Focus on endometrial and cervical cancer

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Introduction

Two of the most common malignancies of the female genital tract are endometrial and cervical cancer. They are separate entities with different epidemiology, pathogenesis, and histology, but are linked by their origin in the uterus. Although they have garnered less attention in developed countries than ovarian carcinoma, it is important to recognize that endometrial carcinoma is the most common female genital tract malignancy in the United States, and that cervical carcinoma is the second most common cancer in women worldwide. Thus, these are highly prevalent cancers, and their further study has the potential for significant beneficial impact on women's health on an international level.

(1) Endometrial cancer

Epidemiology and incidence statistics

Malignancies that arise in the endometrium are collectively referred to as endometrial cancer. However, the term is often used synonymously with endometrial carcinoma, as it accounts for more than 95% of endometrial cancers. Although the data on incidence and outcome refer to endometrial cancer, due to the preponderance of endometrial carcinoma, the numbers largely reflect its incidence and outcome. In the United States, endometrial cancer is the eighth most common cause of cancer deaths and the fourth most common cancer in women. It is also the most common malignancy of the female genital tract. The American Cancer Society predicts that 40,320 women will be diagnosed with endometrial cancer in 2004 and that 7,090 will die of the disease. What is not evident from these data is the large number of hysterectomies performed to treat the noninvasive precursors of endometrial carcinoma, which contribute significantly to the morbidity related to endometrial neoplasia. Overall, endometrial cancer has an 86% five-year survival rate. This almost certainly reflects the fact that the disease usually comes to the attention of women early because of abnormal vaginal bleeding, and they are treated with a hysterectomy before the disease has spread beyond the uterus. Unfortunately, if it has extended outside the uterus, neither radiation nor chemotherapy significantly increases long-term survival.

Clinicopathologic characteristics and therapeutics

The endometrium is a complex tissue that lines the uterine cavity and is composed of both glandular and stromal elements. The cyclic proliferation, differentiation, and sloughing of both components is exquisitely controlled and coordinated by steroid hormones produced by the ovary. In general, estrogen promotes the proliferation and progesterone the differentiaton of both components. Endometrial carcinoma arises from the epithelial component and has historically been thought of as a single disease. However, in 1983, a clinicopathological study suggested that endometrial carcinoma could be broadly divided into two major types, referred to as type I and type II (Bokhman, 1983). These findings have now, for the most part, been accepted and have provided a logical paradigm for studying endometrial carcinoma (Figure 1).

Type I carcinomas are associated with a constellation of clinical findings (obesity, hypertension, diabetes) and a hyperestrogenic state. The tumors are in general low-grade (i.e., well to moderately differentiated), low-stage (confined to the uterus), indolent tumors often associated with endometrial hyperplasia. If left untreated, endometrial hyperplasia, a proliferative process of the endometrial glands, can progress to carcinoma (Kurman et al., 1985). Moreover, Type I tumors are of endometrioid histology, that is, they resemble normal proliferative endometrial glands. These tumors are usually treated with surgery with or without radiation depending on the stage of the tumor. If the tumors are metastatic, adjuvant chemotherapy is used, but this results in little, if any, improvement in survival.

Type II tumors are found in the setting of atrophy and are not associated with unopposed estrogen. They are poorly differentiated tumors and behave in an aggressive manner. The most common histologic tumor type associated with type II tumors is serous carcinoma; however, poorly differentiated endometrioid and clear cell tumors are generally placed in this category. Serous carcinoma is composed of markedly atypical cells that can grow in papillary, glandular, or solid patterns. The precursor of serous carcinoma, endometrial intraepithelial carcinoma (serous EIC), consists of cells resembling those of serous carcinoma that line the surface of atrophic endometrium but do not invade the underlying stroma. The aggressive nature of this neoplastic process is highlighted by the fact that serous EIC, even in the absence of a detectable invasive component, often presents with associated peritoneal metastases. Since the recognition of the similarities between this tumor type and ovarian carcinoma (e.g., early peritoneal spread, p53 mutations, etc.), it is most commonly treated with chemotherapeutic regimens used for ovarian cancer.

