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E-cadherin induces mesenchymal-to-epithelial transition in human ovarian surface epithelium

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ABSTRACT **Ovarian carcinomas are thought to arise in the ovarian surface epithelium (OSE). Although this tissue forms a simple epithelial covering on the ovarian surface, OSE cells exhibit some mesenchymal characteristics and contain little or no E-cadherin. However, E-cadherin is present in metaplastic OSE cells that resemble the more complex epithelia of the oviduct, endometrium and endocervix, and in primary epithelial ovarian carcinomas. To determine whether E-cadherin was a cause or consequence of OSE metaplasia, we expressed this cell-adhesion molecule in simian virus 40-immortalized OSE cells. In these cells the exogenous E-cadherin, all three catenins, and F-actin localized at sites of cell–cell contact, indicating the formation of functional adherens junctions. Unlike the parent OSE cell line, which had undergone a typical mesenchymal transformation in culture, E-cadherin-expressing cells contained cytokeratins and the tight-junction protein occludin. They also formed cobblestone monolayers in two-dimensional culture and simple epithelia in three-dimensional culture that produced CA125 and shed it into the culture medium. CA125 is a normal epithelial-differentiation product of the oviduct, endometrium, and endocervix, but not of normal OSE. It is also a tumor antigen that is produced by ovarian neoplasms and by metaplastic OSE. Thus, E-cadherin restored some normal characteristics of OSE, such as keratin, and it also induced epithelial-differentiation markers associated with weakly preneoplastic, metaplastic OSE and OSE-derived primary carcinomas. The results suggest an unexpected role for E-cadherin in ovarian neoplastic progression.**

Epithelial ovarian carcinomas are the most prevalent and lethal of all gynecological malignancies. Histopathological observations and experimental data strongly suggest that these tumors arise from the ovarian surface epithelium (OSE) (1–3). Despite their epithelial morphology, OSE cells exhibit mesenchymal characteristics that reflect their mesodermal origin. Specifically, normal OSE cells rarely express the epithelial cell-adhesion molecule E-cadherin, they deposit stromal extracellular matrix proteins, and they produce matrix-degrading metalloproteases (4–7). Such characteristics likely contribute to the ability of OSE cells to rapidly undergo mesenchymal transformation in monolayer culture (8). Although a definitive premalignant lesion for ovarian carcinoma has not been identified, OSE cell metaplasia is considered to be weakly preneoplastic, particularly in women who are at increased risk of developing the disease because of hereditary factors (2, 9). Interestingly, metaplastic OSE cells, which are invariably epithelial, often express E-cadherin (5, 6).

E-cadherin is a 120-kDa transmembrane, calcium-dependent cell-adhesion molecule. Adhesion is initiated by the binding of homologous E-cadherin extracellular domains on adjacent cells, and it is stabilized by the association of the intracellular E-cadherin domain with α, β, and γ catenins that are physically linked to F-actin to form functional adherens junctions (10, 11). E-cadherin is required for epithelial histogenesis, tissue stabilization, and differentiated function (12). Developmental down-regulation of E-cadherin, which is initiated by morphogen-mediated signals that cause a translocation of β-catenin to the nucleus, induces an epithelial-to-mesenchymal conversion that is required for cell migration and tissue rearrangements during embryogenesis (13). In solid tumors, abnormal regulation or mutation of E-cadherin and β-catenin contributes to the "mesenchyme-like" phenotype of many late-stage, invasive carcinomas (14–16). Thus, E-cadherin acts as a master regulator of the epithelial phenotype (17).

Although E-cadherin often is scanty or absent in ovarian carcinoma metastases, it is almost always present in primary ovarian carcinomas (18–23). At first glance it seems paradoxical that E-cadherin expression is increased in metaplastic OSE and primary ovarian tumors. However, both lesions often resemble the more complex Mullerian duct-derived epithelia of the oviduct, endometrium, or endocervix. The latter tissues and the OSE share a common embryonic precursor, the urogenital coelomic epithelium. Therefore, the appearance of Mullerian duct characteristics in metaplastic OSE and primary ovarian carcinomas likely reflects an aberrant differentiation toward tissue types of closely related embryologic origin. To determine whether ectopic E-cadherin expression is a cause or consequence of this aberrant differentiation, we expressed the cell-adhesion molecule in normal immortalized OSE cells.

