Release of Estrogen-induced Glycoprotein with a Molecular Weight of 52,000 by Breast Cancer Cells in Primary Culture\(^1\)

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**ABSTRACT**

In an attempt to find estrogen-specific responses in breast cancer, we have established primary cell culture from metastatic pleural effusions of breast cancer and have analyzed the proteins labeled by \(^{35}\)S]methionine and released into the culture medium using sodium dodecyl sulfate-polyacrylamide gel electrophoresis. We show that the synthesis of a M, 52,000 glycoprotein which is released by metastatic breast cancer cells in primary cultures is stimulated by estradiol in four of six patients. This protein is similar to the M, 52,000 protein of MCF7 cells on the basis of its mobility in one- and two-dimensional gel electrophoresis (the molecular weight of this protein was originally found to be 46,000; it is closer to 52,000 using labeled proteins from New England Nuclear as molecular weight markers), its immunoprecipitation by antiserum raised against the M, 52,000 protein, and its binding to concanavalin A. We conclude that, similar to some breast cancer cell lines, some metastatic breast cancers synthesize a M, 52,000 glycoprotein which is regulated by estrogens and exported from the cells into the medium. This study also shows that some primary cultures established from metastatic breast cancer remain responsive to estradiol in vitro for the synthesis of specific proteins. More clinical studies are needed to prove the interest of the M, 52,000 secreted protein as an additional marker of the hormone responsiveness of breast cancer.

**INTRODUCTION**

The hormone responsiveness of human breast cancer is presently predicted from the levels of ER\(^3\) and PGR (15, 17). However, these assays are sometimes of limited value: (a) they require that tissue is accessible for biopsy and surgically removed before these assays are sometimes of limited value: (a) they require that tissue is accessible for biopsy and surgically removed before treatment; (b) the predictive value of ER and PGR levels is limited to prove the interest of the M, 52,000 secreted protein as an additional marker of the hormone responsiveness of breast cancer.

Recently, we have described a M, 52,000 glycoprotein which is induced by estradiol in human mammary cell lines containing ER and PGR (26). The regulation of this protein appears to be more closely related to cell growth than is the PGR since, in contrast to the PGR, the M, 52,000 protein is not induced by antiestrogens in antiestrogen-sensitive cells. Before this protein may be considered as a potential marker of estrogen responsiveness in breast cancer, however, it is important to demonstrate that it is present in breast cancer patients.

One major criterion by which the M, 52,000 protein may be identified is its regulation by estrogens. For ethical reasons, this can be demonstrated only in vitro. We have therefore established primary cultures from metastatic pleural effusions of breast cancer and compared the estrogen-regulated proteins released into the medium to the M, 52,000 protein of MCF7 (and ZR75-1) breast cancer cell lines.

**MATERIALS AND METHODS**

**Primary Cultures.** Primary cultures were established as described by Cailleau et al. (4) except that we used Ham’s F-12, N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (10 mm), NaHCO\(_3\) (2 g/liter), insulin (0.6 \(\mu g/ml\)), and penicillin (25 IU/ml) streptomycin (25 \(\mu g/ml\)) for the preparation of pleural cells and as growth medium.

Briefly, pleural effusions were obtained aseptically in vacuum bottles by thoracocentesis. Heparin was added (Choay; 1000 units/100 ml), and the effusate was centrifuged for 7 min at 1400 rpm in 50-ml conical tubes. A sample of the pellet was processed for cytology. RBC were hemolyzed, if required, by resuspending the pellet in water for 1 min. Concentrated growth medium was then added to give an isotonic solution and centrifuged for 7 min at 1400 rpm. The cell pellet was finally resuspended in growth medium plus 10% FCS-DCC. Approximately 400,000 to 600,000 epithelial cells (generally in clusters) (Fig. 1a) were plated in 1.6-cm-diameter wells (Linbro) either directly on plastic or on attached collagen in 1 ml of growth medium plus 10% FCS-DCC. Collagen gel was prepared as described (9). Briefly, 1 volume of Vitrogen 100 (Flow Laboratories) was mixed with 1 volume of 2-fold-concentrated growth medium and adjusted to pH 7.4 with 5 \(\mu \)molar NaOH. Plastic wells were coated with 400 \(\mu l\) of collagen solution and allowed to polymerize for about 1 hr at 37°. After 24 hr, when the cells were attached, the collagen gels were released from the plastic to give floating collagen membranes (Chart 1).