Molecular pathogenesis

Endometrioid carcinoma. Endometrioid carcinoma accounts for approximately 80% of all cases of endometrial cancer and is divided into three grades based on histologic criteria. Grades 1 (well differentiated) and 2 (moderately differentiated) are the most common and fall into the Type I category, whereas Grade 3 tumors are often thought of as Type II tumors because they portend a poor prognosis.

The *PTEN* tumor suppressor gene is the most frequently altered gene in this tumor type, with mutations present in approximately 50% of tumors (Tashiro et al., 1997a). In addition, mutations have been detected in approximately 20% of hyperplastic lesions, suggesting that mutations in *PTEN* occur relatively early in the pathogenesis of endometrioid carcinoma (Levine et al., 1998; Maxwell et al., 1998). There is a wide spectrum of mutations of *PTEN* in endometrioid carcinoma, which primarily occur

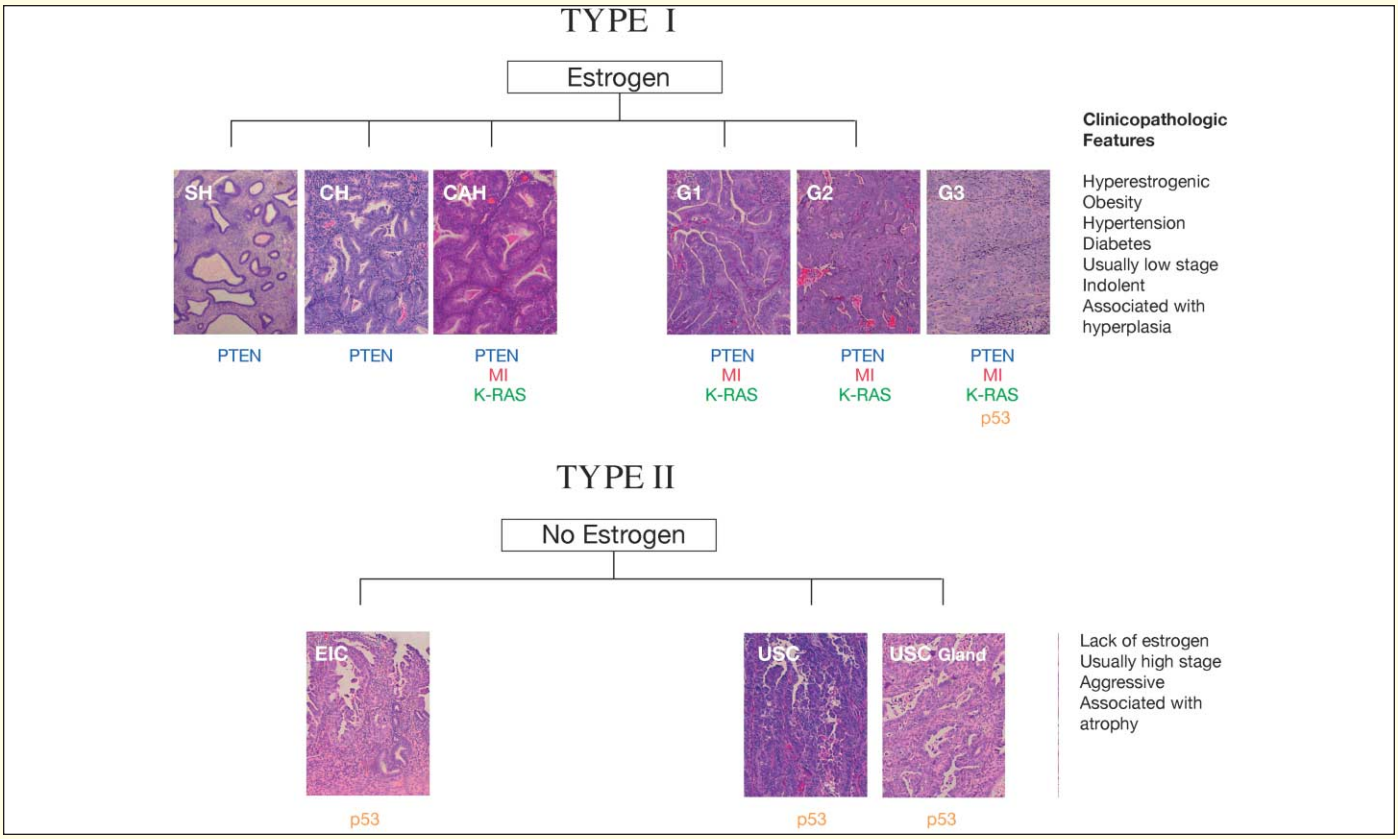

Figure 1. Histological and molecular characteristics of endometrial lesions

The precursor lesions are shown on the left and the invasive tumors on the right. The common molecular genetic alterations found in each morphologic entity are shown directly below each photomicrograph. SH, simple hyperplasia; CH, complex hyperplasia; CAH, complex atypical hyperplasia; G1, Grade 1 endometrioid carcinoma; G2, Grade 2 endometrioid carcinoma; G3, Grade 3 endometrioid carcinoma; EIC, endometrial intraepithelial carcinoma; USC, papillary form of serous carcinoma; USC glandular, glandular form of serous carcinoma.

in exons 3, 4, 5, 7, and 8, targeting the phosphatase domain and regions that control protein stability and localization. Many of the mutations result in decreased or absent expression of PTEN, documented by both Western blot analysis and immunohistochemistry. One study has found loss of PTEN expression in clusters of morphologically benign glands, suggesting that *PTEN* mutation may precede the development of detectable light microscopic lesions (Mutter et al., 2000). Other data have suggested that epigenetic mechanisms (e.g., promoter hypermethylation) and subcellular localization can affect PTEN function in the absence of intragenic mutations.

Microsatellite instability (MI) was first detected in tumors arising in individuals with hereditary nonpolposis colorectal cancer (HNPCC). Since endometrial carcinoma is the second most common tumor in women with HNPCC, MI studies were performed, and MI was detected in approximately 25% of sporadic endometrial cancers (Gurin et al., 1999). Unlike the familial cases in which the affected member carries a germline