Normal immortalized OSE cells transfected with a mouse E-cadherin cDNA lost their mesenchymal characteristics and became epithelialized. E-cadherin, all three catenins, and F-actin were localized at cell–cell borders, indicating the formation of functional adherens junctions. In addition, the transfectants formed tight junctions and re-expressed epithelial cytokeratins found in normal OSE *in vivo*. In two-dimensional culture the transfectants formed cobblestone epithelial monolayers, and in three-dimensional sponge culture they formed simple epithelial linings. Furthermore, the transfectants produced the CA125 tumor antigen and shed it into the culture medium. CA125 is produced by metaplastic OSE *in vivo*, and a sustained, high level of this antigen in a patient's serum is an indicator of ovarian carcinoma (24). However, CA125 also is a normal differentiation product of endometrial, oviductal, and endocervical epithelia, which metaplastic OSE resembles both histologically and cytologically (25). Therefore, E-cadherin expression restored some characteristics of normal OSE that had been lost in culture,

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Abbreviations: CK, cytokeratins; OSE, ovarian surface epithelium.

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and it initiated a metaplastic, epithelialized phenotype that shares some characteristics with neoplastic OSE.

MATERIALS AND METHODS

Cell Culture and Transfection. The IOSE-29 cell line (referred to as IOSE-Mar in previous publications) was generated by transfecting normal human OSE cells in passage 5 with the immortalizing simian virus 40 virus early genes (26). IOSE-29 cells lack keratin because they have undergone epitheliomesenchymal conversion, which is typical of cultured OSE (8). IOSE-29 cells at passage 11 were cotransfected (Lipofectamine; Life Technologies) with pSV2neo (27) and a full-length mouse E-cadherin cDNA under the control of the β-actin promoter (28). The cells were selected under G418 (50 μg/ml), which generated a heterogeneous population of cells, designated IOSE-29pre-EC. A homogeneous epithelial population then was selected by differential adhesion. Briefly, after trypsinization G418-selected cells were allowed to adhere to tissue culture dishes for 30 min and unattached cells were removed by gentle pipette flushing. The adherent epithelial population was designated IOSE-29EC. Parental cells sham-transfected with SV2neo alone were designated IOSE-29neo. Parental and sham-transfected cells yielded no epithelial colonies after differential adhesion. Control cell lines used included human ovarian carcinoma OVCAR-3 cells, which express human E-cadherin and CA125 (29); mouse mammary epithelial scp2 cells, which express mouse E-cadherin (30); human cervical carcinoma C4-II cells (31), which express the cytokeratins 7, 8, 18, and 19 that are found in normal OSE *in vivo* (8); and canine kidney epithelial MDCK cells, which express tight junctional occludin. For monolayer culture, cells were maintained on tissue culture dishes (Falcon; Life Technologies) in a 1:1 mixture of 199/MCDB 105 medium (Sigma) supplemented with 5% FBS. For three-dimensional culture, cells were maintained in pig-skin-derived, dehydrated collagenous sponge matrices (ref. 32; Spongostan, Health Design Industries, Rochester NY) or reconstituted basement membrane gels (Matrigel; Collaborative Research).

Immunofluorescence, Immunocytochemistry, and Immunoblotting. For immunofluorescence, cells were grown on glass coverslips, fixed in cold methanol, and postfixed in cold methanol/acetone (1:1) for all antigens except the catenins, which were fixed in buffered paraformaldehyde followed by permeabilization with digitonin. The cells were incubated with antibodies that recognize both human and mouse E-cadherin (mouse monoclonal; Transduction Laboratories, Lexington, KY), α-catenin (rabbit polyclonal; Sigma), β- and γ-catenins (mouse monoclonals, Transduction Laboratories), or pan-cytokeratin (rabbit polyclonal, Dako). Binding of primary antibodies was visualized by epifluorescence microscopy after incubation with fluorescently labeled, species-specific second antibodies. F-actin was assessed by using rhodamine phalloidin (Molecular Probes). For CA125 immunocytochemistry, cells were fixed in 10% buffered formalin, incubated with the mAb OC-125, and processed as described (33).

