ABSTRACT

A hallmark of breast carcinoma is the deposition of fibrinogen (FBG) without subsequent conversion to fibrin in the tumor stroma. In this study, the ability of the MCF-7 human breast cancer epithelial cell line to synthesize, secrete, and deposit FBG into the extracellular matrix (ECM) was examined. Whereas MCF-7 cells produced low levels of intact FBG, abundant levels of FBG intermediate complexes or degraded Αα, Ββ, and γ chain polypeptides were observed. Most of the Ββ chain was degraded and missing an NH₂-terminal peptide fragment. Reverse transcription-PCR analysis indicated that only γ chain mRNA was present in detectable steady-state levels, although Southern hybridization revealed that the FBG Αα, Ββ, and γ chain genes were intact in MCF-7 cells. Immunostaining showed that extracellular FBG was bound to the surface of MCF-7 cells in a punctate pattern, reminiscent of receptor binding, rather than a fibrillar pattern characteristic of mature ECM. A similar punctate pattern of staining was observed when MCF-7 FBG was added to fibroblasts that normally assemble exogenous FBG into an extensive, fibrillar ECM, suggesting that MCF-7 cells are defective in assembly of a fibrillar ECM. The loss of FBG Ββ chain NH₂-terminal peptides may contribute to the lack of intact FBG assembly in MCF-7 cells, which may further affect its ability to assemble FBG into a fibrillar ECM. Taken together, the data suggest that endogenous synthesis and secretion of FBG is, at least in part, the source of FBG deposition in the ECM of breast cell carcinomas.

INTRODUCTION

The progression of a tumor from benign and localized to invasive and metastatic growth is the major cause of poor clinical outcome in cancer patients. This complex process requires many components for successful dissemination and growth of the tumor cell at secondary sites, including angiogenesis, enhanced ECM degradation via tumor and host-secreted proteases, tumor cell migration, and modulation of tumor cell adhesion (1). Thus, the progression of solid tumors has been likened to “a wound that does not heal.” The importance of angiogenesis to tumor growth is now well recognized; the establishment of a tumor beyond 1–2 mm³ requires the formation of new blood vessels from preexisting vessels (2–5). Much like in a healing wound, the deposition of FBG and/or fibrin, along with other adhesive glycoproteins, into a provisional matrix serves as a scaffold to support binding of growth factors and the cellular responses of adhesion, proliferation, and migration in angiogenesis and tumor cell growth (1, 4, 6–8).

FBG is the major adhesive glycoprotein involved in the final stages of blood clotting in support of platelet aggregation and fibrin formation. FBG is synthesized constitutively by liver epithelium; however, upon induction of a systemic inflammatory response, FBG synthesis is up-regulated 2–10-fold. The increased levels of circulating FBG and resulting fibrin serve to restore homeostasis by augmenting the innate immune response to infection, tissue injury, or neoplasia (2, 4, 5, 9–11). Recently, we showed that the assembly of soluble FBG into the insoluble ECM of fibroblasts and lung alveolar epithelial cells occurs in the absence of proteolytic processing. This matrix-bound FBG is conformationally altered to expose a “fibrin-specific” epitope; however, the FBG protomer remains intact in that it is not converted to fibrin by thrombin cleavage. Furthermore, the matrix-bound FBG coaligns with mature matrix fibrils of both heparan sulfate proteoglycans and FN (12).

