Requirement for Estrogen Receptor α in a Mouse Model for Human Papillomavirus–Associated Cervical Cancer

Sang-Hyuk Chung,¹ Kerri Wiedmeyer,¹ Anny Shai,¹ Kenneth S. Korach,² and Paul F. Lambert¹

¹McArdle Laboratory for Cancer Research, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin and ²Laboratory of Reproductive and Developmental Toxicology, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina

Abstract
The majority of human cervical cancers are associated with the high-risk human papillomaviruses (HPV), which encode the potent E6 and E7 oncoproteins. On prolonged treatment with physiologic levels of exogenous estrogen, K14E7 transgenic mice expressing HPV-16 E7 oncoprotein in their squamous epithelia succumb to uterine cervical cancer. Furthermore, prolonged withdrawal of exogenous estrogen results in complete or partial regression of tumors in this mouse model. In the current study, we investigated whether estrogen receptor α (ERα) is required for the development of cervical cancer in K14E7 transgenic mice. We show that exogenous estrogen fails to promote either dysplasia or cervical cancer in K14E7/ERα−/− mice despite the continued presence of the presumed cervical cancer precursor cell type, reserve cells, and evidence for E7 expression therein. We also observed that cervical cancers in our mouse models are strictly associated with atypical squamous metaplasia (ASM), which is believed to be the precursor for cervical cancer in women. Consistently, E7 and exogenous estrogen failed to promote ASM in the absence of ERα. We conclude that ERα plays a crucial role at an early stage of cervical carcinogenesis in this mouse model. [Cancer Res 2008;68(23):9928–34]

Introduction
Cervical cancer, a virally caused cancer, is the second most common cancer and the second most frequent cause of death by cancer in women worldwide (1). The vast majority of cervical cancers are associated with the so-called high-risk human papillomaviruses (HPV), among which HPV-16 is most common, being found in ~60% of all cervical cancers (2). Compelling epidemiologic and experimental evidence has clearly established a causative role of HPV in this human malignancy (2, 3). Specifically, E6 and E7 oncoproteins expressed by high-risk HPV can immortalize primary human keratinocytes and cause cancers in transgenic mouse models in a cofactor-dependent manner (4–7). In addition, E6 and E7 are required for the continued proliferation of cervical cancer cell lines (8, 9). The tumorigenic potential of HPV E6 and E7 oncoproteins depends, at least in part, on their ability to inactivate p53 and pRb tumor suppressor protein, respectively (10–12).

Despite the robust carcinogenic potential of E6 and E7, HPV infection alone is not sufficient for the development of cervical cancer because only a minor fraction of patients infected with HPV develop cervical cancer (13). Indeed, several cofactors, including long-term use of oral contraceptives and high parity, have been implicated in the genesis of HPV-associated cervical cancer, suggesting a potential role of female steroid hormones, such as estrogen, in cervical carcinogenesis (14, 15). Other studies, however, have concluded otherwise. For instance, one observational study argues that estrogen does not increase risk of cervical cancer (16). This study, however, did not control for HPV, a major factor for cervical cancer. Another clinical study shows that antiestrogen tamoxifen has no beneficial effect on cervical cancer (17). This result is not surprising because tamoxifen has an agonistic rather than antagonistic effect on estrogen function in the human cervix (18). Thus, there remains a poor understanding as to the estrogen dependence of cervical cancers in humans.

An essential role of estrogen in cervical cancer, however, has been clearly defined in mouse models for HPV-associated cervical cancer that make use of transgenic mice expressing HPV-16 E6 or E7, or both, under the control of human keratin 14 (K14) promoter, which drives transgene expression in stratified squamous epithelia, natural HPV infection sites. In these mouse models, either a HPV oncogene or estrogen alone is insufficient to cause cervical cancers, whereas a HPV oncogene in conjunction with physiologic levels of exogenous estrogen can promote the development of cervical cancer (4, 11, 19, 20). The progressive cervical disease that arises in these mouse models recapitulates various aspects of human cervical disease, including the multiple stages of cervical carcinogenesis, anatomic location and histopathologic nature of the cancers, and expression patterns of various biomarkers (4, 21). In both HPV-infected women and these mouse models, cervical cancer is preceded by cervical intraepithelial neoplasia (CIN) of increasing severity that arises preferentially in the transformation zone of the endocervix, at which is found the normal transition from columnar epithelium to stratified squamous epithelium (4, 22). The transformation zone is hypothesized to be the preferential site of carcinogenesis by HPV because therein lie the reserve cells, which are thought to be multipotential progenitor cells from which cervical cancer is argued to arise (23).

