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Estrogens increase the expression of fibulin-1, an extracellular matrix protein secreted by human ovarian cancer cells

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ABSTRACT **Ovarian cancers have a high ability to invade the peritoneal cavity and some are stimulated by estrogens. In an attempt to understand the mode of action of estrogens on these cancer cells and to develop new markers, we have characterized estrogen-regulated proteins. This study was aimed at identifying a protein secreted by ovarian cancer cells whose level was increased by estradiol [Galtier-Dereure, F., Capony, F., Maudelonde, T. & Rochefort, H. (1992) *J. Clin. Endocrinol. Metab. 75,* 1497–1502]. By using microprotein sequencing, the 110-kDa protein was identified as fibulin-1, a protein of the extracellular matrix that binds to fibronectin, laminin, and nidogen. The amount of immunoprecipitated fibulin-1 secreted into the medium and present in the cell extract was increased up to 10-fold by estradiol in three estrogen-responsive ovarian cancer cell lines. By immunohistochemistry fibulin-1 was located in the stroma of several ovarian cancers and cysts. The findings highlight a potential role for fibulin-1 in the spread of ovarian cancer in the peritoneal cavity and/or in distal metastases.**

Epithelial ovarian cancer is the leading cause of death from gynecological cancer in western countries. Contributing to the high mortality is the propensity for local spread of cancer cells within the peritoneal cavity and in lymphatic vessels and their hidden location, which often accounts for late diagnosis (1). Therefore, there is an urgent need to define the components involved in invasion of the peritoneal cavity by ovarian cancer cells in order to develop new therapies and new marker(s) allowing earlier detection of the disease (2).

One approach proven to be effective in defining markers involved in metastasis and invasion of breast cancer has been to identify estrogen-regulated proteins in estrogen-responsive breast cancer cell lines (3, 4). Assays in the primary tumors of some of these proteins (progesterone receptor, cathepsin D, pS2) have proved to be useful as prognostic indicators (4, 5). A similar approach can be used for defining ovarian cancer markers by using the available estrogen receptor-positive epithelial ovarian cancer cell lines (6).

Several factors have been proposed to facilitate the emergence of epithelial ovarian cancers, including the number of ovulations (1). The role of estrogens in ovarian cancers is more controversial than for endometrial and breast cancers. However, several studies point to estrogens having a facilitative role in ovarian cancer. The estrogen receptor, which is mandatory for estrogen action (7), is expressed in >60% of ovarian cancers (8). The growth of human ovarian cancer cell lines containing estrogen receptors is stimulated in culture by estrogen, as is that of breast cancer cell lines (6, 9, 10). Estrogens also increase expression of genes associated with cell proliferation such as c-*myc* (11) or with metastasis such as the gene for cathepsin D, a lysosomal protease (10). Recently, a prospective epidemiological study on 240,000 U.S. postmenopausal women has shown that estrogen replacement therapy increased the risk of ovarian cancer by 40% after 4 years and 70% after 11 years of treatment (12). Since estrogen replacement therapy of menopause is increasingly used in western countries, it is critical to specify the role of estrogen in carcinogenesis. Our approach is to identify the genes that are under estrogen control and to define the function of the corresponding proteins.

A major secreted protein induced by estrogen, but not by other classes of steroids, in the BG1 ovarian cancer cells has been previously described (10). In this manuscript, we report the identification of this protein as fibulin-1, an extracellular matrix and plasma protein (13, 14).

MATERIALS AND METHODS

Cell Culture. The three following estrogen receptor-positive human ovarian adenocarcinoma cell lines used in this study displayed characteristics of epithelial cells. The BG1 ovarian carcinoma cell line was derived from the primary tumor tissue of a stage II, poorly differentiated ovarian adenocarcinoma. Tumor cells were stained with cytokeratin antibody but not with vimentin-specific antibody (9). This cell line obtained from C. E. Welander (Emory University, Atlanta) was cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (vol/vol) fetal calf serum (FCS). The PEO4 cell line, derived from ascites of a stage III serous ovarian adenocarcinoma, displayed ultrastructural characteristics of epithelial cells (15). This cell line, obtained from Thomas Hamilton (Fox Chase Cancer Center, Philadelphia), was cultivated in RPMI 1640 medium supplemented with 10% FCS and 10 μg of insulin per ml (6). The SKOV3 adenocarcinoma cell line, which also contains estrogen receptors (16), was obtained from the American Type Culture Collection and was cultivated in DMEM containing 10% FCS. All culture media contained 0.5% gentamycin.

