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Synthesis and secretion of platelet-derived growth factor by human breast cancer cell lines

D. A. BRONZERT*, P. PANTAZIS†‡, H. N. ANTONIADES†§, A. KASID*, N. DAVIDSON*, R. B. DICKSON*, AND M. E. LIPPMAN*

*Medicine Branch, National Cancer Institute, Bethesda, MD 20892; †Center for Blood Research and ‡Department of Medicine, Boston University School of Medicine, Boston, MA 02115; and §Department of Nutrition, Harvard School of Public Health, Boston, MA 02115

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ABSTRACT **We report that human breast cancer cells secrete a growth factor that is biologically and immunologically similar to platelet-derived growth factor (PDGF). Serum-free medium conditioned by estrogen-independent MDA-MB-231 or estrogen-dependent MCF-7 cells contains a mitogenic or "competence" activity that is capable of inducing incorporation of [^{3}H]thymidine into quiescent Swiss 3T3 cells in the presence of platelet-poor plasma. In addition, the conditioned medium contains an activity that competes with ^{125}I-labeled PDGF for binding to PDGF receptors on normal human fibroblasts. The secretion of PDGF-like activity by the hormone-responsive cell line MCF-7 is stimulated by 17β-estradiol. Like authentic PDGF, the PDGF-like activity produced by breast cancer cells is stable after acid and heat treatment (95°C) and inhibited by reducing agents. The mitogenic activity comigrates with a material of ≈30 kDa on NaDodSO$_4$/polyacrylamide gels. Immunoprecipitation with PDGF antiserum of proteins from metabolically labeled cell lysates and conditioned medium followed by analysis on nonreducing NaDodSO$_4$/polyacrylamide gels identified proteins of 30 and 34 kDa. Upon reduction, the 30- and 34-kDa bands were converted to 15- and 16-kDa bands suggesting that the immunoprecipitated proteins were made up of two disulfide-linked polypeptides similar to PDGF. Hybridization studies with cDNA probes for the A chain of PDGF and the B chain of PDGF/*SIS* identified transcripts for both PDGF chains in the MCF-7 and MDA-MB-231 cells. The data summarized above provide conclusive evidence for the synthesis and hormonally regulated secretion of a PDGF-like mitogen by breast carcinoma cells. Production of a PDGF-like growth factor by breast cancer cell lines may be important in mediating paracrine stimulation of tumor growth.**

A strong link between growth factors and cancer has been established. Production of growth factors by neoplastically transformed cells enables them to grow in culture with diminished serum requirements (1). An autocrine growth model has been proposed for cancer cells based primarily on evidence from transformed rodent fibroblast model systems that they are capable of secreting a multitude of growth factors. Growth factors are active at different points of the cell cycle of fibroblasts (2). It appears that one growth factor is able to modulate the mitogenicity of another (3, 4). Platelet-derived growth factor (PDGF), a potent mitogen in human serum, is one such growth factor. Brief treatment of quiescent fibroblasts with PDGF allows them to become competent, enabling them to respond to other "progression" growth factors such as epidermal growth factor and insulin-like growth factor I (IGF-I) (4). Biologically active PDGF is a 30- to 34-kDa protein consisting of two polypeptide chains, the A chain (PDGF-1) and the B chain (PDGF-2), linked together by disulfide bonds (5, 6). Treatment of PDGF with reducing agents results in biologically inactive molecules of 12–17 kDa (7, 8). PDGF-2 is encoded by the *SIS* protooncogene located on chromosome 22 (9). Biologically active PDGF-2/*SIS* proteins are produced by simian sarcoma virus-transformed cells and by human tumor cells with active *SIS* genes, including glioblastoma, fibrosarcoma, and osteosarcoma cells (reviewed in ref. 10). PDGF-1, with a molecular mass of 31 kDa, is synthesized independently of PDGF-2, and its gene has been shown to be localized on chromosome 7 (11). These findings have suggested that inappropriate expression of PDGF-related genes may play an important role in the processes leading certain cells to malignant transformation.