**Stimulation by Estradiol.** The cells were grown for 2 to 10 days in growth medium plus 10% FCS-DCC to withdraw cells from estrogens or antiestrogens. They were then grown for 2 further days in the same medium with or without 10 \(\mu \)m estradiol. The media were changed every 2 days (Chart 1).

**Labeling and Analysis of Released Proteins.** Cells were labeled in Eagle’s minimum essential medium containing methionine (3 mg/liter) plus \([\text{35}^\text{S}]\text{methionine (80 } \mu \text{Ci/ml; Amersham; specific activity, } \geq 1000 \text{ Ci/}\mu \text{mo})\) for 6 hr. Cells grown on plastic and on floating collagen membranes were labeled in 300 and 600 \(\mu l\) of medium, respectively. The medium was collected and centrifuged at 1400 rpm for 10 min. The incorporation

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\(^1\) This investigation was supported by the Institut National de la Santé et de la Recherche Médicale, la Fondation pour la Recherche Médicale Française, and the Ligue Nationale de Lutte contre le Cancer.

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\(^3\) The abbreviations used are: ER, estrogen receptor; PGR, progesterone receptor; FCS-DCC, fetal calf serum treated with dextran-coated charcoal; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; Con A, concanavalin A; TAM, tamoxifen.

Received June 16, 1982; accepted December 29, 1982.

\(^{16}\) 1861

CANCER RESEARCH 43, 1861-1868, April 1983
0008-5472/83/0043-0000$02.00

0008-5472/83/0043-0000S02.00

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of [35S]methionine into protein was measured in 10 μl of medium by trichloroacetic acid precipitation as described (26). Media containing equal amounts of trichloroacetic acid-insoluble radioactivity were lyophilized, dissolved in 30 μl of 2-fold-diluted sample buffer (10% β-mercaptoethanol-40 mM EDTA-0.01% bromophenol blue-4% sodium dodecyl sulfate-4% glycerol-125 mM Tris, pH 6.8) and heated at 100° for 5 min. [35S]Labeled proteins were electrophoresed on 12% acrylamide gels and then fluorographed (16, 26). Fluorographs were scanned using a scanning densitometer (Vernon, Paris, France). Two-dimensional electrophoresis was as described (20, 26).

**Immunoprecipitations.** Proteins in the media were immunoprecipitated using antibodies against the M, 52,000 protein as described (5) and analyzed by SDS-PAGE (25).

**ER and PGR Assay.** Cytoplasmic and nuclear ER and PGR were assayed using a dextran-coated charcoal technique as described (12, 25).

**Cytology and Histology.** Smears of the centrifuged cells of pleural effusions were stained using May-Grunwald-Giemsa staining. The primary cultures of cells grown on plastic were observed by phase-contrast microscopy. Cells grown on floating collagen membrane were fixed in Bouin’s solution, embedded in paraffin, and stained with hematoxylin and eosin. The morphology of the cultures was classified as described in Ref. 11.

**RESULTS**

**Morphology of Epithelial Cells in Primary Cultures.** Cancer epithelial cells which synthesized proteins were successfully cultured from pleural effusions obtained from 6 of 14 patients. Cytology of the smears of pleural effusions of Patients 3, 4, 5, and 6 showed that 80 to 90% of the cells (RBC excluded) were organized in clusters. These clusters consisted of cell nests, often demonstrating a peripheral and central organization similar to a morula. Nuclei were isonucleic with a finely dispersed chromatin, and the intercellular borders were not visible (Fig. 1a).

The morphologic changes of the cells cultured on plastic varied slightly between patients, but in all cases most of the cells were epithelial-like and organized in monolayer (Fig. 1b). Only a few fibroblast-like spindle-shaped cells could be seen. After 7 days of culture on floating collagen membranes, cells were organized in well-differentiated epitheloida, either duct carcinoma [Patients 3 and 5 (Fig. 1c)] or papillary carcinoma [Patient 4 (Fig. 1d)] according to the criteria of Fisher et al. (11).

