Spontaneous Malignant Transformation of Human Ovarian Surface Epithelial Cells in Vitro

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ABSTRACT

Purpose: Epithelial ovarian cancer has no reliable marker for early detection and no known specific premalignant changes. Human ovarian surface epithelial (HOSE) cells expressing human papillomavirus type 16 (HPV-16) E6/E7 genes undergo crisis, and surviving cells exhibit an immortalized phenotype. Cells show an increasingly invasive phenotype on collagen rafts over time. To ascertain the nature of this aberrant growth, we characterized this spontaneous progression of HOSE cells from a benign to an invasive phenotype using histopathology, immunophenotyping, and tumorigenesis assays.

Experimental Design: At various passages, cells were monitored for growth on collagen, response to tumor necrosis factor α and daunorubicin, immunohistochemistry and Western blot analysis of E-cadherin and β-catenin, growth in soft agar, and tumor formation in immunodeficient mice.

Results: As passage number increased, cells became increasingly aggressive on collagen, with more pronounced focal stratification and invasion. Furthermore, late-passage cells were more resistant to the apoptotic effects of TNF-α and daunorubicin than earlier-passage cells. E-cadherin expression was limited to early-passage cells, whereas β-catenin was expressed regardless of passage. Cells invading collagen formed colonies in soft agar at low efficiency but were not tumorigenic in immunodeficient mice. Some cultures recovered from colonies grew in soft agar at high efficiencies, and one was tumorigenic.

Conclusions: HOSE cells expressing E6/E7, over time, develop characteristics of malignant cells and produce tumors consistent with an ovarian surface epithelium lineage. Progression of HOSE cells from a benign to an invasive phenotype in vitro may provide a model to dissect the progression of ovarian cancer.

INTRODUCTION

Although ovarian cancers are less common than other gynecological tumors, they account for a disproportionate number of fatal cases, being responsible for almost one-half of the deaths from all female genital tract tumors. This is caused, in part, by the decline in the incidence and mortality of advanced cervical and endometrial cancers since the wide use of screening techniques that aid in early detection. In addition, most women with ovarian cancer tend to present with advanced and disseminated disease that is more difficult to manage and often fatal. Currently, little is known about the pathogenesis of sporadic ovarian cancer development, and no screening methods for early detection are available.

Most epithelial cell cancers (cervix, colon, skin, prostate, breast, etc.) develop from precursor lesions that arise from an accumulation of mutations. Cervix cancer develops through a continuum of well-defined stages of intraepithelial neoplasia to invasive disease. These stages have been reproduced in vitro with a high degree of fidelity (1, 2). Such precursor lesions have not been identified for ovarian cancer, but it has been proposed that ovarian cancers arise by a multistep process through increasingly aggressive stages (3). Some histopathological studies have shown an increased incidence of ovarian surface epithelium aberrations in ovaries from women with a family history of ovarian cancer compared with ovaries from normal women (4); however, this has not been a consistent finding (5, 6). Others have reported similar aberrations in normal ovaries of ovarian cancer patients, suggesting these to be precursor lesions (7). There is, however, no evidence that the changes seen in the surface epithelium are precursors to epithelial ovarian cancer.

Progress in the cultivation of HOSE cells has provided a limited model to study the growth characteristics of this epithelium in vitro; however, primary HOSE cells undergo a restricted number of population doublings before senescence (8, 9). Introduction of the SV40 T-antigen or HPV-16 E6/E7 genes extends the life span of these cells (10, 11). These cells undergo crisis, and after several months, continuously growing cells emerge that seem to have an unlimited life span fail to form colonies in soft agar and are not tumorigenic in immunodeficient mice. We
report here, however, that HPV-16 E6/E7-immortalized HOSE cells develop a progressively aggressive phenotype over time when monitored by three-dimensional organotypic culture on collagen rafts. Later passages of these cells readily stratify, form nodules, and invade the collagen gel. This coincides with their ability to form colonies in soft agar and develop resistance to the cytotoxic effects of TNF-α and daunorubicin; however, these cells fail to form tumors in SCID mice. Notwithstanding, some cultures derived from the soft agar colonies can produce aggressive, poorly differentiated tumors in SCID mice.