mutation in one of the DNA mismatch repair genes, *hMLH1* promoter hypermethylation is the predominant cause of MI in sporadic cases. MI can be found in complex atypical hyperplasia associated with invasive cancer, but it has not been found in lesser degrees of hyperplasia (Levine et al., 1998). However, promoter hypermethylation of *hMLH1* has been detected in hyperplasia lacking an associated carcinoma, suggesting that inactivation of mismatch repair precedes the development of MI (Esteller et al., 1998). Although it remains unclear exactly when in the development of endometrial neoplasia the DNA mismatch repair system becomes inactivated, these findings suggest that it may occur early in its pathogenesis. Several studies have reported a statistically significant association between *PTEN* mutations and MI. The mechanism(s) underlying the association is uncertain, but recent data suggest that it has biological significance. The exact mechanisms by which DNA mismatch repair inactivation contributes to the development of endometrioid carcinoma remain largely unknown, but PTEN may represent one possible target. Answers to this question are not only of biological interest, but may also have important clinical ramifications in predicting which lesions are at risk of progression and require future focused investigation.

The *p53* tumor suppressor gene has received considerable attention in endometrial cancer, as it has in most tumor types, and along with other genetic alterations has provided important pathogenetic information for distinction between the two types of endometrial carcinoma. Mutations in *p53* are found in approximately 10%–20% of all endometrioid carcinomas, the majority occurring in high-grade tumors (Lax et al., 2000). Approximately 50% of Grade 3 tumors, and rare Grade 2 tumors, contain p53 mutations, but they have not been identified in Grade 1 tumors or endometrial hyperplasia.

The most commonly altered oncogene in endometrial carcinoma is K-ras, which is mutated in 10%–30% of cases. The mutations have been found in all grades of endometrioid carci-

noma and have been reported in complex atypical hyperplasia, suggesting a relatively early role for K-ras mutations in this tumor type. Most recently, mutations in the *CTNNB1* (β catenin) gene have been found in approximately 13% of endometrioid carcinomas, with an accumulation of the protein found in 38% of cases (Fukuchi et al., 1998). This genetic abnormality is closely associated with squamous metaplasia, and a recent article found an associated increase in p53 and cyclin D1 expression in the areas of metaplasia (Saegusa et al., 2004).