We have reported that breast cancer cell lines are capable of secreting a variety of growth factors including transforming growth factor type β (12), transforming growth factor type α (13), and IGF-I (14, 15). Transforming growth factor type α and IGF-I are produced constitutively in hormone-independent lines, whereas their production is stimulated by 17β-estradiol in the hormone-responsive MCF-7 breast cell line. *In vivo* experiments done in female oophorectomized nude mice show that conditioned medium (CM) from 17β-estradiol-treated MCF-7 cells is capable of stimulating tumor growth of MCF-7 cells (16). Thus, this CM contains factors that allow tumor formation without the estrogen stimulation, a step that had been considered essential.

Cellular transformation in rodent fibroblasts has been shown to occur through the coordinated action of PDGF and transforming growth factor types α and β (17). In human diploid fibroblasts, PDGF is capable of stimulating the production of IGF-I (18). To further define the growth factors that are secreted by breast tumors, we chose to look for a "competence" or PDGF-like activity in breast cancer cell lines. Rozengurt *et al.* (19) have reported that breast cancer cells produce mitogenic activities for 3T3 cells. Here, we report that breast cancer cell lines secrete a PDGF-like activity that can compete with ^{125}I-labeled PDGF for PDGF receptors, that has a molecular weight similar to human plasma PDGF, and that is immunoprecipitated by PDGF antisera. In addition, PDGF-1 and PDGF-2/SIS mRNA transcripts are detected in these lines.

MATERIALS AND METHODS

Cell Culture. The cell lines used in these studies included the breast carcinoma MCF-7 (obtained from the Michigan Cancer Foundation, Detroit, MI) and MDA-MB-231, T47D, ZR-75-1, and Hs578T (obtained from American Type Culture Collection). Swiss mouse 3T3 and human foreskin fibroblasts AG1523 were obtained from American Type Culture Collection and the Human Mutant Cell Repository, respectively.

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Abbreviations: PDGF, platelet-derived growth factor; IGF-I, insulin-like growth factor I; CM, conditioned medium; PDGF-1, PDGF A chain; PDGF-2, PDGF B chain.

Cells were routinely passaged in T-75 flasks in improved minimal essential medium (IMEM; GIBCO) supplemented with 10% (vol/vol) fetal calf serum and gentamicin at 50 mg/liter. For estrogen-stimulation studies, cells were transferred to phenol red-free IMEM supplemented with 5% (vol/vol) charcoal-stripped calf serum and insulin (0.1 unit/ml) for at least 2 weeks prior to the experiments (20). For the collections of serum-free medium conditioned by breast cancer cells, cells were plated in Falcon T-175 flasks and grown in the above medium until 90% confluent. The medium was replaced with 40 ml of serum-free IMEM supplemented with transferrin at 2 mg/liter (Sigma), 10 mM Hepes (pH 7.4), fibronectin at 1 mg/liter (Collaborative Research), and 1× trace elements (GIBCO). After 24 hr, the medium was removed, fresh medium was added, and the cells were incubated at 37°C for 48 hr. The serum-free CM was then collected and filtered through a 0.22-μm filter. The protease inhibitor aprotinin (Sigma) was added at a final concentration of 0.05 trypsin inhibitory unit/ml, and CM was frozen at −20°C until needed. The cells were gently scraped, pelleted by low-speed centrifugation, and assayed for DNA content (21).

Stimulation of DNA Synthesis. Stimulation of [^{3}H]thymidine incorporation into quiescent 3T3 cells was measured essentially as described (22). Briefly, Swiss 3T3 cells were plated in 16-mm wells in IMEM supplemented with 10% (vol/vol) fetal calf serum. After 4–5 days when the cultures were confluent and nondividing, medium was removed, and cultures were incubated for 24 hr with serum-free IMEM supplemented with 0.5% platelet-poor plasma. Test samples of concentrated CM were then added for 24 hr. During the last 2 hr, cells were labeled with [^{3}H]thymidine (81 Ci/mmol; 1 μCi/ml; 1 Ci = 37 GBq). To terminate the assay, cells were washed twice with cold isotonic phosphate-buffered saline (GIBCO), extracted with 10% (wt/vol) cold trichloroacetic acid, washed once with distilled water, and lysed with 10% (wt/vol) $NaDodSO_4$. Radioactivity was determined in a liquid scintillation counter.