This previously unrecognized feature of FBG as a component of the ECM becomes important in light of the many studies showing both FBG and fibrin localization to the tumor-host cell interface (3, 13). Fibrin is abundant in different types of tumors (2, 4, 5, 9, 10), such as primary brain lesions (14) and prostate cancer (15). However, FBG, not fibrin, deposition is a feature of mesothelioma (16), colon cancer (17), lymphoma (18), and a predominant component in breast tumor stroma (13). The primary source of this extracellular FBG is thought to be attributable to exudation of plasma from leaky blood vessels and deposition of fibrin(ogen) into the tumor stroma (2, 4). In contrast, it has been speculated that the presence of FBG in the connective tissue stroma of breast carcinoma may not be attributable to exudation from plasma because other plasma proteins of comparable or smaller size are not found (19). Hereafter, endogenous synthesis and secretion of FBG by breast epithelial tumor cells has not been considered the source of FBG in the stroma of breast carcinomas. In this study, we show that MCF-7 human breast cancer cells synthesize and secrete FBG polypeptides, suggesting that the origin of FBG in the stroma of breast carcinoma may be, in part, attributable to endogenous synthesis and deposition in vivo.
**Western Blotting.** Western blotting was performed as described (21). Blots were incubated with the following mAbs: mAb 134B29, anti-FBG Aα566–580 (a kind gift from Dr. Z. Ruggeri; Scripps Research Institute, La Jolla, CA); mAb 18C6, anti-FBG Bβ1–21 (Accurate Chemicals, Westbury, NY); mAb D73H, anti-FBG Bβ23–30,561 and mAb J88B, anti-FBG Y63–78 (22). The blots were then incubated with horseradish peroxidase-conjugated rabbit antiserum Igs (Jackson, West Grove, PA), and signal development was performed using a chemiluminescence detection kit (DuPont NEN). After exposure to film, the blots were stripped according to kit instructions and reprobed with another mAb.

**Tunicamycin and N-Glycosidase F Treatment.** To analyze the glycosylation of FBG, MCF-7 and HepG2 cells were pretreated for 4 h at 10 μg/ml TUN (Calbiochem, San Diego, CA). Next, the cells were incubated in serum-free medium for 15 min, pulsed with 1 μCi/ml [35S]methionine + [35S]cysteine for 30 min, and then chased in complete media with or without 10 μg/ml TUN for 90 min. FBG was immunoprecipitated from culture media and cell lysates and subjected to SDS-PAGE, followed by fluorography. For N-Glycosidase F treatment, MCF-7 cells and HepG2 cells were metabolically labeled with 40 μCi/ml [35S]methionine + [35S]cysteine for 24 h. Media and cell lysates were collected, immunoprecipitated using anti-FBG pAb, heat denatured at 100°C in 1% SDS, and incubated with 0.5 unit/ml N-glycosidase F (Sigma) for 18 h at 37°C (23).

**Northern, RT-PCR, and Southern Analyses.** FBG mRNAs were analyzed by both Northern hybridization and RT-PCR (20). Total RNA was isolated using TriReagent (Molecular Research Center, Cincinnati, OH), according to the manufacturer’s instructions. Northern blot hybridizations were performed using FBG-specific cDNAs as probes (20). RT-PCR was performed using primer pairs described previously for human FBG Bα and γ chains and the highly conserved primers for rat β-actin, which amplify human β-actin mRNA efficiently (20). Aα primer pairs were: sense, 5′-CTCTAACCCGAGTTGTCAGAGGATCTGAG; and antisense, 5′-CAGAGTTCCAGCT-3′. The human FBG primer pair for amplification of mRNA spans an intron. First-strand cDNA synthesis was primed by oligo(dT) using 15 μg of total RNA from HepG2 or MCF-7 cells. Samples were denatured at 94°C for 2 min, annealed at 55°C for 3 min, and extended at 72°C for 3 min for 35 cycles (24).

Genomic DNA was isolated from MCF-7 and HepG2 cells by SDS-Proteinase K digestion and multiple phenol/chloroform extractions (24). Twenty μg of each genomic DNA were digested to completion with EcoRI and HindIII. After gel electrophoresis through 0.8% agarose, the DNA was denatured and neutralized and then transferred from the gel by capillary blotting onto Zeta-Probe nylon membrane (Bio-Rad, Richmond, CA). Probes were labeled with [α-32P]dCTP using a random primer kit (Life Technologies). After heat denaturation, the probe was added to the hybridization solution and incubated for 16 h at 65°C. The blots were then washed, air dried, and placed against film (24).