For E-cadherin and catenin immunoblotting, whole-cell RIPA lysates (0.1% SDS/1% deoxycholate/1% NP-40) were separated by 10% SDS/PAGE, transferred to nylon membranes (Bio-Rad), and probed with the primary antibodies described above as well as a mouse monoclonal that is specific for human E-cadherin (HECD-1; Zymed) (34). Antibody binding was visualized by using enhanced chemiluminescence (Amersham Pharmacia). For keratin immunoblotting, 30 μg of solubilized cytoskeletal protein was separated by 8.5% SDS/PAGE, transferred to membranes, and probed with either a 1:1 mixture of AE1/AE3 mAbs, which, together, detect all basic and acidic cytokeratins (CKs) except CK-18 (31), or an anti-CK-18-specific mAb (Boehringer Mannheim). For CA125 immunoblotting, cell lysates (100 μg; 0.1% Tween 20) or conditioned medium (300 μg) from subconfluent cultures was separated on nondenaturing 5% SDS/PAGE gels, transferred to membranes, and immunoblotted with OC-125 as described (35). CA125 levels also were assessed by microparticle enzyme immunoassay per the manufacturer's specifications (Abbott).

RESULTS

In monolayer culture, normal human OSE cells, which lack E-cadherin, frequently undergo a mesenchymal conversion (8). The cells become fibroblastic and stop expressing cytokeratins. This mesenchymal phenotype is maintained in OSE cell lines whose lifespan has been extended by the simian virus 40 large T antigen (26). The IOSE-29 line is a typical example, because it lacks E-cadherin (Figs. 1 and 2*A*) and forms fibroblastic monolayers (Fig. 3*A*) that are keratin-negative (Fig. 4*C*).

In the present study, IOSE-29 cells were cotransfected with a mouse E-cadherin cDNA and a pSV2neo selection vector. After selection under G418, the resulting population, designated IOSE-29pre-EC, was heterogeneous, containing mostly fibroblastic cells that were interspersed with small, compact epithelial islands. The epithelial cells, which were slow-growing compared with the fibroblastic cells, were not amenable to limited dilution cloning but they adhered to tissue culture dishes very rapidly after passaging. Therefore, we used a differential adhesion strategy (see *Materials and Methods*) to produce an homogeneous epithelial population, designated IOSE-29EC. Differential adhesion of the parental untrans-

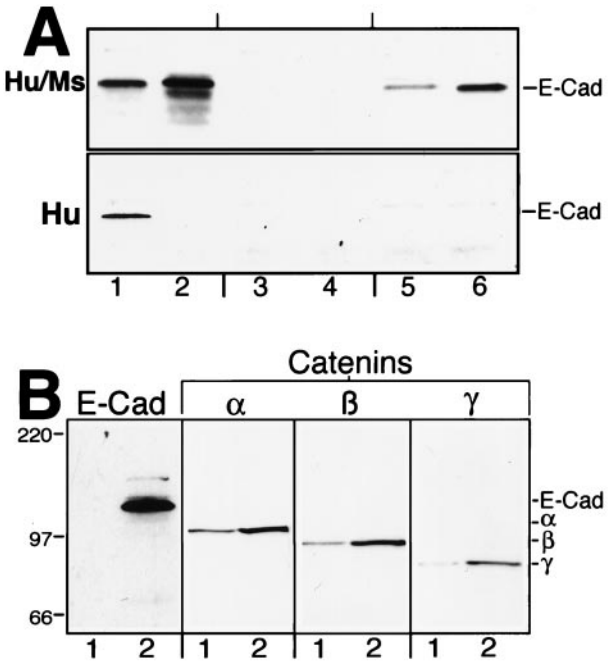

FIG. 1. E-cadherin and catenin expression. (*A*) Whole-cell lysates (37.5 μg total protein) were probed by Western blotting for E-cadherin by using two different mAbs. (*Upper*) An antibody that recognizes both human and mouse (Hu/Ms) E-cadherin was used. (*Lower*) An antibody that recognizes only human (Hu) E-cadherin was used. Lanes: 1, OVCAR-3 human ovarian carcinoma cells (positive control for human E-cadherin); 2, scp2 mouse mammary epithelial cells (positive control for mouse E-cadherin); 3, IOSE-29; 4, IOSE-29neo; 5, IOSE-29preEC; 6, IOSE-29EC. (*B*) Parental IOSE-29 (lane 1) and IOSE-29EC (lane 2) cell lysates were probed by Western blotting for the 120-kDa E-cadherin protein (100 μg total protein) and the 102-kDa α-catenin, 94-kDa β-catenin, and 86-kDa γ-catenin proteins (20 μg total protein). See *Materials and Methods* for details of cell lines and antibodies.

fected IOSE-29 cells and sham-transfected IOSE-29neo cells did not generate epithelial populations.