Competitive Binding Assay for PDGF-Like Activity. PDGF-like activity in CM was measured using the AG1523 human foreskin fibroblast cell line in a competitive binding assay (22). Cells were plated in 25-mm wells in IMEM supplemented with 10% (vol/vol) fetal calf serum. Four days later when cells are confluent and nondividing, the cells were washed with binding buffer (IMEM containing bovine serum albumin at 1 mg/ml and 25 mM Hepes, pH 7.4). Test samples of CM were then diluted in 1 ml of binding buffer and preincubated at 4°C for 3 hr with gentle agitation to allow the PDGF-like activity to bind to PDGF receptors on the cells. Cells were washed three times with 1 ml of binding buffer and incubated with ^{125}I-labeled PDGF (25,000–30,000 dpm; specific activity, 6.7 μCi/μg) in 1 ml of binding buffer for 1 hr at 4°C. The assay was terminated by washing three times in binding buffer and extracting bound ^{125}I-labeled PDGF with 1 ml of lysis buffer (phosphate-buffered saline containing 1% Tween 20 and bovine serum albumin at 1 mg/ml). Nonspecific binding was determined as the amount of ^{125}I-labeled PDGF bound in the presence of unlabeled pure PDGF at 50 ng/ml. PDGF-like activity of samples was converted to ng equivalents of PDGF per ml, using a standard curve constructed from results obtained with known concentrations of pure PDGF (0.2–20 ng/ml).

Immunoprecipitation and Analysis of PDGF-Like Proteins. Breast cancer cells in culture were labeled with [^{35}S]cysteine at 250 μCi/ml (specific activity, >1000 Ci/mmol; Amersham) and in IMEM containing 0.5% fetal calf serum in 10% of the normal cysteine concentration. Incubation continued at 37°C for 24 hr to obtain metabolically labeled, secreted proteins. Preparation of secreted proteins for immunoprecipitation studies was as described (23). Immunoprecipitation of proteins with specific PDGF antiserum that crossreacts with both PDGF-1 and PDGF-2, analysis of precipitates by $NaDodSO_4$/polyacrylamide gel electrophoresis, fluorography, and visualization of labeled proteins on x-ray films were as described (23).

RNA Isolation and Blot-Hybridization Analysis. Total cellular RNA was isolated from MCF-7 and MDA-MB-231 cells by guanidine isothiocyanate solubilization and cesium chloride density centrifugation (24). Polyadenylylated RNA was selected by chromatography on oligo(dT)-cellulose, size-fractionated on a 1.1% agarose/2.2 M formaldehyde gel, and transferred to nitrocellulose. Equivalent amounts of mRNA were loaded in each lane and verified by ethidium bromide staining. Filters were hybridized to ^{32}P-labeled probe overnight at 42°C and washed three times in 1× SSC plus 0.5% $NaDodSO_4$ and once in 0.25× SSC at 60°C for 30 min each wash (25). (1× SSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7.0.) Filters were exposed to Kodak XAR-5 film at −70°C. The PDGF-1 (PDGF-A) probe was a 1.3-kilobase (kb) fragment comprising the complete nucleotide sequence of the human PDGF-1 gene (D-1, a generous gift of C. Betsholtz, University Hospital, Uppsula, Sweden) (11). The PDGF-2/SIS probe was a 1.7-kb *Bam*HI fragment comprising the sixth and seventh exon of human *SIS* (9).

RESULTS

Stimulation of DNA Synthesis by CM. Breast cancer cell lines were screened for their ability to secrete growth factors that are mitogenic to quiescent Swiss 3T3 cells. Serum-free CM derived from different breast cancer cell lines were concentrated 10-fold and then tested for their ability to stimulate [^{3}H]thymidine incorporation into the DNA of Swiss 3T3 cells (Table 1). CM from the estrogen receptor-negative cell line MDA-MB-231 stimulated thymidine incorporation at the lowest volume tested and contained the largest amount of mitogenic activity. The MDA-MB-231 CM retained its ability to stimulate DNA synthesis when heated at 95°C for 15 min but was inactivated by treatment with the reducing agent 2-mercaptoethanol. These properties are identical to those described for human plasma PDGF (26).