**Immunofluorescence Staining.** Immunofluorescence staining of HFF and MCF-7 cells cultured on glass coverslips was carried out as described (25). Purified plasma FBG used in all experiments was depleted of FN by gelatin-Sepharose affinity chromatography. Cells were grown for 10–14 days, washed, overlaid with or without 50 μg/ml purified plasma FBG or Oregon Green (Molecular Probes, Eugene, OR)-labeled purified plasma FBG and incubated for an additional 3 days. The cells were washed, fixed in 3.7% formaldehyde, and stained with pAbs against FBG, followed by rhodamine-conjugated goat antirabbit IgG (Cappel, Durham, NC) or directly visualized by epifluorescence microscopy. Coverslips were mounted inverted onto glass slides with GelMount (Biomed Corp., Foster City, CA). FBG was detected by indirect immunofluorescence staining. MCF-7 cells were grown on glass coverslips for 10–14 days. The cells were washed, fixed, and stained with pAb or mAb, followed by secondary rhodamine-conjugated goat antirabbit IgG or rhodamine-conjugated goat antimouse IgG (Cappel).

**Competitive Inhibition of Nascent FBG Binding to the MCF-7 Cell Surface.** MCF-7 cells were incubated in serum-free medium for 15 min, pulsed with 1 μCi/ml [35S]methionine + [35S]cysteine for 5 min, washed once with PBS, and then chased with and without increasing concentrations of unlabeled purified plasma FBG for 45 min. The 45-min chase allowed ample time for nascent FBG to be synthesized and secreted from MCF-7 cells. After 45 min, the medium was removed, and the cells were washed with PBS and then lysed as described above. The cell lysate for each condition was precipitated in TCA (26), and the protein concentration was measured by Bradford assay according to the kit instructions (Bio-Rad). The total TCA-precipitable cpm was divided by the μg of total protein. The results were graphed as a function of the amount of competing unlabeled FBG added.

**RESULTS**

**MCF-7 Cells Produce FBG Polypeptides.** FBG is composed of two sets of three nonidentical polypeptide chains that are products of three separate genes designated Aα, Bβ, and γ (27). Metabolic labeling and immunoprecipitation of FBG from control HepG2 cells showed intact FBG in both media and cell lysates (Fig. 1A, Lanes 1). Under reducing conditions, all three FBG chains were seen in HepG2 cells at their expected molecular weights of Mα, 66,000 (Aα), Mβ, 52,000 (Bβ), and Mγ, 46,000 (γ; Fig. 1B, Lanes 1). Metabolic labeling and immunoprecipitation of FBG from MCF-7 cells showed barely detectable levels of intact FBG either secreted in the medium or retained intracellularly (Fig. 1A, Lanes 2, arrow). Under reducing conditions, only lower molecular weight immunoreactive FBG species were detected, particularly in the intracellular fraction (Fig. 1B, Lanes 2). None of the detectable polypeptide chains immunoprecipitated from MCF-7 cells using the monospecific anti-FBG pAb corresponded in molecular size to the Aα, Bβ, and γ chain polypeptides in HepG2 FBG. The numerous bands at and below the Mγ, 46,000–48,000 band most likely represent FBG degradation products.