Molecular data now exist which suggest that most Grade 2, and at least some Grade 3, tumors have molecular profiles similar to Grade 1 tumors (e.g., *PTEN* and *K-ras* mutations, and MSI). These findings suggest that although Grade 3 tumors have a poor prognosis, like Type II tumors, they can share a similar genetic profile to Grade 1 and 2 tumors.

Serous carcinoma. Serous carcinoma, the prototype of Type II, accounts for only 10%–15% of all endometrial carcinomas. Although a number of cancer-causing genes have been studied, only the p53 tumor suppressor gene is consistently altered in a significant number of cases. Some studies have detected p53 mutations in almost 90% of serous carcinomas (Tashiro et al., 1997b). The same study found that approximately 75% of endometrial intraepithelial carcinomas, the putative precursor of serous carcinoma, also have mutations in p53, implicating a role for its inactivation early in the development of this aggressive tumor type. This is in contrast to endometrioid carcinoma in which p53 mutations are relatively uncommon and are largely confined to Grade 3 tumors. The early inactivation of p53 in the pathogenesis of serous carcinoma may be a factor in its aggressive behavior. In addition, the fact that p53 mutations occur most commonly in Grade 3 endometrioid and serous carcinomas may explain why overexpression and mutation of p53 has been found to be an independent indicator of poor prognosis in several multivariable analyses. In cases in which the morphology is not certain, immunohistochemical stains for p53 can be used to make the distinction between endometrioid and serous carcinoma, a distinction that has true clinical consequences.

In contrast to endometrioid carcinoma, mutations in *K-ras* and *PTEN* are rare in serous carcinoma. Additionally, microsatellite instability is extremely uncommon. Scattered studies have found amplification and overexpression of c-*myc* and Her-2/neu; however, it is not clear from the literature what percentage of serous carcinoma demonstrate these alterations.

The molecular studies of endometrial carcinoma, like with other epithelial-derived tumors, support their development from preinvasive lesions via the accumulation of genetic alterations providing the cell with the attributes necessary for unregulated growth. In endometrioid carcinoma, *PTEN* alterations and microsatellite instability occur early and set the stage for the acquisition for mutations in other cancer-causing genes (e.g., *K-ras*, *p53*, *CTNNB1*, etc.) in the progression to malignancy. Conversely, p53 mutations appear to be critical in the conversion of relatively quiescent, atrophic endometrium to an intraepithelial form of serous carcinoma that then leads to alterations in yet unidentified cancer-causing genes. Finally, although the dualistic model is valid, it may not adequately encompass the more uncommon types of endometrial carcinoma. Future studies will hopefully increase our understanding of the pathogenesis of endometrial carcinoma and refine the current broad classification system.

Animal models

As described above, several studies have shown that MI and mutations in *PTEN* are restricted, with rare exceptions, to the estrogen-related endometrioid subtype of endometrial cancer. Furthermore, an association between MI and *PTEN* mutations, the most common alterations in endometrioid carcinoma, has been identified, but the nature of the association remains unclear.

Several models have recently been developed using transgenic approaches to regulate the expression of *Pten* and *Mlh1*. These mice develop both hyperplastic as well as invasive carcinoma that closely resemble the human disease. Furthermore, $Pten^{+/-}$ mice lacking a functional DNA mismatch repair system ($Pten^{+/-}/Mlh^{-/-}$) have an accelerated onset of hyperplasia and carcinoma (Wang et al., 2002). This model may provide mechanistic insights into the association between the two most common alterations in endometrioid carcinoma. An analysis of the neoplastic lesions arising in both $Pten^{+/-}$ and $Pten^{+/-}/Mlh^{-/-}$ mice