To rule out the possibility that the IOSE-29EC cells might have represented a minor variant of the parental population that expressed endogenous human E-cadherin rather than the exogenous mouse E-cadherin cDNA, we carried out Western blotting with two different E-cadherin antibodies (Fig. 1*A*). The blots were probed with an antibody that recognizes both human and mouse E-cadherin (Fig. 1*A Upper*). Control cell lines that express human (lane 1) and mouse (lane 2) E-cadherin were both positive when using this antibody, parental IOSE-29 (lane 3) and IOSE-29neo (lane 4) cells were both negative, and IOSE-29pre-EC (lane 5) and IOSE-29EC (lane 6) cells were both positive. In Fig. 1*A* (*Lower*), which was probed with an antibody that specifically recognizes only human E-cadherin (34), none of the OSE cell lines, either untransfected or transfected, was positive. Therefore, we conclude that the IOSE-29pre-EC and IOSE-29EC cells expressed exogenous mouse E-cadherin. Interestingly, however, we found that after repeated passaging, IOSE-29EC, but not untransfected IOSE-29 or the sham-transfected IOSE-29neo cells, began to express human E-cadherin (data not shown). This raises the intriguing possibility that E-cadherin can positively regulate its own expression in OSE cells.

When Western blots were overloaded with 100 μg of total protein we still could not detect the 120-kDa E-cadherin in the parental IOSE-29 cells (Fig. 1*B*), and the protein was not observed by immunofluorescence staining (Fig. 2*A*). In contrast, E-cadherin was present and appropriately localized at areas of cell–cell contact in the IOSE-29EC cells (Figs. 1*B* and 2*B*). Although parental IOSE-29 cells contained detectable levels of the 102-kDa α-catenin and the 94-kDa β-catenin (Fig. 1*B*), both proteins were dispersed throughout the cell and they were only discontinuously located at sites of cell–cell contact (Fig. 2 *C* and *E*). The steady-state levels of α-catenin and β-catenin were increased significantly in IOSE-29EC cells (Fig. 1*B*), and both proteins were localized almost exclusively at cell–cell contacts (Fig. 2 *D* and *F*). Parental IOSE-29 cells contained very little of the 86-kDa γ-catenin (Figs. 1*B* and 2*G*), but this protein was induced in the IOSE-29EC cells and it was also located at cell–cell contacts (Fig. 2*H*). F-actin was localized in prominent mesenchymal cytoplasmic stress fibers in IOSE-29 cells (Fig. 2*I*), but it was redistributed to areas of cell–cell contact in IOSE-29EC cells (Fig. 2*J*). The colocalization of E-cadherin, all three catenins, and F-actin at cell–cell contacts indicates that the IOSE-29EC cells formed functional epithelial adherens junctions (11). At no time did we observe epithelial cell–cell junction formation in the sham-transfected IOSE-29neo cells (data not shown).

Adherens junctions link epithelial cells into cohesive, cobblestone monolayers. The mesenchymal IOSE-29 cells were fibroblastic at low cell density (Fig. 3*A*), and at high cell density the bipolar shape of the cells was accentuated (Fig. 3*C*). On the other hand, IOSE-29EC cells were packed closely and epithelial at low cell density (Fig. 3*B*), and at high cell density they formed cobblestone monolayers with bulging hemicystic domes (Fig. 3*D*). The presence of domes suggested that IOSE-29EC cells formed tight junctions (see Fig. 4).

The mesenchymal characteristics of the parental IOSE-29 line were clearly evident in three-dimensional sponge culture (Fig. 3*E*). The cells migrated into the spaces between the sponge spicules, which likely reflects their ability to deposit a stromal extracellular matrix (4, 32). In contrast, IOSE-29EC cells formed simple epithelia that lined the sponge spicules (Fig. 3*F*). Morphological differences between the parent and transfected lines also were apparent in three-dimensional basement membrane gels. Under these conditions, the IOSE-29 cells formed cell aggregates with radiating branching cords that invaded the gel (Fig. 3*G*) whereas IOSE-29EC cells formed tightly packed epithelial spheres with no evidence of invasion (Fig. 3*H*).