To further characterize the FBG immunoreactive species identified above, Western blotting with a panel of mAbs to FBG Aα, Bβ, and γ chains was performed to identify the chain composition. Purified plasma FBG (Fig. 2A, Lane 1) and FBG immunoprecipitated from HepG2 conditioned media and cell lysates (Fig. 2A, Lanes 2 and 3) showed the expected FBG immunoreactivity at Mγ, 430,000 with all mAbs, as well as with FBG-specific bands between Mγ, 140,000 and Mγ, 170,000 from both HepG2 and MCF-7 cells. These results suggest that α-γ and β-γ complexes, as well as the half-molecule (Aα-Bβ-γ) at Mγ, 170,000, were present (Fig. 2A). Only mAb J88B (anti-γ) identified FBG secreted from MCF-7 cells at the expected Mγ, 340,000 (Fig. 2A, panel γ, Lane 4, arrow), albeit at very low levels compared with the amount identified in the HepG2 cells. The Aαγ566–580 mAb, specific for the RGD cell-binding domain of the FBG Aα chain (28), did not recognize the FBG half-molecule species even in HepG2 cells, suggesting that this epitope is inaccessible. Taken together, these results suggest that MCF-7 cells synthesized and secreted FBG polypeptides which are associated with a non-FBG Aα chain.
created only small amounts of intact FBG, while the majority of FBG remained in half-molecule, unassembled, or degraded form. Under reducing conditions, purified plasma FBG (Fig. 2B, Lanes 1), as well as FBG in the HepG2 media (Fig. 2B, Lanes 2) and cell lysates (Fig. 2B, Lanes 3) showed the expected immunoreactivity with the FBG-specific mAbs Aα566–580 (134B29), Bβ63–78 (18C6), Bβ243–305 (D73H), and γ63–78 (88B) at their appropriate molecular weights. Low levels of intact Aα chain were identified at M₆₆,000 only in the MCF-7 cell lysate (Fig. 2B, panel Aα566–580 Lane 5). Furthermore, in both HepG2 and MCF-7 medium and cell lysate and, to some extent, plasma FBG, lower molecular weight products between M₁₄,000 and M₄₈,000 were identified with mAb 134B29 indicating the presence of FBG RGDS-containing Aα chain degradation products (Fig. 2B, panel Aα566–580 Lanes 1–5). Similarly, mAb 88B reacted with products between M₄₄,000 and M₄₆,000 in plasma FBG and both HepG2 and MCF-7 media and cell lysates, indicative of the FBG γ chain and γ chain degradation products (Fig. 2B, panel γ63–78 Lanes 1–5). Although γ-γ dimers were not detected by mAb 88B in MCF-7 medium and cell lysate, they were identified in plasma FBG and FBG in the secreted fraction of HepG2 cells (Fig. 2B, panel γ63–78 Lanes 1 and 2). FBG Bβ chains of the expected M₅₂,000 were recognized by mAb 18C6 in plasma FBG and in FBG from HepG2 media and cell lysates (Fig. 2B, panel Bβ1–21, Lanes 1–5). However, only a lower molecular weight product was detected with mAb 18C6 in MCF-7 medium and cell lysate (Fig. 2B, panel Bβ1–21, Lanes 4 and 5). These data suggest that this band is a Bβ chain degradation product with an intact NH₂-terminal region but missing an undetermined portion of the COOH terminus. FBG Bβ chain-specific mAb D73H showed significant amounts of immunoreactive products between M₁₄,000 and M₄₈,000 (Fig. 2B, panel Bβ243–305 Lanes 1–5) that were not reactive with mAb 18C6 in the MCF-7 cell lysate (Fig. 2B, panel Bβ1–21, Lanes 4 and 5), suggesting that at least Bβ1–21 is missing from the majority of the Bβ chain produced by MCF-7 cells.

**MCF-7 FBG Is Not Glycosylated.** Plasma FBG is N-glycosylated at Asp₁₃₅₆ on the Bβ chain and Asp₁₅₂ on the γ chain (29). Inhibition of N-linked glycosylation in HepG2 cells using TUN resulted in the expected shift to a lower molecular weight of the FBG Bβ and γ chains but not the Aα chain (Fig. 3). Treatment of MCF-7 cells with TUN did not produce any shift in the molecular weight because the FBG polypeptides isolated from the TUN-treated samples were of the same molecular weight as the untreated MCF-7 cells (Fig. 3). The absence of carbohydrate side chain addition on MCF-7 FBG was confirmed by peptide:N-glycosidase F treatment. N-Linked carbohydrate side chains were cleaved from mature Bβ and γ chains of HepG2 and plasma FBG but not from MCF-7 FBG (not shown). These data indicate that the MCF-7 cells derived FBG Bβ and γ chain polypeptides were not glycosylated, suggesting that appropriate post-translational processing of FBG through the Golgi apparatus does not occur in these breast carcinoma epithelial cells.

