed in cervical cancer. Moreover, inactivation of HPV oncogenes may provide a mechanism to reactivate apoptotic pathways and cellular surveillance, resulting in the elimination of abnormal cancer cells.

Cytogenetic analysis of a small number of cervical carcinomas showed all had undergone loss of heterozygosity (LOH) on the short arm of chromosome 3 [31]. Interestingly, small cell lung carcinoma (another type of cancer where pRB and p53 are frequently mutated) may contain lesions in the same region of chromosome 3p (3p21) [32]. The presence of recessive host cell mutations in cervical carcinomas was also detected by studies with somatic cell hybrids [33], where hybrids of normal human cells and cervical carcinoma cell lines reverted to a non-tumorigenic phenotype. Analysis of tumorigenic revertants of these fusions, as well as microcell fusion studies, have genetically mapped a locus on human chromosome 11 [34,35]. The biological function and importance of this locus in carcinogenic progression is not clear. According to one model, some of these factors may control aspects of HPV transcription [reviewed in 36]. Disruption of this locus may further contribute to the deregulation of HPV gene expression during carcinogenic progression. The long latency period between a precursor lesion and development of cervical carcinoma often observed in patients may therefore be explained by the increased genomic instability in a lesion caused by high-risk HPV E6/E7 gene expression. Only a small number of the resulting chromosomal aberrations provide a growth advantage to a cell and give rise to clonal expansion and tumorigenic progression. Epidemiologic studies have clearly shown that infection with a high-risk HPV type constitutes the major risk factor for developing cervical cancer. The viral oncoproteins E6 and E7, therefore, provide promising drug targets for prevention and therapy of HPV-associated anogenital precursor lesions as well as cancers.

ACKNOWLEDGEMENTS

This work is supported by grants from the American Cancer Society (#VM-97) and the

Council for Tobacco Research (#3859). I am grateful to Ann Frenning for her editorial assistance.

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