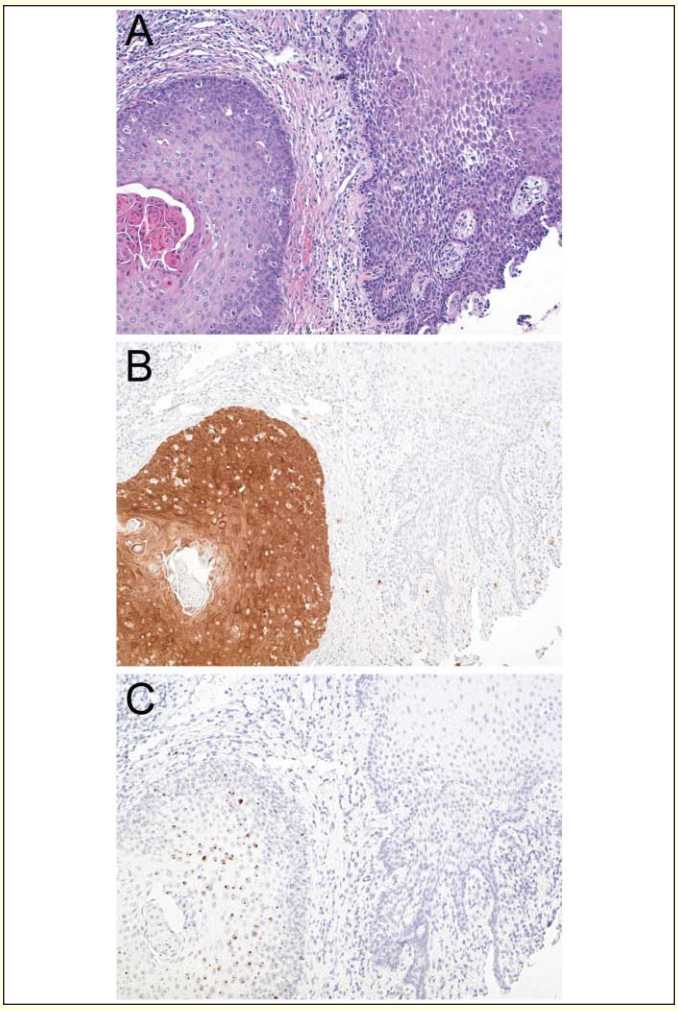

Figure 2. HPV-infected malignant cells displaying upregulated $p16^{INK4A}$

Staining was performed on consecutive sections.

A: H&E stain of a lesion biopsied from a patient with squamous cell carcinoma of the cervix. The biopsy shows an area of malignant tumor cells separated from benign squamous epithelium by a region of normal stromal tissue. The tumor cells show abnormal squamous differentiation.

B: Immunohistochemical stain for $p16^{INK4A}$. p16-positive areas stained brown within the tumor.

C: HPV in situ hybridization. Presence of HPV-16 genomes was indicated by a brown, speckled pattern within the nuclei of tumor cells.

shows that the lesions (hyperplasias and carcinomas) demonstrated decreased expression of Pten by immunohistochemistry. Furthermore, in every lesion detected by light microscopic evaluation, irrespective of size or architecture, phosphorylated Akt was detected by immunohistochemical analysis. Although other downstream targets in the Pten pathway may play a role in the development/progression of endometrial tumorigenesis, these studies indicate a central role for phosphorylated Akt.

(2) Cervical cancer

Epidemiology and incidence statistics

Worldwide, cervical cancer is the second most common malignancy among women, with nearly 80% of cases arising in less developed countries. There are approximately 500,000 diagnoses of cervical cancer per year worldwide, leading to approximately 200,000 deaths each year. Based on the 2002 estimates of the American Cancer Society, approximately 13,000 cases of cervical cancer were diagnosed in the United States, and approximately 4,100 women will die as a result (Jemal et al., 2002). The primary factor in the development of cervical cancer is infection by the human papillomavirus (HPV). Approximately 90% to 98% of cervical cancers are associated with HPV infection, which is common and is acquired mainly through sexual contact (for review see Waggoner, 2003).