**MCF-7 Cell FBG Gene Expression.** Northern blot analysis did not show steady-state levels of any of the FBG mRNAs in MCF-7 cells (not shown); therefore, the more sensitive technique of RT-PCR...
indicated that rapid degradation of the FBG A chain genes are intact in MCF-7 cells. Together, these data suggest that the mRNAs does not accumulate in MCF-7 cells. The inability to detect steady-state populations of Aα and Bβ chain mRNAs were amplified from total RNA isolated from HepG2 cells (Fig. 4A). However, only FBG γ chain mRNA could be reproducibly amplified by RT-PCR from MCF-7 cell total RNA. No products were seen for FBG Aα and Bβ chains, indicating that a steady-state level of these mRNAs does not accumulate in MCF-7 cells.

The inability to detect steady-state populations of Aα and Bβ chain mRNAs suggested that either the mRNAs are unstable or that the Aα and Bβ chain genes may be defective. To investigate whether there were gross rearrangements or deletions of the FBG Aα and Bβ chain genes in the MCF-7 cells, Southern blot analysis was performed. Comparison of restriction digests of HepG2 and MCF-7 genomic DNAs probed with 32P-labeled cDNA probes for FBG Aα and Bβ chains showed that the FBG genes are intact in MCF-7 cells. Together, these data indicated that rapid degradation of the FBG Aα and Bβ chain mRNAs and polypeptides likely contributes to the defective assembly of FBG in MCF-7 cells.

Extracellular Localization of MCF-7 FBG. Indirect immunofluorescence staining using pAb anti-FBG and mAbs for FBG Aα656–580 (134B29), Bβ1–21 (18C6), and γ63–78 (J88B) chains confirmed positive extracellular staining for all three FBG chains (Fig. 5). These results suggest that FBG synthesized and secreted by MCF-7 cells binds to the cell surface. To determine whether this cell surface binding was FBG specific and saturable, the binding of metabolically labeled nascent secreted FBG to the MCF-7 cell surface was performed in the presence of increasing concentrations of unlabeled, purified plasma FBG. The data are presented as the amount of TCA-precipitable cpm/µg of total protein as a function of increasing concentrations of cold competitor FBG in the chase medium. These results show that binding of nascent FBG to the MCF-7 cell surface was inhibited in a dose-dependent and saturable manner with exogenously added FBG, suggesting a receptor-mediated binding event (Fig. 6).

Defective Assembly of MCF-7 FBG in the Extracellular Matrix of Fibroblasts. To determine whether FBG produced by MCF-7 cells could be incorporated into a preformed, heterologous ECM, 7-day-old MCF-7 cell conditioned medium was overlaid onto HFF cells, followed by indirect immunofluorescent staining using anti-FBG pAb. In the absence of MCF-7 conditioned medium, there was no detectable immunostaining with anti-FBG pAb (Fig. 7A). In contrast, in the presence of MCF-7 conditioned medium, the results showed a punctate pattern of positive staining for FBG on the surface of the HFF (Fig. 7B). These results indicated that MCF-7 FBG bound to the cell surface but did not assemble into the ECM of HFFs in the fibrillar pattern indicative of mature matrix, whereas plasma FBG assembled into mature matrix fibrils in the ECM of HFFs, as shown previously (12) and in Fig. 8A.
Defective Assembly of Plasma FBG into MCF-7 ECM. Because plasma FBG assembles into the ECM of heterologous cells such as lung epithelial cells and fibroblasts (12), we wanted to determine whether exogenous plasma FBG incorporates into breast epithelial cell ECM. Medium containing Oregon Green labeled-purified plasma FBG was overlaid onto MCF-7 cells. As a control, HFFs were also overlaid with the FBG-Oregon Green. The results showed the expected fibrillar pattern of plasma FBG in the ECM of HFFs (Fig. 8A). In contrast, FBG-Oregon Green appeared in a punctate pattern of staining (Fig. 8B), indicative of FBG binding to the MCF-7 cell surface. Together, these results indicate a defect in the synthesis and processing of endogenous FBG by MCF-7 cells, as well as a defect in the ability of MCF-7 breast carcinoma cells to assemble exogenously added plasma FBG into mature matrix fibrils.