It is hypothesized that estrogen can contribute to the development of cancers by estrogen receptor (ER)-dependent and ER-independent mechanisms. Estrogen is best known for exerting its physiologic effects by binding and activating its receptors, ERα and/or ERβ (24). The mitogenic effects of estrogen that are mediated through ERα are crucial for the development and maintenance of most breast cancers (25). ERα-positive breast cancers are highly responsive to the therapy with antiestrogen drugs such as tamoxifen and fulvestrant that directly bind and thus inhibit function for ERα (26). The function for ERβ in
breast cancer and other estrogen-dependent cancers is less well understood. The potential ER-independent mechanism involves estrogen metabolites that can function as a direct carcinogen inducing detrimental genetic mutations (27). However, it remains unclear to what extent this mechanism contributes to the role of estrogen in estrogen-dependent cancers.

In the present study, we used ERα knockout (ERα−/−) mice to assess whether ERα is crucial for cervical carcinogenesis in the K14E7 transgenic mouse model. Our results clearly show that ERα is absolutely necessary for the development of estrogen-dependent cervical cancer in this mouse model.

Materials and Methods

Mice. All transgenes in mice used in this study were derived from HPV-16, K14E7 transgenic mice and ERα knockout (ERα−/−) mice were described previously (28, 29). Experimental mice were generated by intercrossing F1 generations of K14E7 (FBV) and ERα−/− (C57BL/6) matings. Female progenies were genotyped by PCR. A slow-releasing 17β-estradiol tablet (0.05 mg/60 days; Innovative Research of America) was inserted s.c. under the dorsal skin every 2 mo beginning at 4 to 6 wk of age. Mice were injected i.p. with 0.3 mL bromodeoxyuridine (BrdUrd; 12.5 mg/mL) 1 h before euthanasia to measure cellular proliferation. Female reproductive tracts were harvested and processed as previously described (20). Mice were housed in McArdle Laboratory Animal Care Unit of the University of Wisconsin Medical School approved by the Association for Assessment of Laboratory Animal Care. All procedures were carried out according to an animal protocol approved by the University of Wisconsin Medical School Institutional Animal Care and Use Committee.

Antibodies and cervical cancer specimens. Antibodies were purchased from Santa Cruz Biotechnology (ERα, E7), Abcam (ERβ), Developmental Studies Hybridoma Bank (K8), NeoMarkers (p63, Mm7), Sigma (β-actin), Calbiochem (BrdUrd), Vector Laboratories (biotinylated horse anti-mouse/rabbit IgG), and Invitrogen (Alexa Fluor 350/488/595–conjugated secondary antibody against rabbit, mouse, or rat IgG). Human cervical cancer specimens used in this study were previously described and genotyped for HPVs (30).

Immunohistochemistry and immunoblot. Immunohistochemical analyses for the detection of Mcm7 and BrdUrd were performed as described previously (7, 10). For staining ERα, ERβ, p63, and K8, standard procedures were followed as previously described (21). Briefly, deparaffinized/rehydrated sections were blocked and incubated with an antibody at appropriate dilution (α-ERα, 1:100 in 5% nonfat milk/5% horse serum; α-ERβ, 1:100 in 3% horse serum; α-K8, 1:50; and p63, 1:150 in 3% bovine serum albumin/10% goat serum). Proteins were visualized by 3,3′-diaminobenzidine (Vector Laboratories) or fluorescent microscopy. Vaginal tissues were lysed in radioimmunoprecipitation assay buffer [50 mmol/L Tris-HCl (pH 8.0), 150 mmol/L NaCl, 1% IGEPAL CA-630, 0.5% sodium deoxycholate, 0.1% SDS] supplemented with protease inhibitors, and immunoblot analyses were performed as described previously (11).