There are more than one hundred known genotypes of HPV, but only some are associated with infection of the genital mucosa. Genital HPV types can be classified into "high-risk" and "low-risk" varieties, based on the frequency of their identification in cervical cancers. Low-risk types (e.g., HPV 6 and 11) are associated with benign proliferative growths such as genital warts. High-risk HPVs can cause cervical intraepithelial neoplasia (CIN) and, if left untreated, may lead to cervical cancer (for review see zur Hausen, 2002). The majority of CIN 2-3 are caused by HPV types 16 and 18, but several other types are also considered high-risk. Most HPV infections are clinically benign and are cleared naturally, but approximately 3%–10% of women cannot clear their HPV infections. These women become HPV carriers and are at high risk for developing cervical cancer.

This strong association between HPV and cervical cancer applies to both squamous cell cancers and adenocarcinomas. In addition, most cases that have initially appeared to be HPV-negative have later been identified as false negatives. These findings have led to the claim that HPV infection is a necessary cause for cervical cancer, providing a strong argument for HPV screening programs and the development of HPV vaccines. While HPV stands out as one of the most important etiological contributors to cervical cancer, studies have identified other factors that may play a role. These include use of oral contraceptives, smoking, multiple pregnancies, and coinfection with chlamydia and/or herpes simplex virus-2 (for review see Monsonego et al., 2004).

Screening, diagnostics, and therapeutics

Identification of cervical precancerous lesions is the primary goal of cervical cancer screening. These precursor lesions of cervical cancer can be classified into low-grade squamous intraepithelial lesions (LSIL), equivalent to CIN1, and high-grade squamous intraepithelial lesions (HSIL), equivalent to CIN2-3. LSIL have a high percentage of spontaneous regression, whereas HSIL are more likely to develop into cervical cancer if left untreated. Therefore, screening methods should focus on detecting squamous intraepithelial lesions, particularly HSIL. However, the diagnostic reproducibility of SIL represents a substantial barrier to execution of robust translational studies of precursor lesions of cervix. The most widely used screening approach to detect HSIL is conventional cervical cytology, usually via Pap smear, followed by investigation of positive women with colposcopy and directed biopsy. A definitive diagnosis of cervical cancer or its precursor lesions requires a biopsy of the suspicious lesion. Once a tissue diagnosis of invasive carcinoma has been confirmed, the patient should be staged. Staging is determined clinically at the time of primary diagnosis and is determined mainly based on the size of the tumor in the cervix or its extension into the pelvis. The Federation Internationale de Gynecologie et d'Obstetrique (FIGO) defines clinical stages of cervical cancer as stages 0, IA1, IA2, IB1, IB2, IIA, IIA, IIIA, IVA, or IVB based on tumor size, extension of the tumor, involvement of regional lymph nodes, and presence of distant metastases.

The treatment options for cervical cancer are based on the outcome of clinical staging and are generally composed of surgery, radiotherapy, chemotherapy, and/or chemoradiotherapy. If the cervical cancer lesion is small and well defined (stages IA1–IB1), surgery and/or radiotherapy are probably sufficient to control the cancer, and the five-year survival rate is very good. For patients with larger tumors and significant vaginal involvement (stages IB2–IIA), more extensive surgery and/or concomitant chemotherapy and radiotherapy (chemoradiotherapy) are necessary to provide a reasonable chance for five-year survival. However, if tumors extend beyond the cervix (stages IIB–IVA), chemoradiotherapy should be considered, as several randomized clinical trials have shown significant improvement in time to progression and survival for patients given chemoradiotherapy compared to radiotherapy or chemotherapy alone (Morris et al., 1999; Whitney et al., 1999). For patients with advanced cervical cancer (stage IVB, recurrent or refractory disease), palliative chemotherapy and/or chemoradiotherapy may provide some chance for five-year survival (for review, see Waggoner, 2003).