DISCUSSION

The origin of the fibrinogen and corresponding degradation products associated with tumors is usually thought to be attributable to exudation of plasma because of the increased vascular permeability and subsequent procoagulant or fibrinolytic activity at the tumor site (6, 13, 30–36). Because FBG deposition in the stroma, but not subsequent fibrin formation, is considered a hallmark of breast carcinoma, we investigated whether the MCF-7 human breast cancer cell line is capable of synthesizing and secreting FBG in vitro. In further support of this line of investigation, it has been shown that FBG is synthesized and secreted from established extrahepatic epithelial cell lines including human uterine cervical (37), intestinal (38), and alveolar (20) carcinoma cells. These extrahepatic epithelial cells synthesize FBG composed of Aα, Bβ, and γ chain polypeptides with the characteristic Mr 340,000. Hepatocytes secrete mainly intact FBG; although, occasionally, very small amounts of intermediate chain complexes, unassembled fragments, or individual chains are observed (39). However, bovine granulosa cells appear to synthesize and secrete only Bβ and γ chains of FBG (40). Furthermore, studies of COS cells transfected with FBG cDNAs show that FBG assembly is not required for its secretion and that intermediates such as α-γ complexes are secreted along with fully assembled FBG (41).

We show in this report that the intracellular assembly of intact, dimeric FBG is limited in MCF-7 cells. Analysis of the chain composition indicates that most of the FBG secreted by MCF-7 cells is intact γ chain with partially degraded Aα and Bβ chains. The absence of intact Bβ chain likely hinders the formation of fully assembled FBG, because these NH2-terminal regions are known to be important in disulfide bond formation between two FBG half-molecules (42–44). Thus, abundant FBG fragments with limited amounts of intact FBG are secreted from MCF-7 breast carcinoma cells. FBG secreted from MCF-7 cells binds to the cell surface instead of assembling into mature ECM fibrils. Together, these data support the hypothesis that breast carcinoma cell FBG and FBG degradation products may contribute to the pathophysiology of breast cancer progression by providing additional adhesive cell binding domains or chemotactic peptides to modulate breast cancer cell responses to the ECM microenvironment.

We investigated the expression of the FBG transcripts in MCF-7 cells by both Northern hybridization and RT-PCR. The FBG Aα and Bβ chain mRNAs were undetectable by both procedures, suggesting that these mRNA species are either expressed at low levels or that they are very unstable and do not reach steady-state levels in MCF-7 breast epithelial cells. One possibility for limited Bβ chain production may involve mRNA instability. Instability of mRNA is mediated primarily by the AU-rich region in the 3’ untranslated region in many genes such as tumor necrosis factor-α (45), granulocyte/macrophage colony-stimulating factor (46), and interleukin 3 (47, 48). The Bβ chain gene contains a degradation signal in the 3’ untranslated region that is homologous to the nonamer described as being the key AU-rich sequence mediating mRNA degradation (49, 50). The presence of this conserved AU-rich motif may lead to rapid degradation of the Bβ chain mRNA in the MCF-7 cells by RNases not present in HepG2 cells. Together, the data indicate that the low levels of the FBG Aα and Bβ chain mRNAs and the rapid degradation of the polypeptides likely contribute to the defective intracellular assembly of FBG into the intact Mr 340,000 molecule in MCF-7 cells.