Metabolic Labeling of Estrogen-Regulated Proteins. The effects of estrogen on metabolically labeled proteins were examined as described (4, 10). Cells were deprived of steroids in phenol red-free medium supplemented with 5% dextran-coated charcoal (DCC)-stripped FCS for 5 days and then treated with 10 nM 17β-estradiol (E_2) or the control ethanol vehicle for 2 days. Proteins synthesized by ovarian carcinoma cells were labeled as

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Abbreviations: E_2, 17β-estradiol; FCS, fetal calf serum; mAb, monoclonal antibody.

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follows: cultured cells were washed four times with phosphate-buffered saline (PBS), and about 10^6 cells were incubated for 6 hr in 2 ml of 9:1 (vol/vol) methionine- and cysteine-free DMEM/complete DMEM containing 200 μCi (1 μCi = 37 kBq) of [^{35}S]methionine/[^{35}S]cysteine (Amersham).

Isolation and Protein Sequencing of p110. BG1 cells grown in T175 culture flasks with serum containing DMEM were washed four times with 10 ml of PBS. Secreted proteins were then collected in 15 ml of DMEM without serum for 6 hr. One milliliter of ^{35}S-labeled conditioned medium was added as a radiolabeled tracer to 15 ml of unlabeled conditioned medium, and the mixture was centrifuged at 1800 × *g* for 5 min and made 1 mM in phenylmethylsulfonyl fluoride. Cell debris was removed by centrifugation with a Beckman ultracentrifuge and a Ti 50 rotor at 20,000 rpm for 10 min at 4°C. Sodium dodecyl sulfate (SDS) was added to the clarified conditioned medium to a final concentration of 0.02%, and the solution was concentrated 320-fold by using a Centriprep that retains material ≥ 30,000 M_r (Amicon). Ten microliters of the concentrated sample in Laemmli sample buffer was electrophoresed in an SDS-containing 8% polyacrylamide gel. The proteins in the gel were electrotransferred onto either Immobilon (Millipore) or nitrocellulose membranes at 2 mA for 2 hr in a Tris glycine buffer containing 20% (vol/vol) methanol (17). The major 110-kDa protein secreted from BG1 cells was located by autoradiography and by Ponceau red (0.5%) staining of the membranes. The region of the Immobilon membrane containing the 110-kDa protein from four lanes of the SDS gel was directly submitted to Edman N-terminal sequential analysis in an on-line 470 A protein sequencer coupled to a 120 A phenylthiohydantoin analyzer (Perkin–Elmer), both operated according to the manufacturer's recommendations. The region of the nitrocellulose membrane containing the 110-kDa protein was submitted to *in situ* tryptic digestion. The polypeptides liberated from the membrane by digestion were fractionated by reverse-phase HPLC on a 2 × 100 mm Brownlee C_8 column (Perkin–Elmer) eluted with a linear acetonitrile gradient in 0.1% trifluoroacetic acid, while monitoring the UV absorbance of the eluate at 220 nm. The resulting peptides were further purified by a Brownlee C_{18} column under the same conditions. Selected peptides were analyzed by the same sequencing system, and sequences were compared to those in the Swiss-Prot protein data bank by using the FASTA algorithm (18).

Immunoprecipitation of Secreted Proteins. ^{35}S-labeled proteins within the conditioned medium or in detergent cell extracts were immunoprecipitated as described (13) with mouse fibulin-1 monoclonal antibody (mAb 3A11) at 25 μg/ml or with affinity-selected rabbit polyclonal (rb2954) fibulin-1 IgG or control antibodies at 25 μg/ml. The 3A11 mAb was generated as part of the studies reported in ref. 13. Its epitope has been mapped to the N-terminal portion of fibulin-1 (data not shown). Immune complexes were analyzed by electrophoresis in an SDS-containing 8% polyacrylamide gel followed by autoradiography of the gel.