CM from other breast cancer cell lines varied in their ability to stimulate [^{3}H]thymidine incorporation in Swiss 3T3 cells. To determine whether the secretion of a PDGF-like factor is estrogen regulated, CM was collected from the estrogen-responsive breast cancer cell line MCF-7 growing in the presence and absence of 10 nM 17β-estradiol for 3 days. In the absence of 17β-estradiol, proliferation was minimal, and the amount of the PDGF-like factor secreted was low. However, cells exposed to 10 nM 17β-estradiol, a concentration capable of stimulating cell growth, secreted substantial amounts of the mitogenic factor. The T47D cell line also secretes measurable PDGF-like factor, whereas the ZR-75-1 cell line does not, when CM is collected in the presence of 17β-estradiol (Table 1).

PDGF Receptor-Binding Activity in Breast Cancer CM. CM from the two breast cancer cell lines that secreted the largest quantities of PDGF-like activity, MDA-MB-231 and MCF-7, were tested for their ability to compete with ^{125}I-labeled PDGF for binding to PDGF receptors on human foreskin fibroblasts. Serum-free CM was harvested from confluent cultures of breast cancer cells after 48 hr, concentrated 20-fold, and analyzed for PDGF competitive binding activity at various concentrations. Both cell lines were found to secrete an activity that competed with PDGF for binding to the PDGF receptor (Table 2). The amount of PDGF-like activity in CM from MCF-7 cells is under estrogen regulation; 17β-estradiol exposure led to a 3-fold increase in PDGF-like activity. The PDGF-like activity is immunologically crossreactive with PDGF antiserum. When CM was incubated with

Table 1. CM stimulation of [^{3}H]thymidine uptake by Swiss 3T3 cells

Cell line	Growth stimulant, μl	[^{3}H]-Thymidine incorporation, dpm × 10^{-2}	[^{3}H]-Thymidine incorporation, dpm × 10^{-5}/unit of CM*
MDA-MB-231	0	3	
	10	65	
	20	101	
	50	174	205
	50 (heated)	156	184
	50 (reduced)	30	32
T47D (10 nM 17β-estradiol)	10	22	
	20	24	
	50	66	41
ZR-75-1 (10 nM 17β-estradiol)	10	8	
	20	10	
	50	10	4
Hs578T	10	28	
	20	26	
	50	39	44
MCF-7 (control)	10	6	
	20	7	
	50	7	5
MCF-7 (10 nM 17β-estradiol)	10	13	
	20	26	
	50	58	77

Collections (40 ml) of serum-free CM from breast cancer cells were concentrated 10-fold and tested for their ability to stimulate incorporation of [^{3}H]thymidine into quiescent Swiss 3T3 cells. Heat-treated samples were heated at 95°C for 15 min. Reduced samples were incubated with 5% (vol/vol) 2-mercaptoethanol for 1 hr then exhaustively dialyzed against IMEM.

*One unit of CM is equivalent to a 48-hr collection of CM from cells containing 1 mg of DNA.

rabbit anti-PDGF IgG in the competitive binding assay, no inhibition of ^{125}I-labeled PDGF binding was seen (Fig. 1). Thus, breast cancer cells secrete a PDGF-like activity that is immunologically similar to authentic PDGF.

Characterization of the PDGF-Like Activity from MDA-MB-231 Cells. To further characterize the mitogenic activity from MDA-MB-231 cells, the cell line secreting the highest amount of PDGF-like activity, 4 liters of serum-free CM was concentrated on an Amicon YM10 membrane and dialyzed against 1 M acetic acid, pH 3. This acid-extracted CM was then chromatographed on Bio-Gel P-100 in 1 M acetic acid,

Table 2. Production of PDGF receptor-binding activity by human breast cancer cells

Cell line	PDGF activity, ng/ml of CM	PDGF activity, ng/unit of CM*
MDA-MB-231	5.0	125
MCF-7 (control)	1.0	27
MCF-7 (10 nM 17β-estradiol)	3.2	88

Serum-free CM (40 ml) from breast cancer cells was concentrated 20-fold and tested for ability to compete with ^{125}I-labeled PDGF in binding to human foreskin fibroblasts. PDGF receptor-binding activity of samples was converted to equivalent ng of PDGF per ml, using a standard curve constructed from results obtained with known concentrations of pure PDGF (0.2–20 ng/ml). Nonspecific binding was determined as the amount of ^{125}I-labeled PDGF bound in the presence of unlabeled PDGF (50 ng/ml).

*One unit of CM is equivalent to a 48-hr collection of 40 ml of CM from cells containing 1 mg of DNA.