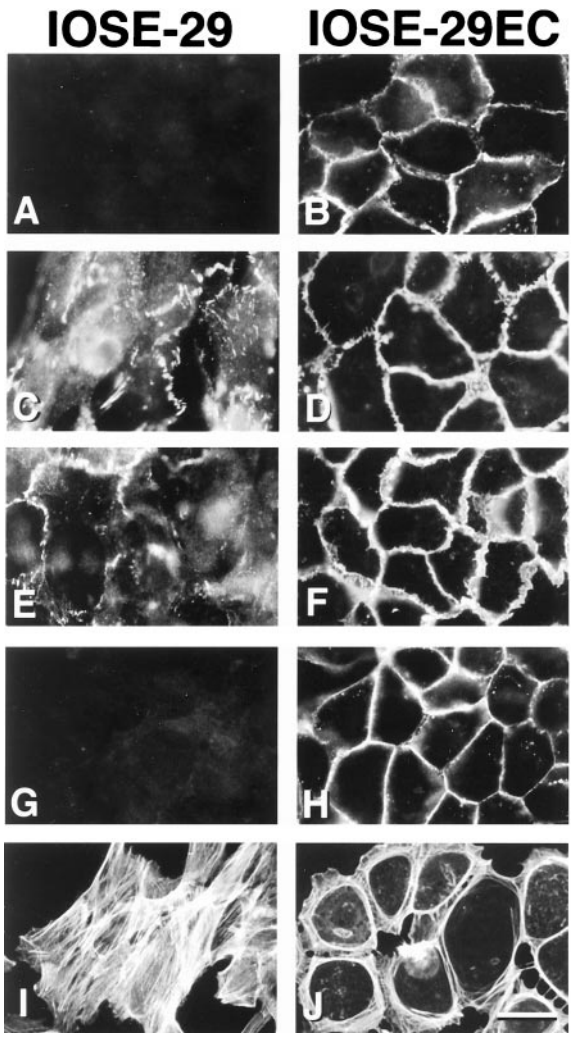

FIG. 2. E-cadherin, catenin, and F-actin localization. IOSE-29 (*A*, *C*, *E*, *G*, and *I*) and IOSE-29EC (*B*, *D*, *F*, *H*, and *J*) cells in subconfluent monolayer culture were stained by immunofluorescence for E-cadherin (*A* and *B*), α-catenin (*C* and *D*), β-catenin (*E* and *F*), and γ-catenin (*G* and *H*). F-actin localization was assessed by rhodamine-phalloidin staining (*I* and *J*). (Bar = 20 μm.)

The morphology of the E-cadherin-transfected cells suggested that they were functionally epithelialized. To confirm this tentative conclusion we examined cytokeratin expression. As expected (8, 26), the mesenchymal IOSE-29 cells did not express CK (Fig. 4 *A* and *C*). The same was true of the sham-transfected IOSE-29neo cells (data not shown). On the other hand, IOSE-29EC cells contained the simple epithelial CK7, CK18, and CK 19 (Fig. 4*A*), which formed classical intermediate filament arrays (Fig. 4*D*). We also examined the tight-junction protein occludin (36). IOSE-29EC cells, but not the parental line, expressed significant amounts of occludin (Fig. 4*B*), which was localized at sites of cell–cell contact (Fig. 4 *E* and *F*). These data, and the formation of domes in monolayer culture, indicated the presence of tight junctions in the epithelialized IOSE-29EC cells.

Unlike Mullerian duct-derived epithelia, normal OSE cells do not express the CA 125 tumor antigen *in vivo* or in culture (25, 33). The same was true of both IOSE-29 (Fig. 5 *A* and *B*) and sham-transfected IOSE-29neo cells (data not shown). In contrast, broad-molecular-mass CA125-containing protein species between approximately 500 and 700 kDa were found in IOSE-29EC cell lysates and conditioned medium (Fig. 5*A*). It