MATERIALS AND METHODS

Cell Culture. The culturing of primary HOSE cells, HOSE-A (96.9.18) and HOSE-B (1.24.96), and extension of their life span by infection with the amphotropic retrovirus LXSN-16E6E7 and organotypic culture of HOSE cells grown on collagen with fibroblast feeder cells has been described previously (12). For histopathological analysis of organotypic cultures, the collagen rafts were overlayed with 2% agarose in RPMI 1640 at 46°C, allowed to gel to stabilize the raft, and transferred to 10% buffered formalin for processing for routine histopathology. Postcrisis 96.9.18 and 1.24.96 cells were maintained as monolayer cultures on standard plastic tissue culture vessels and passed at a 1:10 split ratio at the time of confluence. Passage numbers referred to in the text are the numbers of passages the cells have undergone since crisis.

Growth in Soft Agar. Cells (2 × 10⁶) were suspended in 1 ml RPMI 1640 containing 20% FCS and mixed with 1 ml of 0.7% Bactoagar (Difco Laboratories, Detroit, MI) in RPMI 1640 at 45°C and layered over 5 ml of 0.75% Bactoagar in RPMI 1640 in 60-mm dishes and cultured in 5% CO₂ in a humidified chamber. Colonies were counted 2 weeks after seeding, and their size was determined using a 10 calibrated eyepiece. Colonies were separated into two groups: (a) ≥ 50 μm; and (b) ≥ 10 but < 50 μm. Human ovarian cancer cell line SKOV-3 was used as control.

In Vivo Tumorigenic Assays. Female (4–6 weeks of age) SCID beige mice were obtained from Charles Rivers, Wilmington, MA. Cells were grown to confluency in monolayer, trypsinized, washed, and resuspended in sterile PBS at a concentration of 1 × 10⁷ cells/ml. Each mouse was inoculated with 0.3 ml of cell suspension s.c. on the flank and i.p. Mice were weighed and inspected for tumor growth or ascites for a period up to 8 months before terminating the experiment. Animal care was in accordance with the Wayne State University (Detroit, MI) Division of Laboratory Animal Research guidelines.

Viability Assays. Cells (8 × 10⁴) were seeded onto 96-well dishes and incubated overnight. Cells were treated for 2 h with daunorubicin hydrochloride in PBS (0 to 1.0 μM; Sigma Chemical Co., St. Louis, MO), after which the medium was removed, fresh medium was added, and the cells were incubated for an additional 22 h. Cells treated with TNF-α (0 to 10 ng/ml; Sigma Chemical Co.) were incubated for 24 h. Viability was evaluated by the conversion of tetrazolium bromide to formazan by viable cells [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay] as described by Hansen et al. (13). Differences in viability were calculated using Bonferroni’s multiple comparison test after ANOVA analysis, and Ps < 0.05 were considered significant.

Analysis of Proteins. Western blot analysis for detection of E-cadherin and β-catenin in cell lysates was described previously. Cells were scraped off the plates, and lysed with a hypotonic lysis buffer (1 mM NaCO₃, 0.2 mM PMSF, 1 μg/ml aprotinin, 1 μg/ml leupeptin, and 1 μg/ml pepstatin) for 30 min on ice. After passing through a 20-gauge needle (10 times), the lysates were centrifuged (2,000 × g for 5 min) and the supernatants were removed and centrifuged at 4°C for 30 min at 15,000 × g (14, 15). Protein concentrations were determined by a bicinchoninic acid protein assay kit (Sigma Chemical Co.). Proteins (20 μg/well) were separated on a 12.5% SDS-polyacrylamide gel and transferred to nitrocellulose; β-actin staining was used as the loading control. Membranes were immunoblotted using monoclonal antibodies against E-cadherin (clone HEC-1; Zymed Laboratories, San Francisco, CA) or anti-E-cadherin, (Santa Cruz Biotechnology, Santa Cruz, CA), β-catenin (clone 15B8; Sigma Chemical Co.), and horseradish peroxidase-conjugated secondary antibodies (Sigma Chemical Co.). Bands were visualized by treatment with a chemiluminescent substrate (LumiGLO, Kirkegaard & Perry Laboratories, Gaithersburg, MD) and recorded on Fuji X-ray film. A431 cells were used as positive control. Immunohistochemical staining was done on 3–5-μm sections that were deparaffinized in xylene and rehydrated through graded alcohol. After blocking for 1 h at room temperature with 2% bovine calf serum in PBS containing 0.5% delipidated milk powder, the sections were incubated overnight at 4°C with primary antibodies anti-vimentin (clone V9; Sigma Chemical Co.), anti-Pan cytokeratin (Sigma Chemical Co.) polyclonal anti-β-catenin antibody (Sigma Chemical Co.), monoclonal anti-E-cadherin antibody (clone HECID-1; Zymed) and monoclonal anti-CA125 (clone M11; Dako, Carpinteria, CA) and then rinsed with PBS and incubated with fluorochrome-conjugated secondary antibodies and 4′,6-diamidino-2-phenylindole (1 ng/ml) to stain nuclei (Molecular Probes, Eugene, OR) for 1 h. In some instances, 2% goat serum was used to block nonspecific binding reactions. After intensive washes with PBS, the sections were mounted with Mowiol (Calbiochem, La Jolla, CA) supplemented with 0.2% 1,4-diazacyclo-[2.2.2]-octane (Sigma Chemical Co.) as antifade and evaluated with a NIKON E800 epifluorescence microscope with differential interference contrast optics. Images were optically captured with a SenSys digital charged coupled device camera using ISEE imaging software (Inovision, Raleigh, NC). Images from samples using nonspecific antibody (mouse monoclonal IgG1; BD Pharmigen, San Diego, CA) and/or only secondary antibody served as controls.