H&E staining. H&E staining was performed as previously described (20).

Statistical analyses. Two-sided Fisher’s exact test and Wilcoxon rank sum test were carried out with MSTAT software version 12.0.0.3.

Results

ERα but not ERβ is detectable in cervical cancers of mice and women. Exogenous estrogen is required for the development and maintenance of cervical cancer in mouse models (19, 20). If ERs are necessary for cervical carcinogenesis in mice, then ERα and/or ERβ should be expressed in the cancers and/or the surrounding stroma. To test this prediction, we stained archival paraformaldehyde-fixed and paraffin-embedded female reproductive tracts of K14E6 or K14E7 single or K14E6/K14E7 double transgenic mice treated with exogenous estrogen for 6 or 9 months for ERα or ERβ (11, 19). Expression of ERα was evident in the basal and suprabasal cells of normal cervical epithelia (Fig. 1A) and stromal cells (Fig. 1A, inset). Results also showed that 94% of cancer cells and 57% of stromal cells surrounding the cancers were positive for ERα in both K14E6 and K14E7 single transgenic mice (Fig. 1A). In contrast, we failed to detect ERβ in cancers and normal cervical epithelia as well as the surrounding stroma (Fig. 1A). ERβ was readily detected in mouse ovary (data not

Figure 1. ERα but not ERβ is detected in cervical cancers. A, ERα is expressed in murine cervical cancers. Archival paraformaldehyde-fixed and paraffin-embedded female reproductive tracts of indicated mice treated with 17β-estradiol were stained for ERα (top) or ERβ (bottom). Inset, stromal cells stained for ERα. Shown are representatives of more than six cancers in each transgenic mouse. More than 700 cancer cells in at least three different areas of each cancer were examined for positive staining. Nuclei were counterstained with hematoxylin. Black lines, basement membrane separating epithelium from underlying stroma. B, ERα is expressed in human cervical cancers. Formalin-fixed and paraffin-embedded human cervical cancer sections were stained as described in A. Shown is representative of three HPV-positive and four HPV-negative cervical cancers.
shown). We obtained similar results with female reproductive tracts of K14E6/K14E7 double transgenic mice (data not shown).

To evaluate the potential relevance of ERs in human cervical cancer, we stained formalin-fixed and paraffin-embedded human cervical cancers for ERs or ERα. As observed in the mouse tissues, ERα was expressed in cervical cancers and adjacent normal epithelia as well as the surrounding stroma regardless of HPV status (Fig. 1B). Although the majority of cancer cells expressed detectable levels of ERα, the percentage of ERα-positive cells (85 ± 6.9%) in human cervical cancers was significantly less than that (94 ± 11.4%) observed in murine cervical cancers (P = 0.02, Wilcoxon rank sum test). Nevertheless, the vast majority of cells in both human and mouse cancers were ERα positive, consistent with a potential role for this receptor in cervical cancers in both species. ERβ was not detected in any areas of the human cervix (Fig. 1B) but could be detected in the human endometrium (data not shown). Based on these results and the knowledge that estrogen is a critical cofactor for cervical carcinogenesis in HPV transgenic mice, we hypothesized that ERs is required for cervical carcinogenesis.

Normal cervical structure and cervical reserve cells are retained in ERα knockout mice. To test this hypothesis, we used ERα knockout (ERα−/−) mice. Due to the well-known function for ERα in female reproductive tract biology (29, 31), we first investigated whether overall cervical structure is preserved in ERα−/− mice and specifically whether reserve cells, the cell type from which cervical cancers are thought to arise, are retained in these mice. Female ERα−/− mice were found to retain a normal cervical structure, including a well-defined endocervix (endocervical septum and endocervical canal) and ectocervix, as found in wild-type (wt) ERα+/+ littermates (Fig. 2A). Reserve cells can be identified by their anatomic location just underneath the columnar epithelial lining of the cervix and expression patterns of various marker proteins. Reserve cells express both squamous and columnar epithelial makers: p63, K5, and K14 that are absent in squamous epithelia (32, 33). We found cells that are double positive for p63 and K8, middle, that the reserve cells (arrowheads) are positive for both K8 and ERα; right, note (merge of K8, p63, and ERα staining) that the nuclei of the reserve cells (arrowheads) are purple indicative of double positivity for both p63 and ERα (these cells again are also positive in the cytoplasm for K8).