Glycosidase Treatment and Reduction of Secreted Proteins. The ^{35}S-labeled proteins in an aliquot of conditioned medium from BG1 cells were incubated for 24 hr at 37°C with 1 unit of endoglycosidase F (Boehringer Mannheim) per ml.

For analysis under reducing conditions, 10 μl of ^{35}S-labeled protein-containing conditioned medium was reduced with 20 mM dithiothreitol and alkylated with iodoacetamide as described (17). For preparation of the nonreduced sample, the labeled conditioned medium was added to an equal volume of Laemmli sample buffer without reducing agent. All samples were analyzed by SDS/PAGE (8% polyacrylamide gel) followed by autoradiography of the gels.

Fibronectin-Conjugated Sepharose Affinity Chromatography. An affinity matrix was prepared by coupling human plasma fibronectin (Sigma) to CNBr-activated Sepharose (Pharmacia). The resulting matrix contained about 3 mg of fibronectin per ml of Sepharose. ^{35}S-labeled conditioned medium (100 μl) from BG1 cells was preadsorbed on Sepharose 4B and then incubated with fibronectin-Sepharose by mixing overnight at 4°C. The column was washed with 20 volumes of TBS (150 mM NaCl/50 mM Tris, pH 7.4), and bound proteins were sequentially eluted with 2 column volumes of 10 mM EDTA followed by two column volumes of 8 M urea as described (13). The fractions were then concentrated 25-fold by using a Centriprep and were analyzed by SDS/PAGE.

Tissue Samples. Ovarian tissue samples were obtained from the Department of Gynecology (F. Laffargue) after surgical treatment of primary ovarian tumors. They were immediately fixed in FAAM [formaldehyde (40%) (0.1 vol/1 vol), acetic acid (100%) (0.1 vol/1 vol), and methanol (100%) (0.4 vol/1 vol) in distilled water] for at least 24 hr and embedded in paraffin (in the Department of Pathology; P. Baldet). Paraffin sections were pretreated with Pronase before being stained by using a standard avidin–biotin-enhanced immunoperoxidase technique (Vectastin Elite kit, Vector Laboratories) and counterstained with 5% Harris hematoxylin. The mouse mAb 3A11 (2 μg/ml in PBS) was incubated with tissue sections for 1 hr at room temperature. The specificity of immunostaining was verified by using the same concentration of mouse mAb of the same subclass (IgG2a, UPC10; Sigma). In some cases, tissues were immediately frozen in liquid nitrogen and stored at −80°C. Frozen sections were stained by using the 3A11 antibody at 1 μg/ml.

RESULTS

A 110-kDa E_2-Induced Protein Is Secreted by Different Ovarian Cancer Cell Lines. To determine whether the E_2-regulated protein previously described in BG1 ovarian carcinoma cells (10) is commonly found, we examined other estrogen receptor-positive ovarian carcinoma cell lines. The level of a secreted protein of about 110 kDa was increased in PEO4 cells (6) treated with E_2 (Fig. 1). Its electrophoretic mobility was similar to that of the protein released by BG1 cells. The E_2-responsive protein released from PEO4 and BG1 cells had a similar pattern of glycosylation, since endoglycosidase F treatment caused a similar shift in migration of about 5 kDa (Fig. 1*b*).