RESULTS

Characteristics of HOSE Cells. Previously we have reported the growth of primary and extended-life span HOSE cells in organotypic culture (12). In our hands, 96.9.18 (previously termed HOSE-A) and 1.24.96 (previously termed HOSE-B) transduced with HPV-16 E6/E7 underwent about 20 population doublings before the cells abruptly stopped replicating (crisis). Cultures were maintained with fresh medium changes biweekly for about 3 (96.9.18) to 12 (1.24.96) months, when cells began...
to replicate. These cells were expanded, and both cultures have undergone >120 passages at a split ratio of 1:10 and are likely immortalized. Both 96.9.18 and 1.24.96 maintain a cobblestone appearance in monolayer culture similar to precrisis cells, as depicted in Fig. 1. These cells continue to express HPV-specific transcripts (data not shown) as shown previously by others for HPV oncogene-transduced HOSE cells (11).

**Organotypic Culture.** Both 96.9.18 and 1.24.96 primary cultures grew as a monolayer on collagen rafts, but extended-life span cells stratified into 2–4-cell thick layers; however their phenotypes in monolayer culture were similar (12). Regardless of passage, postcrisis cells maintained similar phenotypes in monolayer. Because previously we had observed phenotypic changes when cells were grown on collagen rafts, we tested the phenotype of postcrisis cells at various passages. Early-passage (10–30 passages) postcrisis cells grown on collagen showed a single-cell layer phenotype with only focal stratification (Fig. 2, A and B). Intermediate-passage cells (40–70 passages) grew as a monolayer on collagen but with increased areas of focal stratification. Of interest is the occasional presence of cells embedded within the collagen (Fig. 2, C and D). Late-passage cells (>70 passages) showed, in addition to the monolayer pattern, distinct nodules of large epithelioid cells with hyperchromatic, pleomorphic nuclei, and small nucleoli with abundant vacuolated and amphophilic cytoplasm (Fig. 2, E and F). Some of the nodules formed small papillary structures, and others aggressively invaded the underlying collagen with wide margins reminiscent of peritoneal implants of serous ovarian carcinoma (Fig. 2, G and H). The cells stained positive for both keratin and vimentin, indicating their ovarian surface epithelial origin (data not shown; Ref. 16). Numerous mitotic figures were seen, and many were atypical. Karyorrhexis was also abundant.

**Growth in Soft Agar.** Both early- and intermediate-passage 96.9.18 and 1.24.96 cells were unable to form colonies in soft agar. Human ovarian cancer cell line SKOV-3 readily formed large (>50-μm) colonies in soft agar. Late-passage 96.9.18 and 1.24.96 cells were also capable of forming >50-μm colonies in soft agar but at diminished efficiency compared with SKOV-3. In addition, both cell cultures also gave rise to smaller colonies at higher efficiency (Table 1). A number of large colonies were selected from the soft agar, but only cells from 96.9.18 colonies grew (designated LC1-LC4). Retesting these cells in soft agar after expansion revealed the cultures possessed pleomorphic phenotypes with respect to colony-forming efficiency and colony size (Table 1).