HPV-16 E7 and exogenous estrogen fail to promote cervical cancers in ERα−/− mice. In a prior study making use of K14E6 and K14E7 transgenic mice treated with exogenous estrogen, the HPV-16 E7 oncogene was determined to be more potent in promoting cervical carcinogenesis (11, 20). Therefore, to test whether ERα contributes to cervical carcinogenesis in mice, we bred K14E7/ERα−/− mice to ERα−/− mice. Offspring were genotyped for the E7 transgene and ERα status. Nontransgenic (NTG) and K14E7 transgenic female offspring that were either ERα−/− or...
ERα+/− were treated with 17β-estradiol (using slow-release pellets that deliver 0.05 mg/60 days, a physiologic dose sufficient to induce continuous estrus) for 6 months, a treatment period sufficient for the development of cervical cancers in the majority of K14E7 transgenic mice (4, 20). Reproductive tracts were harvested, fixed in paraformaldehyde, and embedded in paraffin. Every tenth 5-μm section was stained with H&E and histopathologically scored to identify the worst grade of cervical/vaginal disease present in each animal. Consistent with previous results (20), the majority (67%) of K14E7/ERα+/+ mice treated with estrogen developed cervical cancer and the remainder developed high-grade dysplasias (CIN3). As expected, none of NTG/ERα+/+ mice treated with estrogen had cervical cancers and only one developed a low-grade dysplasia (CIN1; Table 1). This difference in cervical cancer incidence was statistically significant (P = 0.001) and recapitulated our prior findings that E7 synergizes with exogenous estrogen to induce cervical cancer (20). A strikingly different result was observed on the ERα/C0/C0 background. K14E7/ERα/C0/C0 mice treated with estrogen failed to develop cervical cancers or any grade of dysplasia (Table 1). This result shows that ERα is absolutely necessary for cervical carcinogenesis in HPV transgenic mice. Not surprisingly, no cervical disease was evident in the NTG/ERα/C0/C0 mice treated with estrogen. Vaginal disease, including vaginal cancer, was also absent in K14E7/ERα/C0/C0 mice unlike in K14E7/ERα+/+ mice (P = 0.00003, Wilcoxon rank sum test).

Cervical cancers are associated with atypical squamous metaplasia in mice. Because human cervical cancers are always accompanied by atypical squamous metaplasia (ASM), it has been proposed that such an aberrant metaplasia is a precursor for cervical cancer (2). For this reason, we investigated whether K14E7/ERα−/− mice treated with estrogen display ASM. We found that K14E7/ERα−/− and NTG/ERα−/− mice treated with estrogen have ASM (K14E7/ERα−/− versus NTG/ERα−/−, P = 0.49, Fisher’s exact test), but not K14E7/ERα−/− or NTG/ERα−/− mice, as evidenced by the presence of multiple patches of squamous epithelium within the columnar epithelium of the endocervix (Fig. 3A). These results clearly show that ERα is necessary for the development of cervical cancers in HPV transgenic mice.

### Table 1. State of lower reproductive tract disease in K14E7/ERα−/− mice

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No dysplasia</th>
<th>Dysplasia</th>
<th>Cervical (vaginal) cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CIN1 (VIN1)</td>
<td>CIN2 (VIN2)</td>
</tr>
<tr>
<td>NTG/ERα+/+</td>
<td>12</td>
<td>1 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>K14E7/ERα+/+</td>
<td>0</td>
<td>0 (1)</td>
<td>0 (2)</td>
</tr>
<tr>
<td>NTG/ERα−/−</td>
<td>10</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>K14E7/ERα−/−</td>
<td>11</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