By using an indirect immunoperoxidase technique, cells of Patients 3, 4, and 6 cultured on floating collagen were shown to react specifically with 2 monoclonal antibodies (HMFG-1 and HMFG-2) raised against human milk fat globule antigens (in collaboration with Dr. J. Taylor-Papadimitriou; data not shown). These data suggest that cultured cells are epithelial cancer cells rather than mesothelial cells (2, 10). In these 3 patients, the reactivity with HMFG-2 was greater than that with HMFG-1, in agreement with earlier observations that many breast tumors react specifically with 2 monoclonal antibodies (HMFG-1 and HMFG-2) raised against human milk fat globule antigens (2, 10). In these 3 patients, the reactivity with HMFG-2 was greater than that with HMFG-1, in agreement with earlier observations that many breast tumors show a more marked positive reaction with HMFG-2 (2, 24).

**Evidence for a M, 52,000 Estradiol-induced Protein.** The radioactive proteins, which were synthesized and released into the culture medium following 5 days of withdrawal from hormone and 2 days of treatment with or without 10 nM estradiol, were then analyzed by SDS-PAGE. Fig. 2 shows the pattern of released proteins (Patients 3 and 4) (Fig. 2). In both cases, a M, 52,000 protein was prominent, and the labeling of this protein was stimulated by estradiol in 4 (Patients 1, 3, 4, and 6) of 6 patients (Table 1). The M, 52,000 protein released by both the MCF7 cells and the cells in primary culture (Fig. 2, Tracks f and h) bound to Con A-Sepharose, but not to Sepharose 4B, confirming that this protein is glycosylated. The percentage of the M, 52,000 protein appeared to be higher when cells were grown on floating collagen membranes than when grown on plastic. This was found for Patients 3, 4, and 6 (Fig. 2, Tracks f, g, h, and i; Table 1). Table 1 summarizes the results obtained from the 6 patients. The M, 52,000 protein could not be induced in the cells from Patients 2 and 5. This might be related to the concentrations of ER and PGR sites which in the cells of Patient 2 were low (<10 fmol/mg protein in both cytosol and nuclear extracts).

The best induction of the M, 52,000 protein by estradiol was obtained in primary cultures established on floating collagen membranes from Patients 3 and 6 who had not been treated with TAM. The primary tumor of Patient 3 was ER positive (45 fmol/mg protein) and PGR negative (<10 fmol/mg protein). The cells of the pleural effusion of Patient 6 were ER positive (372 fmol/mg protein) and PGR positive (322 fmol/mg protein). The synthesis of the M, 52,000 protein was also stimulated by
Comparing between the M, 52,000 Protein Released by Cells in Primary Cultures and by MCF7 Cells. To further demonstrate that the M, 52,000 protein released from the cells in short-term cultures was identical to that released from MCF7 cells, the labeled proteins of the medium were analyzed by 2-dimensional gel electrophoresis. Fig. 3 shows that, for Patients 3 and 4, the M, 52,000 protein was resolved into 6 or 7 spots which had the same pHs and molecular weights as did the components of the M, 52,000 protein released by MCF7 (26). When equal amounts of labeled proteins from MCF7 cells and from Patient 4 cells were coelectrophoresed, the same 7 spots were seen, showing that the M, 52,000 protein from the 2 cell types migrate identically, under the same analytical conditions. The microheterogeneity of the M, 52,000 protein has been suggested previously to result from different numbers of sialic acid residues (26), and the identical pattern obtained for this protein types migrate identically, under the same analytical conditions. The microheterogeneity of the M, 52,000 protein has been suggested previously to result from different numbers of sialic acid residues (26), and the identical pattern obtained for this protein modes of culture (collagen or plastic). In some cases, estradiol appeared to increase the total production of proteins released into the medium; however, the difference was not significant (Table 1).

Comparison between the M, 52,000 Protein Released by Cells in Primary Cultures and by MCF7 Cells. To further demonstrate that the M, 52,000 protein released from the cells in short-term cultures was identical to that released from MCF7 cells, the labeled proteins of the medium were analyzed by 2-dimensional gel electrophoresis. Fig. 3 shows that, for Patients 3 and 4, the M, 52,000 protein was resolved into 6 or 7 spots which had the same pHs and molecular weights as did the components of the M, 52,000 protein released by MCF7 (26). When equal amounts of labeled proteins from MCF7 cells and from Patient 4 cells were coelectrophoresed, the same 7 spots were seen, showing that the M, 52,000 protein from the 2 cell types migrate identically, under the same analytical conditions. The microheterogeneity of the M, 52,000 protein has been suggested previously to result from different numbers of sialic acid residues (26), and the identical pattern obtained for this protein released from primary cultures suggests that it is similarly glycosylated.