Disease mechanism and molecular targets

Numerous studies have indicated that HPV infection is necessary for the development of cervical cancer. It is therefore important to understand the biology of HPV infection in order to describe the molecular pathogenesis of cervical cancer. HPV initially infects basal cells of mucosal or epidermal epithelium. All HPV types have a circular, double-stranded DNA genome containing about 8,000 base pairs and encoding two classes of proteins: "early" proteins, which regulate viral DNA replication (E1, E2), RNA transcription (E2), and cell transformation (E5, E6, E7); and "late" proteins (L1, L2), the structural components of the viral capsid. The expression of these proteins is tightly regulated and coupled with the differentiation status of the infected squamous epithelial cells. As differentiating cells move toward the more superficial cell layers, virus assembly takes place. The viral DNA ordinarily replicates extrachromosomally. In HPV-associated malignant transformation, viral DNA may be integrated into the cellular DNA, and integration often results in deletion of large sectors of the viral genome. Late genes (L1 and L2) and some early genes (such as E2 and E5) are usually lost, leaving E6 and E7 as the principal open reading frames found in carcinomas (for review, see zur Hausen, 2002).

Among the early genes, E6 and E7 are the two most renowned, oncogenic proteins, since they are consistently expressed by HPV-associated malignancies and are responsible for stimulation of proliferation. Evidence has shown that they can immortalize cells efficiently when expressed in concert dur-

ing natural HPV infection. This is supported by data showing that inhibition of E6 and E7 expression can block the malignant phenotype of cervical cancer cells. Expression of E6 leads to unregulated cell growth by enhancing degradation of the tumor suppressor p53 and proapoptotic BAK. E6 expression has also been shown to contribute to stimulation of growth through activation of telomerase and inhibition of degradation of SRC family kinases. E7 expression leads to degradation of pRb, resulting in upregulation of $p16^{INK4A}$, a cell cycle regulatory gene. Figure 2 shows HPV-infected malignant cells displaying upregulated $p16^{INK4A}$. Furthermore, E7 stimulates the S phase genes cyclin A and cyclin E and appears to block the function of cyclin-dependent kinase inhibitors KIP1 (p27) and WAF1 (CIP1 or p21). These functions of E7 further break down regulation of cell growth. E7 has also been shown to induce aneuploidy in E7-expressing cells, which contributes to tumorigenesis. E6 and E7, capable of inducing cellular immortalization individually, function more efficiently when simultaneously expressed. E7 is able to counteract $p16^{INK4A}$ inhibition of E6, and E6 prevents E7-triggered apoptosis. Thus, coexpression of E6 and E7 significantly increases transforming activity (for review see zur Hausen, 2002).

Because E6 and E7 are vital for oncogenesis and are constantly expressed in most HPV-associated cervical cancers, they represent ideal molecular targets for development of strategies to intervene in the progression and treatment of cervical cancer, including therapeutic vaccine development. There may be other molecular targets, however, that are important for development of effective intervention strategies. Most HPV infections regress spontaneously, and the reasons for progression or regression of lesions remain unclear at this point. Identification of mechanisms determining progression or regression of lesions will not only provide us with a better understanding of this disease but also provide potential molecular targets for intervention.

Animal models

There are several HPV-16 E6/E7 transgenic mouse models currently available (Arbeit et al., 1996; Lambert et al., 1993). For example, Elson et al. have reported that using a low dose of estrogen in E6/E7-transgenic mice led to carcinogenesis in the transformation zone of cervix, resembling carcinogenesis of cervical cancer as observed in humans (Elson et al., 2000). These transgenic mice could serve as a valuable preclinical model for preventive and therapeutic intervention aimed at these potential molecular targets.

Recent advances in screening and HPV vaccine development

Although the Pap smear is an important procedure historically and has had an enormous impact on the incidence of cervical cancer, studies suggest that the sensitivity of the Pap smear is low (50% to 60%) and that the procedure has a high propensity for error (Fahey et al., 1995). Two cervical screening methods have recently been developed that may compensate for this lack of sensitivity. One of these is liquid-based cytology, also known as a thin-layer preparation. Recent reviews have concluded that liquid-based cytology is more cost-effective and more sensitive than conventional smear-based cytology and that it should therefore be adopted for cervical cancer screening (for review, see Monsonego et al., 2004). Another alternative to the Pap smear is HPV testing. Recent studies have demonstrated HPV testing to be more sensitive than cytology alone and that the combination of cytology and HPV testing results in a negative predictive value of >99% (Petry et al., 2003; Sherman et al., 2003). As a result, HPV testing has recently been approved for use in some clinical settings in the United States. HPV testing may one day supplant cytology as the primary screening method for cervical cancer and its precursor lesions.