NOTE: Mice were scored histopathologically for the worst state of disease present in the cervix or, in parentheses, the vagina. The numbers of mice with the indicated state of disease are indicated in each column. For Wilcoxon rank sum test (see text), each lesion was given the following arbitrary score: no dysplasia = 1; CIN1 (VIN1) = 2; CIN2 (VIN2) = 3; CIN3 (VIN3) = 4; cancer = 5. Abbreviation: VIN, vaginal intraepithelial neoplasia.
It was noted that ASM was more prevalent in a promote the development of ASM in an ER that HPV oncogenes and endogenous estrogen cooperate to the absence of ASM in progressive cervical disease observed in women. We conclude that the presence of functional E7 (7, 21). Whereas Mcm7 expression was primarily restricted to the basal cells in NTG/ER \(^{-/-}\) cervical epithelium, virtually all of the epithelial cells in K14E7/ER \(^{-/-}\) cervix were found to express Mcm7 (Fig. 4B), as previously described (35). More importantly, whereas only some cells in cervical epithelium of NTG/ER \(^{-/-}\) mice were positive for Mcm7, nearly all of the epithelial cells in K14E7/ER \(^{-/-}\) cervix expressed high levels of Mcm7, consistent with E7 retaining the ability to inactivate the tumor suppressor pRb in ER \(^{-/-}\) cervical epithelial cells.

Another readout for E7 function is its ability to induce hyperproliferation of epithelial cells within the cervix of K14E7 mice (19). To investigate whether E7 also induces hyperproliferation in the ER \(^{-/-}\) cervix, we carried out BrdUrd staining on paraffin sections of female reproductive tracts from estrogen-treated mice of indicated genotypes. In the absence of E7, the proliferation index of the ER \(^{-/-}\) cervical epithelia was significantly lower than that of the ER \(^{-/-}\) cervical epithelia (NTG/ER \(^{-/-}\) versus NTG/ER \(^{-/-}\), \(P = 0.007\), Wilcoxon rank sum test), suggesting that E7 is required for the normal proliferative state of the cervical epithelium (Fig. 4C and D). Nevertheless, E7 retained an ability to induce cell proliferation in the absence of ER \(\alpha\) (compare the proliferation index of K14E7/ER \(^{-/-}\) with NTG/ER \(^{-/-}\) cervical epithelium, \(P = 0.047\), Wilcoxon rank sum test; Fig. 4C and D).

**Discussion**

E7 has been shown to either promote or inhibit the development of cancers in mouse models. For instance, ER \(\alpha\) inhibits APC-dependant colon carcinogenesis (36), whereas it is necessary for the development of ASM. We further analyzed archival parafomaldehyde-fixed and paraﬁn-embedded female reproductive tracts of mice either untreated or treated with estrogen for the presence of ASM (19, 20). Similar to mice treated with estrogen on the ER \(^{-/-}\) background described above, all examined tissues of NTG, K14E6, and K14E7 mice having wt ER \(\alpha\) and treated with estrogen for 6 months showed atypical metaplastic changes (Fig. 3B, top). In contrast, none of untreated NTG mice had ASM and 54% of K14E6 and 100% of K14E7 untreated mice developed ASM, which are statistically signiﬁcantly different (NTG versus K14E6, \(P = 0.044\); NTG versus K14E7, \(P = 0.0003\); K14E6 versus K14E7, \(P = 0.046\), Fisher’s exact test; Fig. 3B, middle). We also observed that ASM was absent in ovariectomized K14E7 mice (\(P = 0.006\), compared with intact K14E7, Fisher’s exact test; Fig. 3B, bottom). These results strongly suggest that HPV oncogenes and endogenous estrogen cooperate to promote the development of ASM in an ER \(\alpha\)-dependent manner. It was noted that ASM was more prevalent in K14E7 than K14E6 mice, correlating with more potent tumorigenic potential of E7 than E6 in the mouse cervix (11, 20) and thus further supporting the hypothesis that ASM is a prerequisite for the development of cervical cancer in mice (4, 20). As such, this mouse model recapitulates the progressive cervical disease observed in women. We conclude that the absence of ASM in K14E7/ER \(^{-/-}\) mice treated with estrogen is primarily responsible for the lack of subsequent stages in the progressive disease leading to cervical cancers.