The polyclonal antibodies which have been developed against MCF7 cell proteins (5) were then tested against proteins released into the medium from the primary cultures. Fig. 4 shows that this antisera immunoprecipitates 2 major proteins (M, 52,000 and M, 66,000) which are released from MCF7 cells (Tracks d, e, and f) and from primary cultures established from Patients 3 and 4 (Tracks a, b, c, g, h, and i). The specificity of this immunoprecipitation is shown in Tracks a, d, and g, where preimmune rabbit serum was tested at twice the concentration of immune rabbit serum (Tracks b, e, and h). In contrast, this antisera did not immunoprecipitate any M, 52,000 or M, 66,000 protein from the culture medium of human fibroblasts (not shown) or of 2 rodent rat mammary tumor cell lines, indicating the specificity of this antisera. Fig. 4 (Tracks j, k, and l) shows the results obtained with the protein released by the rat RBA cell line derived from 9,10-dimethyl-1,2-benzanthracene-induced mammary tumors (6). Only a M, 160,000 protein was precipitated by the antisera against the human MCF7 proteins. Similar results were obtained with the NMU rat mammary cell line which was established from a nitrosomethylurea-induced mammary tumor (5).

These results demonstrate that the M, 52,000 protein released from primary culture of breast cancer is immunologically related to the M, 52,000 protein released from MCF7 cells and therefore provide further evidence for the identity of the 2 M, 52,000 proteins.

To eliminate the possibility that the primary cultures had become contaminated by MCF7 cells, we have analyzed the proteins released after primary culture for a short period of time. Fig. 5 shows that the pattern of proteins released by the cells of Patient 5 was similar whether the cells were cultured for 3 days (Tracks a and b) or 10 days (Tracks d and e). In this patient, a M, 52,000 protein was immunoprecipitated by our polyclonal anti-MCF7 antibodies (not shown). This result suggests that the protein pattern was stable for at least 10 days and representative of the proteins released in vivo by these cells.

This evidence strongly suggests that the M, 52,000 protein of the MCF7 cell line can also be regulated by estrogen and released by pleural metastatic cells in vivo.

DISCUSSION

We have looked at the effects of estradiol on the synthesis of proteins released into the culture medium from primary cultures established from pleural metastases of human breast cancer. A
M, 52,000 glycoprotein which is similar to the protein released by ER-positive human mammary cell lines (26) was released by metastatic breast cancer cells in primary cultures. Phase-contrast microscopy of cells grown on plastic and histopathology of the cells grown on floating collagen membranes showed that the cultures contained predominantly epithelial cells. The following evidence suggests that the cultured cells originated from mammary adenocarcinoma. Smears of the pleural effusions showed that most of the cells were epithelial cancer cells (Fig. 1a) (7).

Furthermore, after 7 days of culture on floating collagen membranes, the cells were organized as duct or papillary breast carcinoma (Fig. 1, c and d). The presence of some inflammatory and mesothelial cells was unlikely since the cells were responsive to estrogen and reacted with 2 specific antibodies derived against human fat globule antigens (2, 10). Finally, the M, 52,000 protein was released and regulated by estradiol as it is in 2 breast cancer cell lines.

Contamination of the primary cultures by MCF7 cells is extremely unlikely since MCF7 cells were not being cultured in the same place while this work was in progress. Moreover, the M, 52,000 protein was released by pleural metastatic cells after only 3 days in primary culture (Fig. 5).

Estradiol increased the synthesis of the M, 52,000 protein in cultures established from 4 of the 6 patients. More proteins were released and the effect of estradiol was more pronounced in Patients 3, 4, and 6 when cells were cultured on floating collagen membranes instead of plastic. It is possible that cells growing on floating collagen remain more differentiated and display more secretory activity, as has been shown previously for the regulation of casein synthesis by prolactin in mouse mammary cells (8, 9). The best induction of the M, 52,000 protein was observed in Patients 3 and 6, who had not been treated by TAM and whose primary or metastatic breast tumors were ER positive (Table 1). In contrast, in 2 patients (Patients 2 and 5), the labeling of the M, 52,000 protein was low and not stimulated by estradiol. Cells of the pleural effusion obtained from Patient 2 contained neither ER nor PGR sites and might therefore be considered as unresponsive to estrogen.