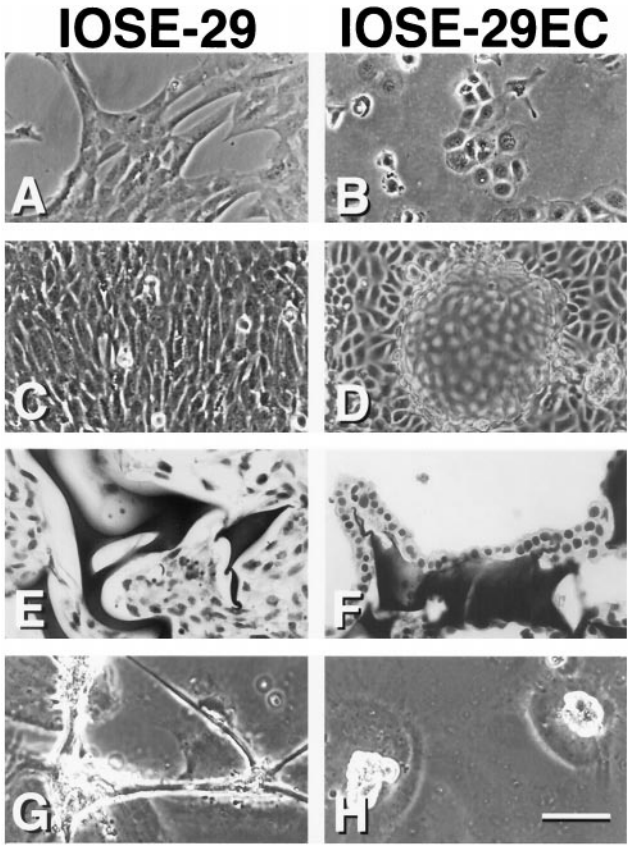

FIG. 3. Culture morphology. IOSE-29 (*A*, *C*, *E*, and *G*) and IOSE-29EC (*B*, *D*, *F*, and *H*) cells were maintained in two-dimensional monolayer culture at low cell density (*A* and *B*), two-dimensional monolayer culture at high cell density (*C* and *D*), in three-dimensional sponge culture (*E* and *F*), and in three-dimensional culture in Matrigel (*G* and *H*). All cultures were photographed live under phase microscopy except *E* and *F*, which were fixed, sectioned, stained with hematoxylin and eosin, and photographed under bright field. (Bar = 100 μm.)

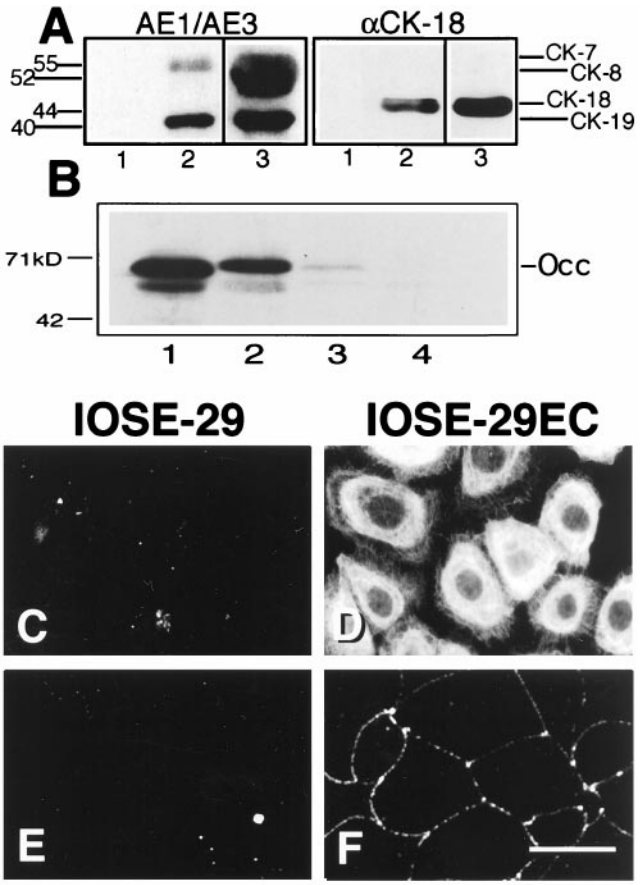

FIG. 4. Cytokeratin and occludin expression and localization. (*A*) Cytoskeletal preparations were probed by Western blotting using a combination of AE1/AE3 antibodies that recognize all cytokeratins except CK-18, or with a specific anti-CK 18 antibody. Lanes: 1, IOSE-29; 2, IOSE-29EC; 3, C4II cervical carcinoma cells (positive control). (*B*) Whole-cell lysates were probed for the 65-kDa tight-junction protein occludin. Lanes: 1, MDCK kidney epithelial cells (positive control); 2, IOSE-29EC; 3, IOSE-29; 4, 3T3 fibroblasts (negative control). Subconfluent monolayer cultures of IOSE 29 (*C* and *E*) and IOSE-29EC (*D* and *F*) cells were stained by immunofluorescence for all cytokeratins by using a pan keratin antibody (*C* and *D*) and for occludin by using a mAb (*E* and *F*). (Bar = 20 μm.)

should be noted, however, that the CA125 shed into the medium by IOSE-29EC cells was qualitatively different from that produced by OVCAR-3 carcinoma cells, which suggests that there may be differences in CA125 glycosylation by the two cell lines. The CA125 tumor antigen also was detected on the surface of IOSE-29EC cells by immunocytochemistry (Fig. 5*C*) and in cell lysates and conditioned medium by using the microparticle enzyme immunoassay (Table 1). The latter levels were comparable to those of OVCAR-3 carcinoma cells. Therefore, the evidence was overwhelming that CA125 was being produced by the epithelialized IOSE-29EC cells, indicating that exogenous E-cadherin expression induced an aberrant Mullerian duct-like differentiation that is a hallmark of OSE metaplasia and neoplasia.