Since HPV infection precedes and plays an essential role in the development of cervical cancer, vaccines targeting HPV-encoded proteins may serve as feasible approaches to controlling cervical cancer (for review see Frazer, 2004; Roden and Wu, 2003). There are two approaches to HPV vaccine development. The first seeks to prevent the virus from establishing infection in the epithelium, mainly through the induction of neutralizing antibodies against viral capsid proteins. A prophylactic vaccine should effect the complete neutralization of free virus upon exposure before infection can occur. However, this may be of lesser benefit to individuals who have an established infection and HPV-associated lesions. The second approach to vaccine development addresses the needs of these patients by attempting to induce a cellular immune response, which may be able to prevent neoplastic lesions and induce their regression. This is antigen-specific immunotherapy, in which effector cells, particularly T cells, are primed against HPV antigens expressed by the neoplastic cells (tumor-specific antigens), such as E6 and E7. Finally, prophylaxis and therapy can be combined into one vaccine, in an attempt to provide total coverage for people newly exposed to high-risk virus and for people with current infections and HPV-associated lesions. Preventive vaccines currently in phase II/III trials include subunit vaccines composed of HPV virus-like particles (VLPs) generated by self-assembling of the major capsid protein L1. These trials reveal highly encouraging data without severe side effects (Harro et al., 2001). High titers of type-specific neutralizing antibody were observed in vaccinated individuals. More importantly, the trials suggest that a VLP vaccine can prevent establishment of HPV infection and therefore has the potential to prevent cervical cancer (Koutsky et al., 2002). HPV therapeutic vaccine trials have shown that E6 and E7 proteins, and some peptides derived from these proteins, are immunogenic in humans. No significant side effects were observed in these trials, but no definitive therapeutic efficacy has been observed in patients receiving the vaccines.

Future challenges

Endometrial cancer

In the future, a combination of approaches will be necessary to further advance our knowledge of endometrial carcinoma so that we can have a meaningful impact on women who are faced with this diagnosis. Not only do we need to develop novel effective therapeutic approaches for women with advanced stage disease through the model systems that are currently being created, but the interaction of genetics and hormones needs to be thoroughly elucidated so that we can determine who is at risk for the development of invasive disease. Again, the mouse models will play a unique role in this undertaking. However, eventually, what is learned in the mouse models must be confirmed in humans through the continued study of primary human tumors and the women with this disease. It is only with the combination of approaches that we will be able to change the incidence and outcome of this common malignancy of women.

Cervical cancer

Significant progress has been made toward understanding of HPV pathogenesis, innovative screening techniques, vaccine development, and effective cervical cancer therapies. However,

many challenges related to research into HPV pathogenesis remain to be addressed: (1) characterization of risk factors leading to persistent HPV infection, (2) identification of biomarkers that contribute to the risk of HPV-positive women for progression to HSIL and cervical cancer, (3) elucidation of the roles of known and suspected environmental cofactors, (4) determination of the relevance of the different strains and variants of HPV viral types, and (5) characterization of other important factors, such as the immune responses against HPV infections and the interaction between the host and virus. Regarding screening for cervical cancer, further research should be focused on determining how new cervical screening technologies (such as HPV testing and liquid based cytology) compare to conventional screening systems for reducing cervical cancer incidence and mortality in multiple international settings. In terms of HPV vaccine development, more extensive prophylactic vaccine (such as VLPs) trials are necessary to confirm efficacy, duration of protection, and the possibility of crossprotection. Furthermore, therapeutic HPV vaccines should be tested in randomized placebo-controlled clinical trials to demonstrate therapeutic efficacy. Increased understanding of these issues could lead to better management of cervical cancer through control of HPV infection and HPV-associated lesions.

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