**In Vivo Tumorigenicity Assays.** None of the late-passage monolayer-derived cultures formed tumors in SCID mice when 3 × 10^6 cells were injected either i.p. or s.c. within 8 months, whereas 3 × 10^6 SKOV-3 cells produced tumors at injected sites in 3–4 weeks. Cultures LC2 and LC3 were also tested for tumor formation in SCID mice. LC2 produced tumors
Table 1  HOSE cell colony formation in soft agar and tumorigenicity in SCID mice

<table>
<thead>
<tr>
<th>Cell culture</th>
<th>Colony size</th>
<th>Tumorigenicity*</th>
<th>No. sites</th>
<th>pos/no. sites</th>
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<td>0</td>
<td>0</td>
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<td>0</td>
<td>0/8</td>
<td></td>
</tr>
<tr>
<td>Passage 106</td>
<td>40</td>
<td>&gt;200</td>
<td>0/8</td>
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</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Passage 60</td>
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<td>0</td>
<td>ND</td>
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</tr>
<tr>
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<td>&gt;200</td>
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<tr>
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<td>&gt;200</td>
<td>ND</td>
<td></td>
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<td>LC2</td>
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<td>194</td>
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* Number of colonies/plate is the average of three experiments.

at 2 of 2 sites (s.c. and also i.p.), with $3 \times 10^6$ cells at each site within 8 weeks with no evidence of ascites; no tumors were detected in 10 weeks in the mouse injected with $3 \times 10^6$ LC3 cells. The s.c. and peritoneal tumor nodules produced in the mouse injected with LC2 were made up of epithelial cells that showed focal glandular differentiation and numerous mitotic figures. The cells were large, had pleomorphic and hyperchromatic nuclei with prominent nucleoli, and abundant vacuolated and amphophilic cytoplasm (Fig. 3). There was strong cytoplasmic staining with broad-spectrum antikeratin and anti-vimentin antibodies, similar to the known immunophenotypic staining of epithelial ovarian tumors (data not shown; Ref. 16).

Viability Assays. Postcrisis cells from 1.24.96 (low and high passage) and 96.9.18 (intermediate and high passage) were tested for their sensitivity to the cytotoxic agent TNF-α and daunorubicin, a chemotherapeutic agent. Late-passage (p105) cells from 1.24.96 were significantly more resistant to the cytotoxic effects of daunorubicin and TNF-α than the early (p33)-passage cells ($P < 0.001$; Fig. 4A). Only at the highest concentration of daunorubicin tested did the high-passage cells show a difference to untreated cells ($P < 0.001$). Likewise, the resistance of 96.9.18 high-passage cells (p121) was significantly greater than intermediate (p55)-passage cells ($P < 0.001$). As with 1.24.96 high-passage cells, 96.9.18 high-passage cells showed sensitivity at the highest concentration of daunorubicin tested. Only the intermediate-passage 96.9.18 cells showed sensitivity to TNF-α when compared with untreated cells, whereas high passage cells were resistant to TNF-α (Fig. 4B).

Analysis of Proteins. Recent reports have suggested that the levels and/or localization of β-catenin and E-cadherin may be linked to tumor cell progression and invasiveness. To determine whether there were any direct correlations between β-catenin and E-cadherin expression and/or subcellular localization and the passage number of our HOSE cells, we analyzed the cells by Western blot analysis and indirect immunofluorescence assay.

Immunohistochemical staining of both cell cultures showed no evidence of E-cadherin staining from cells grown on collagen rafts or in monolayer culture. In contrast, positive membrane staining of SKOV-3 cells on collagen rafts was observed, albeit very weak, when probed with anti-E-cadherin antibody. These results were not confirmed by Western blot analysis of the isolated membrane fractions of these cells. Detectable levels of E-cadherin were only observed in the earliest passages of postcrisis 1.24.96 cells tested (p38 and less in p78) but not in 96.9.18 cells. It should be noted that detection was only possible in 1.24.96 cells when 50 μg of protein was probed (Fig. 5). Cytosolic E-cadherin was not detected in either HOSE culture regardless of passage number.