We have shown that nascent FBG assemblies into the ECM of the alveolar epithelial cells as fibrils indicative of mature matrix (12). Therefore, we wanted to determine whether nascent secreted FBG assembled into the ECM of MCF-7 cells. Indirect immunofluorescence staining using mAbs for each of the chains confirmed that the Aα, Bβ, and γ chains of nascent FBG, or their fragments, bound to the cell surface of MCF-7 cells but did not assemble into mature matrix fibrils. This cell surface binding is likely receptor mediated, because the binding of nascent FBG to the MCF-7 cells was competitively inhibited with unlabeled, purified plasma FBG. The majority of MCF-7 cell-derived FBG is missing the NH2-terminal domain containing Bβ1–21. The loss of Bβ1–21 also results in disruption of the heparin binding domain defined by Bβ1–42 (51), which may participate in FBG incorporation into a fibrillar ECM (52). Thus, the loss of Bβ1–21 sequences in MCF-7 FBG may contribute to its inability to assemble into mature ECM through heparan sulfate proteoglycan-dependent mechanisms.

Fig. 7. Conditioned MCF-7 medium overlay onto HFF cells. HFF cells were grown on glass coverslips for 14 days to establish a mature ECM. The cells were washed, and MCF-7 conditioned medium was overlaid onto HFF cells and incubated for an additional 3 days. The cells were stained under nonpermeabilized conditions with pAb anti-FBG antibodies, followed by rhodamine conjugated-goat antirabbit secondary antibodies and visualized by fluorescence microscopy. A, HFF cells stained with anti-FBG pAb only. B, HFF cells with MCF-7 conditioned medium overlay and stained with anti-FBG pAb.

Insets, secondary antibody staining only; bar, 15 μm.

Fig. 8. Assembly of plasma FBG into ECM of HFF and MCF-7 cells. HFF (A) and MCF-7 (B) cells were grown on glass coverslips for 10 days. Purified plasma FBG labeled with Oregon Green was added to cells and incubated for 24 h. The cells were washed extensively, fixed, mounted, and directly visualized for Oregon Green-FBG by epifluorescence microscopy. Bar in B, 15 μm.
Both alveolar epithelial cell FBG and plasma FBG assemble as mature matrix fibrils into the ECM of heterologous cell types such as HFFs (12). However, the extracellular association of MCF-7 FBG by heterologous cells was illustrated by a punctate pattern of FBG staining; no fibril formation was observed. Because HFFs do not synthesize FBG endogenously, the positive staining was attributable to extracellular binding of FBG from the MCF-7 conditioned medium overlay. Although purified plasma FBG showed the characteristic fibrillar pattern of mature matrix in the ECM of HFFs, the punctate pattern of staining observed with plasma FBG on the MCF-7 cell surface indicates that MCF-7 cells are capable of binding intact plasma FBG but not assembling plasma FBG into a fibrillar matrix. These results suggest that although the MCF-7 cells are capable of binding FBG, they lack the additional cell surface and/or matrix constituents that support the assembly of FBG into mature matrix fibrils. Preliminary studies in our laboratory indicate that FBG assembly into mature matrix fibrils requires cell surface integrin receptors, matrix heparan sulfate proteoglycans, and active matrix assembly of FN.5

Knowing that the functions of FBG are important during wound healing, its role can be extrapolated and expanded to other pathological processes including tumorigenesis. The novel finding that breast tumor cells synthesize and secrete FBG that becomes extracellularly as a specialized ECM molecule that, in turn, affects migration, and degradation of FBG, and its inability to assemble into a fibrillar protein, may signify the production of a specialized ECM molecule that, in turn, affects cellular processes of adhesion, proliferation, migration, and angiogenesis that modulate the progression of breast cancer.

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Fibrinogen Assembly, Secretion, and Deposition into Extracellular Matrix by MCF-7 Human Breast Carcinoma Cells

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