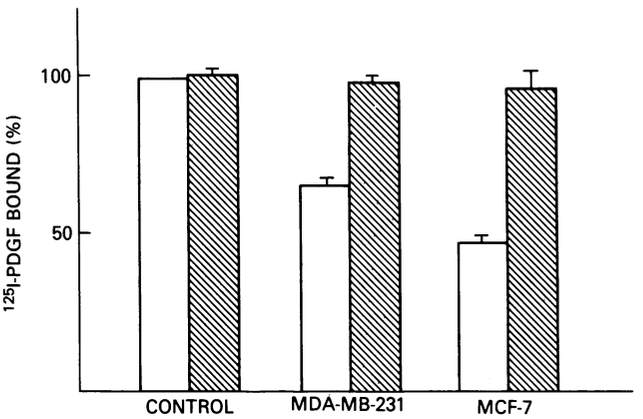

FIG. 1. Effect of PDGF antiserum on the PDGF receptor-binding activity in CM. Serum-free CM from MDA-MB-231 and MCF-7 cells treated with 10 nM 17β-estradiol was concentrated 20-fold on an Amicon YM10 membrane. The PDGF-like activity in CM was determined by a competitive binding assay. CM was preincubated with 50 μg of nonimmune rabbit IgG (open bar) or 50 μg of anti-PDGF IgG (hatched bar) for 3 hr with foreskin fibroblasts. After several washes, cells were incubated with ^{125}I-labeled PDGF and bound, labeled PDGF was quantitated. Data was expressed as a percentage of control. The control was 20-fold concentrated serum-free IMEM medium.

pH 3. A peak of mitogenic activity, as demonstrated by the ability to stimulate [^{3}H]thymidine incorporation in 3T3 cells, was found between 30 and 35 kDa. This peak was further analyzed by electrophoresis on NaDodSO$_4$/polyacrylamide gels. Material was eluted from the gel slices and analyzed for mitogenic activity on Swiss 3T3 cells. In Fig. 2, a single peak of activity that stimulated [^{3}H]thymidine incorporation is found at ≈28 kDa. This is within the molecular weight range of plasma human PDGF.

Immunoprecipitation of PDGF-Like Proteins Secreted by Breast Cancer Cells. Detection of PDGF-like activities in breast cancer cell CM was concomitant with the synthesis and secretion of PDGF-like proteins. For identification, material from the cell lysates and CM of metabolically labeled

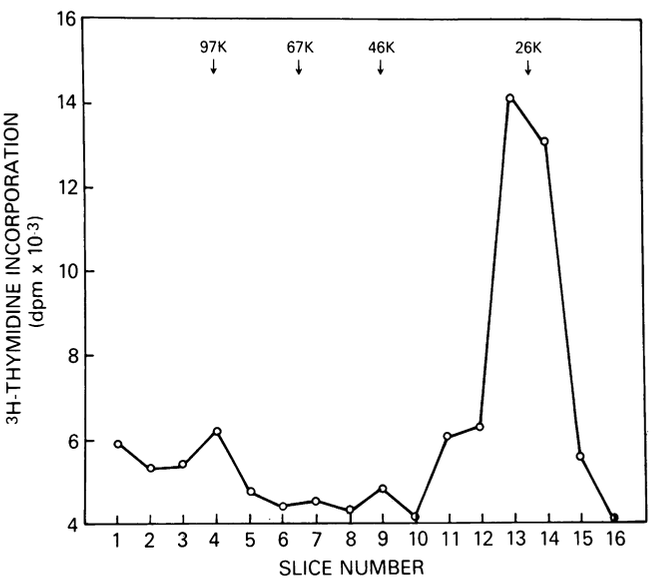

FIG. 2. Gel electrophoresis of CM from MDA-MB-231 cells. CM from MDA-MB-231 cells that had been partially purified on a Bio-Gel P-100 column was electrophoresed on a 10% NaDodSO$_4$/polyacrylamide gel. Gel slices were dialyzed overnight against IMEM containing 0.1% bovine serum albumin and 10 mM Hepes (pH 7.4), and the ability of material from each slice to stimulate [^{3}H]thymidine incorporation in quiescent 3T3 cells was determined. K, kDa.

MDA-MB-231 and MCF-7 cells was immunoprecipitated with PDGF antiserum and analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis under nonreducing and reducing conditions. The results are shown in Fig. 3.

