Microenvironmental Regulation of Membrane Type 1 Matrix Metalloproteinase Activity in Ovarian Carcinoma Cells via Collagen-induced EGR1 Expression

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Late stage ovarian cancer is characterized by disseminated intraperitoneal metastasis as secondary lesions anchor in the type I and III collagen-rich submesothelial matrix. Ovarian carcinoma cells preferentially adhere to interstitial collagen, and collagen-induced integrin clustering up-regulates the expression of the transmembrane collagenase membrane type I matrix metalloproteinase (MT1-MMP). Collagenolytic activity is important in intraperitoneal metastasis, potentiating invasion through the mesothelial cell layer and colonization of the submesothelial collagen-rich matrix. The objective of this study was to elucidate a potential mechanistic link between collagen adhesion and MT1-MMP expression. Our results indicate that culturing cells on three-dimensional collagen gels, but not thin layer collagen or synthetic three-dimensional hydrogels, results in rapid induction of the transcription factor EGR1. Integrin signaling through a SRC kinase-dependent pathway is necessary for EGR1 induction. Silencing of EGR1 expression using small interfering RNA abrogated collagen-induced MT1-MMP expression and inhibited cellular invasion of three-dimensional collagen gels. These data support a model for intraperitoneal metastasis wherein collagen adhesion and clustering of collagen binding integrins activates integrin-mediated signaling via SRC kinases to induce expression of EGR1, resulting in transcriptional activation of the MT1-MMP promoter and subsequent MT1-MMP-catalyzed collagen invasion. This model highlights the role of unique interactions between ovarian carcinoma cells and interstitial collagens in the ovarian tumor microenvironment in inducing gene expression changes that potentiate intraperitoneal metastatic progression.

Epithelial ovarian carcinoma is the leading cause of death from gynecologic malignancy (1), due primarily to the fact that the majority of patients are diagnosed at late stage (III and IV) when metastasis has already occurred. Approximately 10% of all epithelial ovarian carcinomas are hereditary and may be facilitated by gene mutations, whereas the remaining 90% are sporadic (2–6). Tumors are thought to arise from the single cell layer of the ovarian epithelium and metastasis occurs via direct extension into the peritoneal cavity, where shed cells from the primary tumor are found as a component of malignant ascites. Secondary tumors arise as a consequence of CD44– and integrin-mediated intra-peritoneal adhesion and localized invasion into the interstitial collagen-rich submesothelial matrix. Proteolytic activity is important at multiple stages in intraperitoneal metastasis, including disruption of cell-cell interactions, migration and invasion into the mesothelial cell layer, and the submesothelial matrix to anchor secondary lesions, and for subsequent tumor-mediated angiogenesis (7).

Multiple studies have shown that microenvironmental contacts with stromal cells or extracellular matrix elements may play a major role in tumor progression by inducing epigenetic changes in transformed cells (8, 9). Metastasizing ovarian cancer cells encounter a collagen-rich environment, as the submesothelial matrix is comprised primarily of interstitial collagens (types I and III) and ovarian tumors induce a fibro-proliferative response characterized by increased synthesis of collagen in the peritoneal cavity (10–12). Malignant ovarian epithelial cells preferentially adhere to type I collagen via α2β1 and α3β1 integrins (13, 14). Model experiments using microbeads containing immobilized integrin subunit-specific antibodies, to mimic matrix-induced integrin aggregation, showed that clustering of β1-integrins induced expression of the transmembrane collagenase designated membrane type 1 matrix metalloproteinase (MT1-MMP, MMP-14) as well as MT1-MMP-mediated activation of pro-MMP2 (14). As invasion of three-dimensional collagen matrices by ovarian cancer cells is potentiated by MT1-MMP collagenolytic activity (14), these data support integrin-mediated collagen adhesion as an important microenvironmental regulator of ovarian cancer metastasis.