Purification and Peptide Sequencing of p110 Secreted from BG1 Cells. To identify the 110-kDa protein by microprotein sequencing, it was purified as follows: 30 ml of medium from BG1 cells conditioned by about 40 million cells for 6 hr was

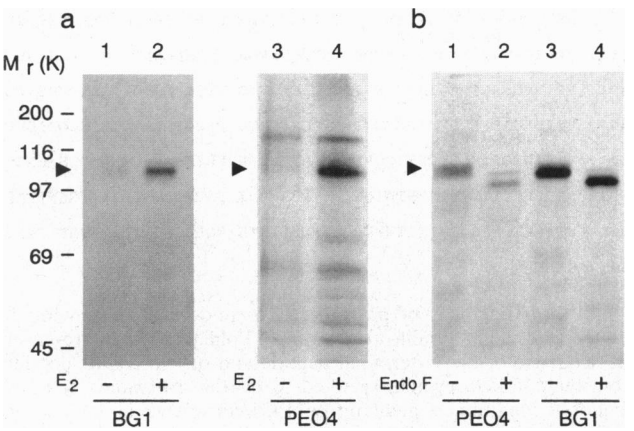

FIG. 1. [^{35}S]Methionine-labeled proteins secreted by two ovarian carcinoma cell lines (BG1 and PEO4) and analyzed by SDS/PAGE and autoradiography. The arrowheads mark the position of the 110-kDa secreted protein in this and subsequent figures. (*a*) Effect of E_2 treatment of cells versus control (ethanol vehicle-treated cells). (*b*) Effect of endoglycosidase F treatment of the conditioned medium. Sizes are shown in kDa.

clarified, concentrated, and resolved in an SDS-containing polyacrylamide gel, and the proteins from the gel were electroblotted onto Immobilon or nitrocellulose. The region containing p110 was clearly resolved as visualized by Ponceau red staining and detected by autoradiography of the radiolabeled tracer. An estimated 3 μg of p110 was obtained per ml of conditioned medium. Amino acid sequences from the N terminus and two internal peptides were obtained and compared with known sequences in the Swiss-Prot data base (Fig. 2). The N-terminal sequence indicated that the protein recovered from Immobilon was a single homogeneous protein. All three peptide sequences were identical to sequences of the mature secreted human fibulin-1 (14).

Properties of p110 Are Similar to Those of Human Fibulin-1. A characteristic property of fibulin-1 is its ability to bind fibronectin (19). To examine whether p110 released from BG1 cells bound specifically to fibronectin, we incubated ^{35}S-labeled conditioned medium with Sepharose or with fibronectin coupled to Sepharose. While there was no ^{35}S-labeled protein eluted from the Sepharose (Fig. 3, lane 2), the p110 was eluted specifically from fibronectin-Sepharose (Fig. 3, lanes 3 and 4). Another characteristic feature of fibulin-1 is its electrophoretic response to reduction (14, 19). Lanes 3 and 4 in Fig. 3 show increased electrophoretic migration in gels of the protein in the nonreduced state compared with the reduced and alkylated form of the protein. Therefore, p110 is stabilized by intramolecular disulfide bonds (14, 19).

Immunoprecipitation Analysis of Secreted Proteins from Ovarian Carcinoma Cells. To confirm the identity of p110 released from ovarian carcinoma cells as human fibulin-1, we examined the immunological cross reactivity of p110 with monoclonal and polyclonal fibulin-1 antibodies (13, 14). Both types of antibodies were shown to specifically immunoprecipitate a ^{35}S-labeled protein secreted from the BG1 cells with electrophoretic mobility corresponding to p110 (Fig. 4*a*). Moreover, the level of immunoprecipitated fibulin-1 increased in both the medium and the cell extracts in response to E_2, indicating that synthesis of fibulin-1 was also stimulated (Fig. 4*b*). In PEO4 and SKOV3 ovarian carcinoma cell lines, the secreted fibulin-1 was also found to be up-regulated by E_2 (Fig. 4*c*). The extent of induction by E_2 was about 10-fold in BG1 and PEO4 cell lines and less in SKOV3. However, the maximal level of fibulin-1 secreted from PEO4 and SKOV3 cells was about 1/10th that from BG1 cells. These data confirmed the