**E7 oncoprotein enhances Mcm7 expression and cellular proliferation in cervical epithelium of ER \(^{-/-}\) mice.** As E7 expression in the K14E7 transgenic mouse is driven by the human K14 promoter (28), the presence of K14-positive cells in ER \(^{-/-}\) cervix (data not shown) implies expression of E7 therein, which was verified by immunoblot with an E7-speciﬁc antibody (Fig. 4A). A well-known cellular target of E7 is the pRb tumor suppressor that negatively regulates E2F transcription factors (34). Therefore, induction of E2F target genes, such as Mcm7, is indicative of expression of functional E7 (7, 21). Whereas Mcm7 expression was primarily restricted to the basal cells in NTG/ER \(^{-/-}\) cervical epithelium, virtually all of the epithelial cells in K14E7/ER \(^{-/-}\) cervix were found to express Mcm7 (Fig. 4B), as previously described (35). More importantly, whereas only some cells in cervical epithelium of NTG/ER \(^{-/-}\) mice were positive for Mcm7, nearly all of the epithelial cells in K14E7/ER \(^{-/-}\) cervix expressed high levels of Mcm7, consistent with E7 retaining the ability to inactivate the tumor suppressor pRb in ER \(^{-/-}\) cervical epithelial cells.
required for hormone-induced prostatic carcinogenesis (37). Similar to the latter, we showed in the present study that ERα is absolutely necessary for cervical carcinogenesis in the context of HPV transgenic mice (Table 1), which require estrogen for the development of cervical cancer (4, 11, 19, 20, 38). We also observed that ASM is associated with cervical carcinogenesis in our HPV transgenic mouse models like in women and that HPV oncogenes as well as estrogen and its receptor ERα contribute to ASM development (Fig. 3; ref. 2). We speculate that HPV oncogenes alter host gene expression in such a way as to induce squamous metaplasia that itself is reliant on estrogen and ERα, and this metaplasia renders the tissue more prone to carcinogenesis.

A model for the role of ERα in cervical carcinogenesis. Based on our and other studies, we propose a model, in which the cooperation between the HPV-16 oncogenes, estrogen, and its receptor ERα leads to the progressive disease that initiates with ASM and ends in cervical cancer (Fig. 5). In this model, reserve cells are the origin of ASM. This is consistent with the fact that cervical cancers preferentially arise at the transformation zone of the endocervix, the site of reserve cells, and the hypothesis that reserve cells are the progenitor cell type for cervical carcinogenesis (4, 23, 39, 40). Our results showed that ERα and likely estrogen are necessary for the development of ASM and that HPV oncogenes also contribute to this process (Fig. 3). Role of HPV E6 and E7 in the development of ASM is also supported by studies showing the ability of HPV-16 to induce squamous metaplasia of colon and lung adenocarcinoma cells in vivo (41, 42). We also argue that the same three factors, HPV oncogenes, estrogen, and ERα, are essential for the subsequent steps in the progressive cervical disease and the maintenance of cervical cancer. This is supported in part by the fact that HPV transgenic mice develop progressively worse disease as treated for longer period of time with exogenous estrogen and its withdrawal from cancer-bearing HPV transgenic mice leads to reduction not only in the cancers but also in the severity of dysplastic lesions in the cervix (4, 19). In addition, HPV-16 oncogenes (E6 and E7) are required for the continued proliferation of cell lines derived from human cervical cancer (8, 9). The continued role of E6/E7 and ERα in the later steps of progressive disease downstream of ASM has not been directly shown but could be with the evaluation of mice in which the expression of these viral oncogenes and ERα can be temporally regulated. Nonetheless, facts that exogenous estrogen, the ligand for ERα, is required for the later steps in cervical carcinogenesis as well as cancer maintenance and that HPV oncogenes are expressed in all stages of human cervical cancer are reasonable basis for predicting that these factors also are required (19, 22, 39).