Western blot analysis of β-catenin expression revealed both a membrane and cytosolic localization (Fig. 5). There was no significant change in the amount of cytosolic β-catenin with respect to cell passage in either cell culture. β-catenin positive degradation bands were evident in the cytosolic fractions, suggesting that β-catenin is being degraded in the cytosol. The ratio between β-catenin and β-actin staining showed little or no change in levels during the transition to the malignant phenotype (Fig. 5). Although the ratios of β-catenin/β-actin seemed high in 96.8.18 cells at p69 and seemed to increase in 1.24.96 cells with passage number, these were not consistent findings. Indirect immunofluorescence assay analysis of raft cultures showed similar levels of cytosolic and membrane-associated β-catenin staining regardless of passage number (data not shown).

DISCUSSION

Previously we have shown that HPV-16 E6/E7 gene expression in primary HOSE cell results in an extended life span and aberrant growth in organotypic culture (12). The use of viral oncogenes to disrupt cell cycle regulation is likely a valid approach to modify HOSE cell behavior. This rationale is based on the high frequency of p16INK4A/MTS1 dysregulation in early stage ovarian cancers (17, 18). The p16 tumor suppressor gene is a cdk inhibitor that deaccelerates the cell cycle by inactivating the cdks that phosphorylate the retinoblastoma tumor suppressor gene protein (pRB). pRB acts by binding and inhibiting the cellular transcription factor E2F, among others. Phosphorylation of pRB by cdks leads to release of E2F, which mediates progression through the cell cycle. HPV-16 E7 binds to pRB and dissociates the E2F-pRB complex (19). The effect is similar to the dysregulation of p16INK4A/MTS1 gene expression, the effect of which also abrogates the binding of E2F by pRB. Similarly, p53 is frequently mutated in early ovarian cancers, suggesting that this gene may contribute to the development of ovarian cancers (20, 21). HPV-16 E6 binds to and promotes the ubiquitination-mediated degradation of p53. Thus, E7 and E6 expression would likely create a similar and appropriate premalignant platform as that created by loss of p16 and p53 function, respectively, in HOSE cells in vivo.

HOSE cells transduced with HPV-16 E6/E7 enter crisis within a few population doublings. Here, we report the growth characteristics in organotypic culture of HOSE cells derived from two different patients that survived crisis. Both cell cultures underwent, over time, three distinct phases of growth on
collagen, exhibiting: (a) no invasion with rare focal stratification; (b) single-cell invasion with focal stratification; and (c) robust stratification with papillary formation and intense invasion of the collagen bed. Furthermore, the stage of development of the invasive phenotype on collagen paralleled the ability of the cells to form colonies in soft agar. This progression to an invasive phenotype was dependent on the number of population doublings, because both HOSE cultures exhibited the phenotype at roughly the same number of passages. Human keratinocytes expressing high-risk HPV E6/E7 genes also grow aberrantly on collagen rafts (1). These cells recapitulate the continuum of cellular changes that represent the transition at early passage

Fig. 3 Histopathology of i.p. tumor from SCID mouse injected with LC2. A, large tumor cells are seen infiltrating adjacent pancreatic tissue (×20). B, poorly differentiated large tumor cells with focal glandular formation (arrow; ×40).

Fig. 4 Viability of 1.24.96 cells (A) and 96.9.18 cells (B) treated with TNF-α and daunorubicin.
from mild to moderate to severe dysplasia/carcinoma in situ at late passage. Cells eventually become tumorigenic in nude mice after many months in culture (22).

The lack of tumorigenicity of late-passage HOSE cells that show the invasive phenotype on collagen may be attributable to heterogeneity within the cell population, which thus limits the number of truly malignant cells. This is supported by the ability of cells derived from some colonies formed in soft agar to produce tumors in SCID mice, whereas cells from other colonies showed low colony-forming efficiency and were not tumorigenic. This is not surprising, because tumor cells are genetically heterogeneous (23).

With increased passage numbers, the sensitivity of both ovarian cell lines to daunorubicin, a chemotherapeutic agent, and TNF-α, a multifactorial cytokine, is significantly reduced. These two compounds were used because both induce apoptosis in cell lines but use different cell death signaling pathways. Daunorubicin, an anthracyclin widely used as an antitumor agent, causes DNA damage and induces apoptosis, the latter possibly an independent pathway involving the generation of reactive oxygen species, ceramide synthesis, and the activation of c-Jun NH2-terminal kinase (24, 25). TNF-α is an important mediator of inflammation, but has also been shown to induce apoptosis in some cell lines by binding to the TNF receptors and in epithelial cells, mostly the p60 receptor (26), thereby activating the apoptotic cascade. It has been shown, however, that TNF-α selectively inhibits proliferation of epithelial cells derived from normal cervix, normal ovarian epithelium, keratinocytes, or myeloid cells but has no effect or even stimulates cell growth in immortal, dedifferentiated, or malignant cells (27–31). This correlates well with our findings that cytotoxicity of TNF-α or daunorubicin is restricted to lower noninvasive passages of HOSE cells, whereas those passages with a more malignant phenotype are resistant. Furthermore, it has been shown that sensitive immortalized keratinocytes do not form tumors in nude mice, but nonsensitive cells are tumorigenic (32).