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    DVLLEAXXAD GHRMATHQK
  1 DVLLEACCAD GHRMATHQKD CSLPYATESK ECRMVQEQCC HSQLEELHCA
 51 TGISLANEQD RCATPHGDNA SLEATFVKRC CHCCLLGRAA QAQGQSCEYS
101 LMVGYQCGQV FRACCVKSQE TGDLDVGGLQ ETDKIIEVEE EQEDPYLNDR
151 CRGGGPCKQQ CRDTGDEVVC SCFVGYQLLS DGVSCEDVNE CITGSHSCRL
201 GESCINTVGS FRCQRDSSCG TGYELTEDNS CKDIDECESG IHNCLPDFIC
251 QNTLGSFRCR PKLQCKSGFI QDALGNCIDI NECLSISAPC PIGHTCINTE
                  GYHLNE EGTR
301 GSYTCQKNVP NCGRGYHLNE EGTRCVDVDE CAPPAEPCGK GHRCVNSPGS
        TGYY FDGI8R
351 FRCECKTGYY FDGISRMCVD VNECQRYPGR LCGHKCENTL GSYLCSCSVG
401 FRLSVDGRSC EDINECSSSP CSQECANVYG SYQCYCRRGY QLSDVDGVTC
451 EDIDECALPT GGHICSYRCI NIPGSFQCSC PSSGYRLAPN GRNCQDIDEC
501 VTGIHNCSIN ETCFNIQGAF RCLAFECPEN YRRSAATRCE RLPCHENREC
551 SKLPLRITYY HLSFPTNIQA PAVVFRMGPS SAVPGDSMQL AITGGNEEGF
601 FTTRKVSPHS GVVALTKPVP EPRDLLLTVK MDLSRHGTVS SFVAKLFIFV
651 SAEL
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FIG. 2. Alignment of p110-derived peptide sequences with the sequence of human fibulin-1 (isoform C). Bolded characters represent the sequence of the N-terminal peptide and of two tryptic peptides from the 110-kDa protein secreted by ovarian carcinoma cells. An aliquot of conditioned medium from BG1 cells grown in the presence of E_2 was resolved in SDS-containing polyacrylamide gels and electrotransferred onto nitrocellulose or Immobilon filters. The ponceau red-stained bands corresponding to p110 were excised, and the membrane-bound protein was subjected to *in situ* tryptic digestion. The enzymatically released peptides were resolved by HPLC and sequenced. From N-terminal sequence determination, an Immobilon membrane containing p110 was directly subjected to Edman degradation.

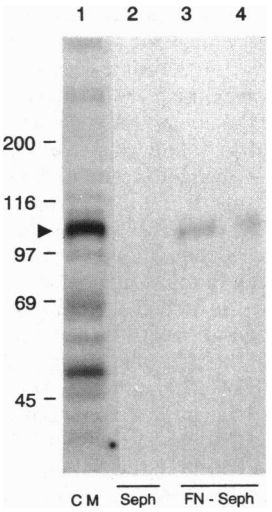

FIG. 3. Fibronectin-Sepharose affinity chromatography selects p110 from the conditioned medium of BG1 cells. Shown in lane 1 is the SDS/PAGE profile of an aliquot of conditioned culture medium (CM) from BG1 cells metabolically labeled with [^{35}S]cysteine/[^{35}S]-methionine. The conditioned medium was preabsorbed on a column of Sepharose 4B and then applied to a column of fibronectin-Sepharose. Bound proteins that were eluted with 8 M urea from the Sepharose 4B column (lane 2) and the fibronectin-Sepharose column (lanes 3 and 4) were electrophoresed on SDS-containing polyacrylamide gels in the absence (lanes 2 and 3) and presence (lane 4) of reducing agent. Sizes are shown in kDa.