In 3 other patients (Patients 1, 3, and 4), the labeling of the M, 52,000 protein was stimulated by estradiol, but its basal level was high in unstimulated cells. To explain this high basal level of M, 52,000 protein, different mechanisms are possible: (a) The M, 52,000 protein may be synthesized and secreted constitutively in the absence of endogenous hormones or antihormone treatment; (b) The cells may have been stimulated by endogenous estrogens, and 7 days of hormone withdrawal in culture may be insufficient to reduce the level of synthesis of the M, 52,000 protein. Studies in cell culture have established that physiological concentrations of estrone (26) and some adrenal androgens such as 5-androstene-3ß,17ß-diol or dehydroepiandrosterone (1) are sufficient to induce the M, 52,000 protein. These levels may be reached in postmenopausal patients; (c) We have recently shown that TAM is able to stimulate the release of the M, 52,000 protein from an antiestrogen-resistant clone (R27). This clone was derived from the MCF7 cell line and its growth, in contrast to that of MCF7, is not inhibited by TAM (19). Patients 1 and 4 were treated by TAM for 1 to 2 years prior to thoracocentesis, and it is therefore possible that antiestrogen-resistant cells in which antiestrogen behaves like full estrogens had been selected. The time of withdrawal of the cells from TAM and its metabolites was probably not sufficient (22) to reduce the level of synthesis of the M, 52,000 protein.

Many attempts have been made to predict the estrogen responsiveness of breast cancer (14) in vitro. They have generally relied on the total incorporation of labeled precursors (leucine, thymidine, etc.) or histochemical techniques, and the results are controversial. In this study, we show for the first time that estradiol can stimulate the production of a specific released protein in some metastatic breast cancer cells in primary culture. Since primary culture of breast cancer cells appears to be easier and more reproducible to establish from pleural effusion than from primary tumors or lymph nodes (23), we cannot presently ascertain whether the M, 52,000 protein is limited to metastatic cancer cells or is also present in primary solid tumors. On the basis of the limited number of patients, we cannot specify whether the M, 52,000 protein regulation is related to the clinical hormone responsiveness of breast cancer.

We have previously attempted to characterize the proteins released into the culture medium from pieces of breast tumor which had been collected before and after TAM treatment. This study showed that the labeling of M, 52,000 released proteins was regulated by TAM treatment in some patients. The pieces of tumor, however, released much less protein than the primary cultures used in this study, and this prevented complete characterization of the M, 52,000 protein.

The M, 52,000 protein which is released from primary cultures of metastatic human breast cancer is similar by several criteria to the one released by human breast cancer cell lines (26). They have the same migration in 1- and 2-dimensional gels, reactivity with antisera raised against MCF7 proteins, binding to Con A, and regulation by estradiol. These findings reinforce the view that the MCF7 and ZR75.1 cell lines provide a valuable in vitro system for studying the hormonal regulation of human breast cancer.

The nature and function of the M, 52,000 protein are presently unknown; however, it is not one of the major milk proteins, casein or -lactalbumin. It is not related to the M, 60,000 estrogen-induced protein which is present in the T47D cell line but which is not immunoprecipitated by the MCF7, M, 52,000 antibodies (5). Its relationship with the protein which reacts with antibodies to the M, 52,000 glycoprotein of the mouse mammary tumor virus (21) or with plasminogen activators (3) is under current study.

From this study, which was performed on a limited number of patients, we conclude that some metastatic human mammary cancer cells synthesize and export an estrogen-regulated M, 52,000 glycoprotein. These results also indicate that metastatic human breast cancer cells remain estrogen responsive for specific proteins in primary culture.

We are now developing a radioimmunoassay to detect the M, 52,000 protein in the plasma of breast cancer patients. The possibility that this protein will provide a circulating marker of the hormone responsiveness, evolution, and malignancy of human breast tumors would be of considerable importance.


ACKNOWLEDGMENTS

The technical assistance of D. Deroq and C. Rougeot is acknowledged. ER and PGR assays were performed by J. L. Borgna and J. Jamal. The paper was typed by E. Barrie. We are grateful to Dr. D. Westley for editing the manuscript; to Drs. Lasfargues, Gary-Bobo, and Dyoko for providing pleural effusions; and to Drs. Emmerman, Taylor-Papadimitriou, Hollowes, Vignon, and Nicolas for their advice and suggestions and to J. Gilbert and Dr. J. Taylor-Papadimitriou for HMFG-1 and HMFG-2 antibodies and immunoperoxidase staining of cells.