DISCUSSION

The genetic basis for familial predisposition to epithelial ovarian cancer is now being elucidated (36–39). However, in both familial and sporadic ovarian cancers, definitive premalignant lesions have not been identified. As a result, at the time of diagnosis the disease often has progressed to its later stages and the prognosis is invariably poor. In the present study we examined the basis of the metaplastic and early neoplastic changes that occur in the OSE, the presumptive target tissue in ovarian carcinogenesis, in an effort to identify early predictive markers of these neoplasms. The results present an unexpected role for E-cadherin in carcinogenesis and identify a differentiation-related step that seems to contribute to early ovarian neoplastic progression.

The OSE arises from the urogenital coelomic epithelium, as do the Mullerian duct-derived epithelia that line the oviduct, endometrium, and endocervix. However, unlike Mullerian duct-derived epithelia, normal OSE rarely contains the epithelial cell–cell adhesion molecule E-cadherin (5–7). Normal OSE do express N-cadherin (23, 40), which is a cell-adhesion molecule that can cause cell–cell junction disassembly and cell migration when it is expressed inappropriately (41). Thus, the presence of N-cadherin in normal OSE cells may contribute to their propensity to undergo mesenchymal conversion in culture. In this study, the mesenchymal characteristics of the normal IOSE-29 line were readily apparent in three-dimensional sponge culture in which fibroblastic, bipolar cells migrated into the spaces between the sponge spicules.

In vivo, Mullerian metaplasia of OSE is common, and it usually has little or no pathological consequence (2). However, the incidence of metaplasia is increased in women with a familial predisposition to ovarian cancer, and both dysplasia and rare cases of carcinoma have been observed within these lesions (9). In contrast to normal OSE, metaplastic OSE and primary ovarian carcinomas both contain E-cadherin (5–7). When we produced cells that expressed high levels of E-

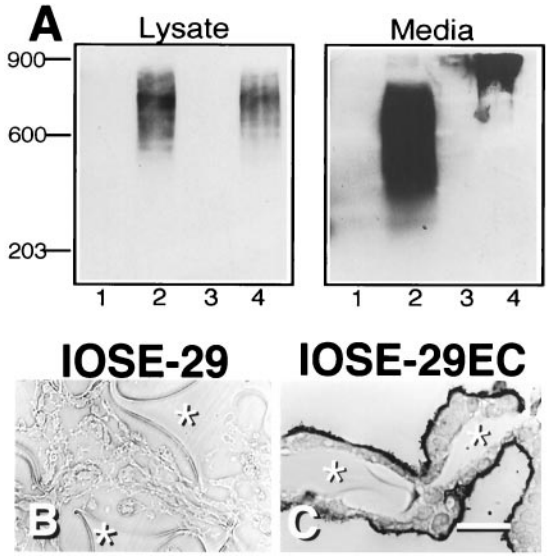

FIG. 5. CA125 expression and localization. (*A*) Whole-cell lysates or conditioned media were assayed by Western blotting for the CA125 antigen under nondenaturing conditions. Lanes: 1, IOSE-29; 2, IOSE-29EC; 3, 3T3 fibroblasts (negative control); 4, OVCAR-3 ovarian carcinoma cells (positive control). IOSE 29 (*B*) and IOSE-29EC (*C*) cells were maintained in three-dimensional sponge culture and stained for CA125 by immunocytochemistry. Note that IOSE-29 cells, which filled the spaces between the sponge spicules (asterisks), were CA125-negative whereas IOSE-29EC cells, which lined the spicules, were CA125-positive at their apical surfaces. (Bar = 40 μm.)

cadherin, they were no longer fibroblastic. Instead, these cells, designated IOSE-29EC, formed closely packed epithelial monolayers. Several lines of evidence indicate that the epithelialization of IOSE-29EC cells was caused by expression of the transfected mouse E-cadherin cDNA and not by the selection of a preexisting subpopulation of metaplastic cells present in the parental line: (*i*) no E-cadherin positive cells were ever observed in the parental IOSE-29 or sham-transfected IOSE-29neo lines; (*ii*) differential adhesion of IOSE-29 and IOSE-29neo cells never yielded epithelial colonies; (*iii*) cytokeratins and/or CA125 were never observed in IOSE-29 and IOSE-29neo lines, although these markers characterize the IOSE-29EC line (see below); (*iv*) low levels of E-cadherin were present in IOSE-29preEC cells before differential adhesion; and (*v*) the E-cadherin present in IOSE-29pre-EC and IOSE-29EC cells was the exogenous mouse, not the endogenous human, protein.