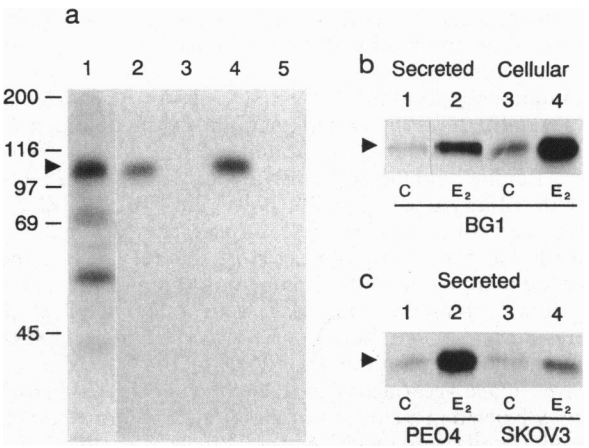

FIG. 4. Immunoprecipitation of ^{35}S-labeled proteins from ovarian carcinoma cells with anti-fibulin-1 antibodies. (*a*) Medium conditioned by BG1 cells was analyzed directly (10 μl) (lane 1), or 100 μl was subjected to immunoprecipitation with anti-fibulin-1 polyclonal antibody from a nonimmunized rabbit (lane 2), control polyclonal antibody (lane 3), anti-fibulin-1 mAb (3A11) (lane 4), or control mAb (lane 5). (*b*) Immunoprecipitation by anti-fibulin-1 polyclonal antibodies of ^{35}S-labeled protein from the conditioned medium (150 μl) (lanes C) or cell extract (10^5 cells) of BG1 cells treated or not with E_2 (lanes E_2). (*c*) One milliliter of conditioned medium from PEO_4 cells (lanes 1 and 2) or $SKOV_3$ cells (lanes 3 and 4) treated (lanes E_2) or not (lanes C) with E_2 was subjected to immunoprecipitation with anti-fibulin-1 polyclonal antibodies. Washed immunocomplexes were analyzed by SDS/PAGE and autoradiography. Sizes are shown in kDa.

identity of the estrogen-stimulated p110 secreted by ovarian cancer cells as the extracellular matrix protein, fibulin-1.

Immunohistochemical Staining of Fibulin-1 in Ovarian Cancer Tissue. mAb 3A11 to fibulin-1 was used to stain frozen and paraffin-embedded sections from human ovarian tumor samples. Fibulin-1 immunostaining was exclusively localized to stroma surrounding islets of ovarian cancer cells (Fig. 5 *a* and *c*). In the four ovarian serous adenocarcinomas examined, no staining was observed in epithelial cancer cells. In two serous benign cystadenoma, stroma was also exclusively stained. Staining was less than in ovarian cancer. Maximal staining was often observed at the basal membrane level (Fig. 5*d*).

DISCUSSION

We have found in three hormone-responsive ovarian carcinoma cell lines that E_2 stimulates the secretion of fibulin-1, an extracellular matrix and blood protein. The identification is based on peptide sequencing of the 110-kDa protein secreted by BG1 cells and its specific immunoprecipitation by antibodies to human fibulin-1.

Together with fibulin-2, fibulin-1 belongs to an emerging family of extracellular matrix and blood proteins located in a variety of connective tissues of the embryo and adult (20–22). Fibulin-1 is a modular glycoprotein with two types of cysteine-containing repeat elements—one element that is repeated three times, which is similar to complement anaphylotoxins, and nine elements that are homologous to epidermal growth factor (13). Molecular cloning has revealed alternative transcripts that encode A, B, C, and D forms that differ in their C-terminal regions (13, 22, 23). The peptide sequences obtained from fibulin-1 secreted by BG1 cells and the antibodies used here did not allow us to determine which form is expressed in the ovarian carcinoma cells. However, recently, RNA hybridization to specific cDNA probes prepared by reverse transcriptase–PCR showed that BG1 cells mostly