REFERENCES

Fig. 1. Morphology of human metastatic breast cancer cells of pleural effusion before (a) and after (b to d) 7 days of primary culture. Cells were cultured as described in “Materials and Methods” on plastic (b) or on floating collagen membranes (c, d). a, cytology of the pleural effusion of Patient 4. × 400. Cells stained using the May-Grunwald-Giemsa procedure are organized in morula-like clusters. b, Patient 2. Phase-contrast light micrograph. × 100. Epithelial-like cell cultivated on plastic for 8 days and growing in monolayer. c, Patient 3. × 250. Cells were grown on floating collagen membrane type I. The cells are organized like a duct carcinoma according to the criteria of Fisher (11). d, Patient 4. × 250. Cells were cultured as for Patient 3. The cells are organized as in a papillary carcinoma according to the criteria of Fisher (11).
Fig. 2. SDS-PAGE of total and Con A-reactive proteins released by estradiol-treated and withdrawn metastatic human breast cancer cells in primary culture. Cells obtained from pleural effusions were cultured, either untreated or treated with 10 nm estradiol (E2), and labeled by [35S]methionine as described in Chart 1. Labeled proteins of the medium were analyzed by SDS-PAGE under conditions which revealed only the high-molecular-weight (MW) proteins (Tracks a to l) or the total proteins analyzed using the usual conditions (Tracks j and k). Tracks a to d, proteins labeled and released by cells (C) from Patient 3 with (Tracks c and d) or without (Tracks a and b) estradiol. Track e, proteins released by MCF7 cells with estradiol. Tracks f to l, proteins released by cells from Patient 4 after estradiol treatment. Cells were cultured on floating collagen (FC) membranes (Tracks f and g) or on plastic (P) (Tracks h and i). Track j, Con A-reactive fraction of the proteins released by cells from Patient 4 (Track k), grown on floating collagen and treated by estradiol. k on ordinate, thousands.

Fig. 3. Two-dimensional gel electrophoresis of proteins released by estradiol-treated metastatic human breast cancer cells in primary culture. Cells obtained from pleural effusions of Patients 3 (B) and 4 (D) were cultured on floating collagen membranes and MCF7 cells on plastic. Cells were then treated by 10 nm estradiol (E2) and labeled by [35S]methionine, and the media (24,000 cpm of trichloroacetic acid-precipitable material) were analyzed as described in "Materials and Methods." The fluorographs are represented for MCF7 (A), Patient 3 (B), Patient 4 (D), and a 1:1 mixture of trichloroacetic acid-precipitable cpm of the labeled medium of Patient 4 and MCF7 cells (C). MW, molecular weight; k, thousands.
Fig. 4. Immunoprecipitation of released proteins using antibodies against the MCF7, M, 52,000 protein. Cells from Patients 3 (Tracks a to c) and 4 (Tracks g to i) were grown on floating collagen membranes, treated by 10 nm estradiol, and labeled by [³⁵S]methionine as described in Chart 1. Human MCF7 cells (Tracks d to f) and rat RBA (6) cells (Tracks j to l) were cultured, stimulated with estradiol, and labeled as described previously (4, 26). Immunoprecipitation was carried out using preimmune (Ig) and immune (lg) rabbit serum raised against the M, 52,000 glycoprotein of MCF7 cells, as described (5). Patient 3, immunoprecipitation with preimmune (Track a), immune (Track b), and total released proteins (Track c); MCF7 cells, immunoprecipitation with preimmune (Track d), immune (Track e), and total released proteins (Track f); Patient 4, immunoprecipitation with preimmune (Track g), immune (Track h), and total released proteins (Track i); RBA cells, immunoprecipitation with preimmune (Track j), immune (Track k), and total released proteins (Track l). MW, molecular weight; k on ordinates, thousands.

Fig. 5. SDS-PAGE of total proteins released by estradiol (E₂)-treated and withdrawn metastatic human breast cancer cells after 3 days (3 d) and 10 days (10 d) of primary culture. Cells obtained from pleural effusion of Patient 5 were cultured on floating collagen membranes, either untreated or treated with 10 nm estradiol, and labeled by [³⁵S]methionine as described in Chart 1. Labeled proteins of the medium were analyzed by SDS-PAGE. Tracks a and b, proteins released by cells from Patient 5 after 3 days of culture with (Track b) or without (Track a) estradiol; Track c, proteins released by MCF7 cells with estradiol; Tracks d and e, proteins released by cells from Patient 5 after 10 days of culture with (Track e) or without (Track d) estradiol. MW, molecular weight; k, thousands.
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