Exogenous E-cadherin expression causes a partial epithelial transformation of true fibroblasts, at least with respect to cell–cell junction formation (42, 43). In contrast, epithelial cell junctions were fully developed in IOSE-29EC cells as judged by the following criteria: f-actin was found in plasma membrane-associated circumferential rings; E-cadherin, α-catenin, β-catenin, and γ-catenin all colocalized with the F-actin rings in adherens junctions; and occludin was up-regulated and localized to sites of cell–cell contact, indicating the formation of secondary tight junctions. Functionally, these epithelial cell junctions supported vectorial ion transport as IOSE-29EC formed hemicystic domes in high-density monolayer culture. Such domes are never found in cultures of normal mesenchymal OSE, but they are common in ovarian carcinoma cultures. IOSE-29EC cells also reexpressed cytokeratins, which are found in normal OSE *in vivo* (8), and they formed epithelial linings on sponge spicules that were reminiscent of metaplastic OSE trapped within surface invaginations and inclusion cysts.

Table 1. CA125 levels in cell lysates and conditioned medium

Cell lines	CA 125, units/ml	
	Cell lysates	Cell medium
3T3 (negative)	<4	<4
OVCAR-3 (positive)	60,000	7,000
IOSE-29	<4	41
IOSE-29EC	82,000	6,000

Cells were grown to subconfluence and lysed (cell lysates), or the medium was changed and collected 1 day later (culture medium). One hundred micrograms of cell lysate and 300 μg of medium then were assayed for CA125 by microparticle enzyme immunoassay.

In many types of epithelial tumors, down-regulation or mutation of E-cadherin is associated with an increased invasiveness that can be prevented by the forced expression of the cell-adhesion molecule. This suggests that E-cadherin is a late-stage tumor suppressor that prevents invasion and metastasis (14). Because E-cadherin is expressed in most epithelia, it is assumed that its presence in primary, differentiated tumors is a characteristic of the target tissue that has been retained during the early stages of carcinogenesis. However, the frequent presence of E-cadherin in primary ovarian carcinomas (18–23) must be acquired during carcinogenesis because it is rarely present in normal OSE (5–7). Although the subsequent loss of E-cadherin undoubtedly plays a role in late-stage ovarian metastasis, our data suggest that ectopic expression of the cell-adhesion molecule initiates early preneoplastic changes by committing OSE cells to a more complex epithelial phenotype that restricts their mesenchymal potential. From a mechanistic point of view this phenotypic modulation could be mediated by changes in the subcellular localization of β-catenin (11–13).

An important phenotypic consequence of OSE epithelialization in IOSE-29EC cells was the secretion of the CA125 tumor antigen. *In vivo*, CA125 is produced by metaplastic OSE, and high levels of the antigen in a patient's serum is an indicator of ovarian carcinoma (25). We recently have produced a second E-cadherin-transfected IOSE line derived independently from a separate normal human donor that also produces CA125 (data not shown). Thus, the E-cadherin-induced CA125 tumor antigen production observed in IOSE-29EC cells was not a unique, isolated event.

The CA125 tumor antigen is a normal differentiation product of oviductal, endometrial, and endocervical epithelia. Therefore, our finding that E-cadherin-induced CA125 production in OSE cells puts to rest any lingering doubts regarding this tissue's ability to acquire the Mullerian characteristics observed in metaplastic OSE and ovarian carcinomas. The mechanism by which E-cadherin induces CA125 production in OSE cells remains unclear because the gene that codes the core protein has not yet been cloned, probably because, until now, it has not been possible to regulate the gene's expression. Taken together, our results demonstrate that the expression of exogenous E-cadherin in mesenchymal IOSE-29 cells not only restored the epithelial properties of normal OSE but, in addition, induced novel characteristics of metaplastic and neoplastic OSE such as tight junctions and CA125 production. Thus, as is not the case in the great majority of other epithelial tissues, the ectopic expression of E-cadherin frequently observed in metaplastic and neoplastic OSE may play a functional role in the earliest stages of ovarian tumor development.

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