Under nonreducing conditions, the immunoprecipitates derived from the lysates of the MDA-MB-231 cells contained proteins with molecular sizes of 31 kDa and 34 kDa (Fig. 3). These proteins are within the molecular size range of biologically active, unreduced PDGF (7, 8). In addition, proteins with molecular sizes from 16 to 24 kDa can be detected in the lysates of the cells. These proteins may represent further processed products, similar to those reported in the lysates of simian sarcoma virus-infected cells and human glioblastoma and fibrosarcoma cells (10). Upon reduction, the 31- and 34-kDa proteins present in the cell lysates were converted to their monomeric forms of 15 and 20 kDa, which is consistent with the disulfide-linked dimeric nature of PDGF (7, 8). The major secretory PDGF-like protein immunoprecipitated from the CM of the labeled cells displayed a molecular size of about 31 kDa under nonreducing conditions (Fig. 3). Upon reduction, it was converted to two monomers of 15 and 16 kDa. These polypeptides were not precipitated with nonimmune serum and were not precipitated by antiserum in the presence of excess PDGF (lanes c).

Expression of PDGF-1 and PDGF-2/*SIS* mRNA in Breast Cancer Cell Lines. PDGF-1 and PDGF-2 mRNA species have been shown in human glioma and sarcoma cells but not in human carcinoma cells or normal fibroblasts (10, 11). Transcripts from MCF-7 and MDA-MB-231 cells were analyzed by RNA gel blot hybridization using probes for both human PDGF-1 and PDGF-2/*SIS* chains. RNA blot analysis in Fig. 4 shows a 4.1-kb band in the MCF-7 and MDA-MB-231 cell lines similar to the 4.0- to 4.2-kb v-*sis*-related species reported (10). When cell lines were probed for PDGF-1 mRNA, 1.9- and 2.3-kb bands were found similar to those reported in glioma and sarcoma cells (11). To test for estrogen regulation of PDGF, MCF-7 cells were grown with or without 10 nM 17β-estradiol for 3 days. A 2- to 3-fold increase in mRNA transcripts was seen in the 17β-estradiol-treated cells with both the PDGF-1 and PDGF-2 probes.

DISCUSSION

The present data provide direct evidence that certain breast cancer cell lines synthesize and secrete PDGF or a closely related substance. The PDGF-like activity in breast cancer CM is capable of stimulating [^{3}H]thymidine uptake by 3T3 cells. This activity is inhibited by reducing agents but not by heating to 95°C for 15 min, similar to authentic PDGF. CM from MCF-7 and MDA-MB-231 cells contains an activity that is capable of binding to the PDGF receptor and competing with ^{125}I-labeled PDGF for binding to human foreskin fibroblasts. It is estrogen regulated in that CM from MCF-7 cells treated with 17β-estradiol show increased secretion of a PDGF-like activity in a PDGF receptor binding competition assay and by stimulation of [^{3}H]thymidine incorporation in 3T3 cells. This activity can be blocked from binding to the PDGF receptor by incubation with PDGF antiserum. The molecular size of the PDGF-like activity purified from MDA-MB-231 cells was estimated at ≈30 kDa, judged after elution from preparative $NaDodSO_4$/polyacrylamide gels.

Further evidence for the synthesis and secretion of a PDGF-like factor is derived from the immunoprecipitation studies using PDGF antiserum. CM and lysates from metabolically labeled MDA-MB-231 breast cancer cells contain PDGF-like proteins that are immunoprecipitated by PDGF antiserum and have molecular sizes of 30–34 kDa under nonreducing conditions. These findings are consistent with the disulfide nature of PDGF and are similar to the results reported for glioblastoma and fibrosarcoma cells (10). In addition, PDGF antiserum immunoprecipitated proteins with molecular sizes of >45 kDa. High molecular weight components may represent precursors or other intracellular proteins that coprecipitate with PDGF antiserum (10).