This model for cervical cancer (Fig. 5) puts forth that the reserve cells are the progenitor cell from which HPV-associated cervical cancers arise. HPV-associated cancers, however, can also be found in other sites of the female reproductive tract both in the HPV transgenic mouse models and in women, where reserve cells are not known to be present. Nevertheless, these cancers seem to rely on the same three factors. In mice, the development and maintenance of cancers in the vagina are also dependent on HPV oncogenes and exogenous estrogen (19, 20). Furthermore, based on the current study, these cancers must also be dependent on ERα, as we failed to see any tumors or dysplasia in the lower reproductive tracts of K14E7/ERα−/− mice treated with estrogen (Table 1). It remains to be understood what the progenitor cell is for cancers arising in these tissues. Perhaps, it remains to be reserve cells and such reserve cells reside within these tissues but simply have not been detected to date, or the initiated cancer precursor cells for these lower reproductive tract cancers actually are the reserve cells at the transformation zone and then migrate. Alternatively, these cancers might arise not from multipotent precursor cells, such as reserve cells, but some other, perhaps more committed, cell type (e.g., a basal cell). Were this the case, then one might predict that these lower reproductive tract cancers, which are rarer than cervical cancer in women and in mouse models, might require different and/or additional genetic/epigenetic changes that arise less frequently.

Potential ERα target genes that are crucial for cervical carcinogenesis. It will be difficult to identify which estrogen-responsive genes are critical for cervical carcinogenesis, as ERα is known to activate or repress a myriad of genes, many of which have been implicated in tumorigenesis. In addition, it is not clear whether known ERα target genes [up-regulated or down-regulated by treatment with estrogen for a short period of time (hours to several days)] are likewise regulated by this ERα in mice treated with estrogen for 6 months. Nonetheless, it is interesting to note that ERα up-regulates proto-oncogenes, such as c-myc, cyclin D1, epidermal growth factor receptor (EGFR), and insulin-like growth factor-I (IGF-I), and represses proapoptotic genes (43, 44). With regard to a potential role of these genes in HPV-associated cervical carcinogenesis, it has been shown that normal human cervical keratinocytes expressing HPV E6 and E7 acquire tumorigenic potential on c-myc overexpression and cyclin D1 is overexpressed in cervical cancers (45, 46). It is also relevant that EGFR inhibitor treatment is marginally effective for controlling recurring cervical cancers and high serum IGF-I levels are correlated with higher risk for CIN (47, 48).

About the possible role of the second ER, ERβ in cervical carcinogenesis, we failed to detect this isoform in cervical cancers and surrounding stroma in both mice and human (Fig. 1). In addition, the level of ERβ in ERα−/− mice is comparable with that in ERα+/− mice (24), yet K14E7/ERα−/− mice did not develop any cervical disease (Table 1), indicating no major role of ERβ in cervical carcinogenesis. Furthermore, other lesions in mouse models induced by estrogen or diethylstilbestrol are also dependent on ERα but not ERβ (49, 50). Therefore, it is unlikely that ERβ plays a crucial role in cervical carcinogenesis. The demonstration herein that ERα is required for cervical carcinogenesis provides support for the hypothesis that drugs

---

**Figure 5.** Model for cervical carcinogenesis. See the text for details.
that can interfere with the function of this specific nuclear receptor will be effective therapeutic agents in preventing and/or treating cervical cancers. Preclinical studies directed toward using this hypothesis could provide a strong basis for treatment of such drugs in treating human cervical disease.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

Requirement for Estrogen Receptor $\alpha$ in a Mouse Model for Human Papillomavirus–Associated Cervical Cancer

Sang-Hyuk Chung, Kerri Wiedmeyer, Anny Shai, et al.


Updated version  Access the most recent version of this article at:  
http://cancerres.aacrjournals.org/content/68/23/9928

Cited articles  This article cites 50 articles, 26 of which you can access for free at:  
http://cancerres.aacrjournals.org/content/68/23/9928.full#ref-list-1

Citing articles  This article has been cited by 14 HighWire-hosted articles. Access the articles at:  
http://cancerres.aacrjournals.org/content/68/23/9928.full#related-urls

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, use this link  
http://cancerres.aacrjournals.org/content/68/23/9928. 
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.