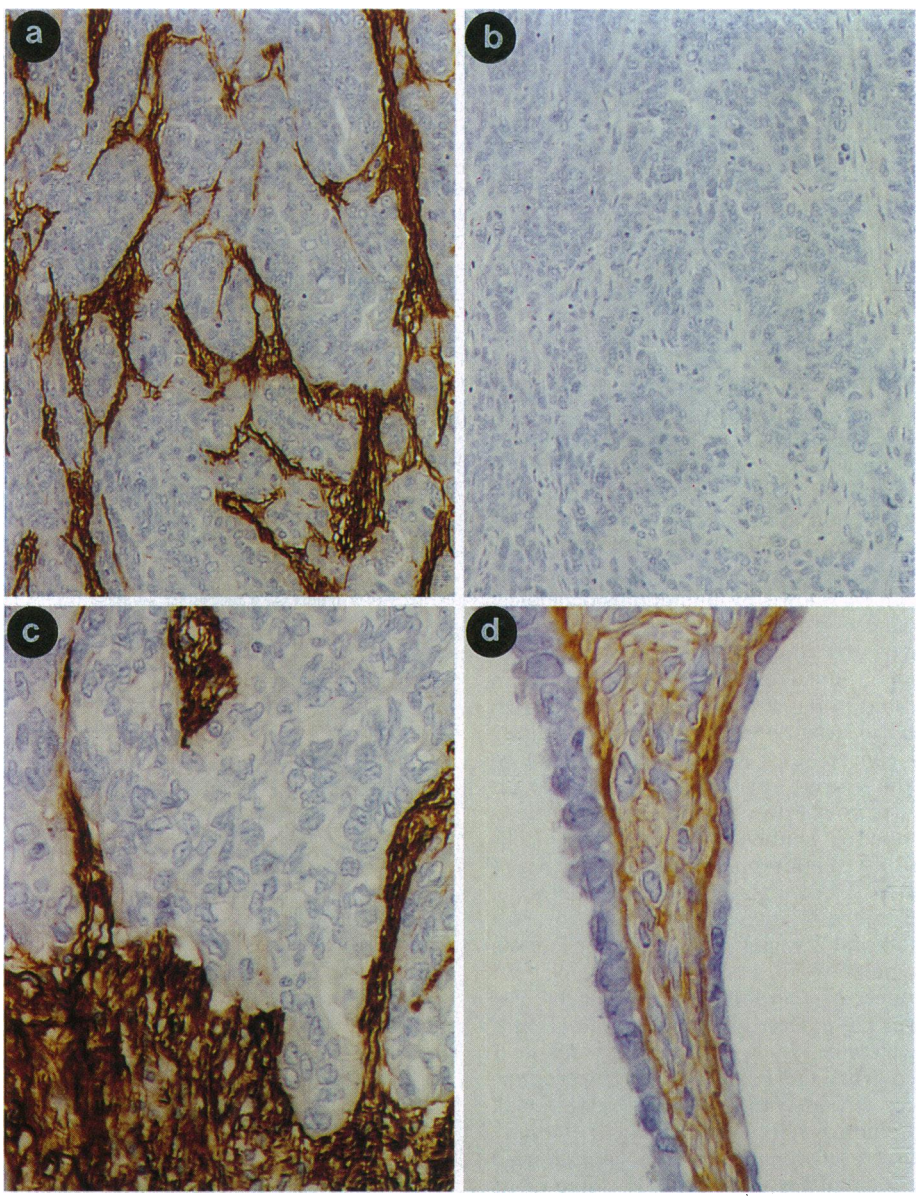

FIG. 5. Immunoperoxidase staining of fibulin-1 in paraffin-embedded sections of a representative ovarian epithelial cancer (serous adenocarcinoma) at stage III (*a*, *b*, and *c*) and a benign serous cystadenoma (*d*). (*a*) Strong immunostaining, in brown, of the peritumoral stroma with the 3A11 anti-fibulin mAb. Fibulin-1 was not detected in epithelial cancer cells, which were counterstained in blue by hematoxylin. (×150.) (*b*) An adjacent section treated with control mAb of the same class as 3A11 anti-fibulin-1 mAb (IgG2a, κ chains) showed no staining. (×150.) (*c*) High magnification of the same ovarian cancer as in *a*, showing fibulin-1 staining in stroma but not in cancer cells. (×295.) (*d*) Benign ovarian (serous cystadenoma) showing two cysts. Staining for fibulin-1 is only observed in stroma with stronger staining at basement membrane level. (×590.)

(95%) produced form C fibulin-1 (P. Pujol, C.R., and H.R., unpublished data).