Since the precursor of PDGF-2 is encoded by the *SIS* gene, the breast cancer cell lines were studied for production of RNA transcripts. MCF-7 cells contained a 4.1-kb RNA species, while MDA-MB-231 cells showed minimal hybridization with the SIS probe. Heldin and coworkers (27) have reported on a human osteosarcoma cell line that secretes an active homodimer of PDGF-1. A number of glioma and sarcoma tumor cell lines that secrete a PDGF-like activity contain only PDGF-1 RNA (11). When MCF-7 and MDA-MB-231 cells were probed with a cDNA encoding the PDGF-1, both cell lines contained 1.9- and 2.3-kb mRNA species. The presence of the PDGF-2/SIS and PDGF-1 mRNA transcripts in these cell lines is consistent with their ability to synthesize and secrete PDGF-like mitogen.

The significance of the production of PDGF-like growth factors by breast cancer cells is not fully understood. This

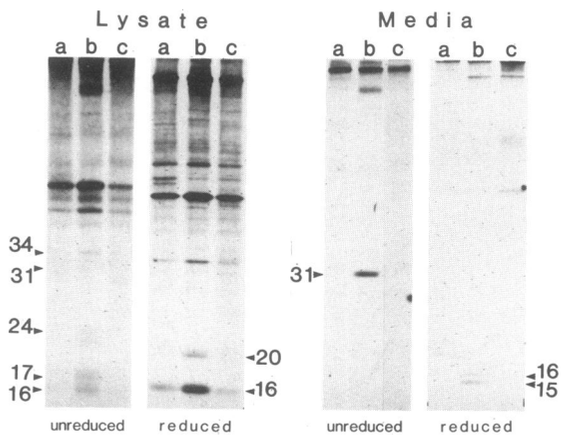

FIG. 3. Immunoprecipitation of PDGF-like proteins secreted by breast cancer cells. Culture medium was conditioned by metabolically labeled MDA-MB-231 cells. Immunoprecipitated cell lysates and CM were analyzed on 16% $NaDodSO_4$/polyacrylamide gels under nonreducing and reducing conditions. Lanes: a, control nonspecific serum; b, specific PDGF antiserum; and c, PDGF antiserum and excess purified PDGF (competition experiment). Molecular sizes of PDGF-like proteins are indicated in kDa.

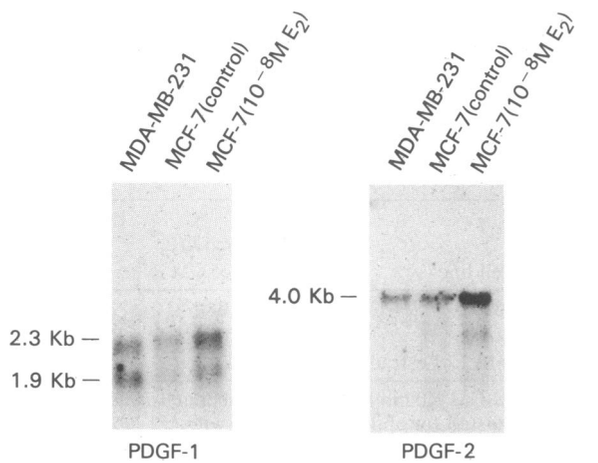

FIG. 4. Polyadenylylated RNA from MCF-7 and MDA-MB-231 cells was size-fractionated on a formaldehyde gel and transferred to a nitrocellulose membrane. Equivalent amounts of RNA were loaded in each lane and verified by ethidium bromide staining. Filters were hybridized to ^{32}P-labeled probes for PDGF-1 and PDGF-2/*SIS* then washed under stringent conditions. E_2, 17β-estradiol.

growth factor may operate in a paracrine manner causing a fibrotic response in the surrounding tissue (28). Our laboratory has reported that breast cancer cell lines secrete significant quantities of IGF-I (13) and transforming growth factor type α (14) that may stimulate the surrounding fibroblasts. In the rodent system, breast epithelial cells promote an estrogen-dependent increase in fibroblast DNA synthesis (29). PDGF can stimulate fibroblasts to secrete IGF-I, which acts as a mitogen for breast cancer cells (18). Other investigators have evidence for a paracrine model with data suggesting that the estrogen growth response of rodent mammary epithelial cells requires physical interaction with mammary stroma (29, 30). In addition, fibroblasts may promote proliferation and differentiation of breast epithelial cells through the production of a collagen matrix (31). Thus a complex relationship exists between breast epithelial cells and fibroblasts such that both cells may be necessary for tumor growth. Breast cancer cells like MCF-7 whose secretion of PDGF and other growth factors is hormonally regulated may provide an excellent model system for the study of paracrine growth.

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