Apoptosis has been shown to play an important role in the regulation or limitation of cell populations. Disruption of apoptotic pathways has been implicated in tumor formation and metastasis and may account for the uncontrolled cell proliferation (33–35). It has yet to be determined whether mutations in tumor suppressor proteins, expression of oncogenes, or development of multidrug resistance or a specific resistance to daunorubicin (by inactivation of the active compound Ref. 36) or TNF-α (by releasing endogenous soluble type I TNF-α receptor Ref. 37) play a role in the differences in HOSE cell sensitivity.

β-catenin is a cell adhesion protein, primarily localized at the junctional complexes in the lateral membranes bound to E-cadherin. Cytosolic β-catenin is rapidly ubiquitinated and degraded in the proteasomes. A disruption of this pathway had been described extensively in colonic tumors that carry an adenomatous polyposis coli mutation or truncation of β-catenin. However, the disruption of the adenomatous polyposis coli-β-catenin pathway seems to be involved in a variety of other cancers (38). It has been shown that mutations to β-catenin do occur within the phosphorylation sites on the NH2 terminus, preventing ubiquitination and degradation, in ovarian cancers, but seem to be limited to ovarian endometrioid tumors (39). Such mutations stabilize β-catenin for interaction with the T-cell factor family of transcription factors for nuclear transport and transactivation of a number of genes, most notably c-myc.
and cyclin D1 (38). β-catenin expression in cytosolic fractions were similar in all passage levels of the HOSE cells, confirming reports that the expression of cytosolic β-catenin is an early event in carcinogenesis (39). Whether this protein plays a role in malignant transformation of the cells is unknown.

The progressive loss of E-cadherin expression from early- (noninvasive) to late- (invasive) passage HOSE cells correlates well with recent findings that the loss of E-cadherin is not only associated with increased invasiveness of tumor cells, but also that these changes may be an early event in carcinogenesis (40). Using immunocytochemistry, Durai et al. (41) showed that E-cadherin expression was homogeneous in benign ovarian tumors but was heterogeneously expressed or undetectable in most borderline and malignant tumors. Davies et al. (42) showed, also by immunocytochemistry, that only one of five ovarian cancer cell lines and 56% of ovarian cancers expressed E-cadherin at appreciable levels. In addition, the majority of poorly differentiated tumors were E-cadherin-negative. Forced expression of murine E-cadherin in SV40 large T-antigen immortalized HOSE cells (which normally express a fibroblastic phenotype in monolayer and are keratin negative and nontumorigenic) induces a differentiated epithelial phenotype (43). These cells now are tumorigenic in SCID mice and produce poorly differentiated adenocarcinomas (44). Murine E-cadherin was expressed at high levels in monolayer cell cultures and was detectable in resultant mouse tumors by immunofluorescence. The discrepancy in expression of E-cadherin between these cells and invasive 1.24.96 cells may be attributable to the heterogeneous nature of epithelial ovarian cancer, which may be reflected in differential gene expression. For example, K-ras overexpression is almost limited to mucinous ovarian cancer (45).

Efforts have focused on understanding the role of various known oncogenes, tumor suppressor genes, and cytological aberrations that might contribute to ovarian carcinogenesis. However, most studies have dealt with carcinomas, and it is unknown whether chromosomal or gene expression changes are the result or have a causal relationship to the cancer. This paper demonstrates the spontaneous progression of HOSE cells from a benign to an invasive phenotype using histopathology, immunophenotyping, and tumorigenesis assays. We propose that intermediate in this transition is a putative preneoplastic lesion that undergoes genetic changes leading to the invasive phenotype. Analysis of chromosomal or gene expression changes throughout the continuum described here should provide insights into the nature of this transition and yield valuable information that would help explain the steps leading to ovarian carcinogenesis.

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