Since we do not yet know the function of fibulin-1 *in vivo*, we can only speculate as to the significance of increased expression of fibulin-1 by ovarian cancer lines in response to estrogen. Fibulin-1 is known to be a component of basement membranes and connective tissue matrix fibers (20–22). The ability of fibulin-1 to associate with matrix structures such as elastic fibers or basement membranes, perhaps through its ability to interact with extracellular matrix proteins such as fibronectin (19), laminin, and nidogen (23), may act to modulate the formation, stabilization, and function of such structures and consequently to facilitate locoregional invasion by cancer cells. In addition, fibulin-1 may also promote tumor cell extravasation at the distance of the metastatic embolus, since it has recently been shown to increase platelet adhesion by interacting with fibrinogen (24).

Several secreted proteins induced by estrogen have been implicated in breast cancer growth and metastasis on the basis of their value as prognostic markers and of their functions as proteases, protease inhibitors, and growth factors (4, 5, 25). Half of the familial breast and ovarian cancers have a common genetic alteration: the BRCA1 gene located on chromosome 17 in q21 (26). However, the human fibulin-1 gene is located on chromosome 22 in q13.3 (27).

Fibulin-1 is an estrogen-induced extracellular matrix protein secreted by ovarian cancer cells. Another extracellular matrix protein, the matrix Gla protein, has been reported to be also induced by estrogen in breast cancer cell lines (28). However, fibulin-1 secretion was not detected in all breast cancer cell lines that we studied. The mechanism of increased secretion of fibulin-1 by E_2 is most likely due to increased gene expression. First, the level of fibulin-1 is increased in the cell extract as well as the culture medium (Fig. 4); second, Northern blot analysis using a fibulin-1 cDNA probe showed an increased mRNA level in BG1 cells treated with E_2 as early as 24 hr (P. Pujol, D. Chalbos, and H.R., unpublished observations).

Regulation of fibulin-1 by estrogen shown here in metastatic ovarian cancer cells also highlights a potential role for fibulin-1 in ovarian cancer for developing new therapies and as a marker for early detection of the disease or for hormone responsiveness. Importantly, we have shown by immunohistochemical staining of ovarian cancer sections that fibulin-1 was also accumulated *in vivo* in ovarian tumors. The staining pattern of the first tumors examined indicated that fibulin-1 was, as in other tissues (22), located in the stroma surrounding ovarian epithelial cancer cells. The lack of immunostaining of fibulin-1 within the epithelial cancer cells should not be taken to indicate that *in vivo* the ovarian cancer cells do not express fibulin-1. In fact, recent *in situ* RNA hybridization analysis showed that in the tumors, epithelial ovarian cancer cells expressed fibulin-1 mRNA at a low but similar level to that of stromal cells (our unpublished data). However, additional work is needed to specify the relative contribution of ovarian cancer cells, of peritumoral stromal cells, and of blood in fibulin-1 stromal accumulation.

Fibulin-1 is not produced by all types of cancer cells. For example, its mRNA was not detected in lymphomyeloid cells, melanoma, or colon cancer cell lines (22). Very low fibulin-1 secretion was detected from the PEO14 estrogen receptor-negative ovarian cancer cell line (unpublished observations). Therefore, of the carcinoma cell lines examined, fibulin-1 appears to be mostly expressed by estrogen-responsive ovarian cancers. Since fibulin-1 circulates in blood (13), some of its forms may prove to be a circulating marker for monitoring estrogen or antiestrogen treatment in patients. While ovarian cancers, after failure of other therapies, are generally poorly responsive to antiestrogen therapy (8), these cancers may be estrogen responsive at earlier stages of the disease as suggested both by epidemiological data (ref. 12 and references therein) and their estrogen responsiveness *in vitro*.

To conclude, we show that fibulin-1 is secreted by several estrogen receptor-positive ovarian cancer cell lines and that its production is stimulated by estrogens. We propose that, by secreting fibulin-1, the estrogen-stimulated ovarian cancer cells may modulate their interaction with the extracellular matrix and consequently their invasiveness. During normal embryonic development, fibulin-1 has been found to be expressed at sites undergoing epithelial–mesenchymal transition, often surrounding migratory cells (20, 21). This has also led to speculation that fibulin-1 might play a role in cellular motility.

Our finding should stimulate further studies on the function of fibulin-1, on its potential value in monitoring human ovarian cancers, and/or as a general circulating marker for estrogen responsiveness.

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