The abbreviations used are: MT1-MMP, membrane type 1 matrix metalloproteinase; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase; EGR1, early growth response protein 1; RT, reverse transcriptase; siRNA, small interfering RNA; MAPK, mitogen-activated protein kinase.
Because of its role as an in vivo collagenase, the activity of MT1-MMP is stringently regulated via multiple mechanisms including transcriptional and post-translational control (15). Previous data from our laboratory suggested that collagen-induced expression of MT1-MMP in ovarian cancer cells was regulated, in part, at the level of transcription (5); however, in contrast to other members of the MMP family, relatively little is known regarding transcriptional control of MT1-MMP expression (20–23). The current study was undertaken to elucidate a potential mechanistic link between collagen adhesion and MT1-MMP expression. Our results support a model wherein β1 integrin signaling via a SRC kinase-dependent pathway can potentiate intra-peritoneal metastasis by rapid induction of the early growth response protein (EGR1), inducing transcriptional activation of the MT1-MMP promoter and subsequent MT1-MMP-mediated collagen invasion.

**EXPERIMENTAL PROCEDURES**

**Immunohistochemistry**—Immunohistochemical analysis was done retrospectively on tumor tissue microarrays prepared by the Pathology Core Facility of the Robert H. Lurie Comprehensive Cancer Center at Northwestern University assembled from tissue originally taken for routine diagnostic purposes. The microarray tissue specimens included 149 human ovarian carcinomas (77 serous, 45 endometroid, 9 mucinous, and 18 clear cell). Samples were cut 3–4 μm thick and deparaffinized. The cores were 1 mm in diameter. Antigen retrieval was accomplished by heat induction at 99 °C for ~45 min. Immunohistochemical staining with antibodies to MT1-MMP (Neomarkers, Kalamazoo, MI, clone RB-1544B raised against a peptide from the second quarter of MT1-MMP) at 1:100 dilution was done according to standard procedures. Breast carcinoma was used as a positive control for MT1-MMP. Analysis of tissue sections was done by light microscopy by an anatomic pathologist (B. P. A.) without prior knowledge of the clinical variables. Scoring of MT1-MMP was assigned according to the average overall intensity of the staining and was graded as follows: 0, no staining; 1, fine granular staining; 2, somewhat coarse staining, but less than positive control tissue (human placenta); 3, very coarse staining, similar to positive control tissue. Staining <10% of tumor cells, regardless of intensity, was considered negative. Staining of between 10 and 75% of tumor cells was considered focal positive, and staining of greater than 75% of tumor cells was considered diffuse positive. Statistical analyses were performed by the Biostatistics Core Facility of the Robert H. Lurie Comprehensive Cancer Center.

**Materials**—Polyclonal anti-early growth response protein 1 (EGR1) recognizing 58-kDa human EGR1 was purchased from Aviva Systems Biology (San Diego, CA). Monoclonal anti-β-tubulin (clone TUB2.1), polyclonal anti-matrix metalloproteinase-14 (hinge region), anti-rabbit and anti-mouse immunoglobulin G horseradish peroxidase-conjugated antibodies, and bacterial collagenase were obtained from Sigma. Phospho-SRC family (Tyr-416) and c-SRC polyclonal antibodies were from Cell Signaling Technology and Santa Cruz Biotechnology (Santa Cruz, CA), respectively. Rat tail type I collagen and BDPuraMatrix Peptide Hydrogel were purchased from BD Biosciences. The kinase inhibitors PP2, PP3, PD98059, SB203580, wortmannin, and LY294002 were purchased from Calbiochem. The broad-spectrum inhibitor of metalloproteinases GM6001 and a function blocking mouse anti-human integrin β1 monoclonal antibody (mAb 1959) were obtained from Chemicon International (Temecula, CA).

**Cell Culture**—The ovarian carcinoma cell line DOV13 was provided by Dr. R. Bast, Jr. (M. D. Anderson Cancer Center, Houston, TX). DOV13 cell culture was maintained in minimal essential medium (Invitrogen), 10% fetal bovine serum (Invitrogen), penicillin/streptomycin (Cellgro, Mediatech), amphotericin B (Cellgro, Mediatech), nonessential amino acids (Cellgro, Mediatech), sodium pyruvate (Cellgro, Mediatech), and insulin from bovine pancreas (10 mg/liter; Sigma) at 37 °C in 5% CO₂ (16). Normal immortalized ovarian epithelial cell line IOSE398 was provided by Dr. N. Auersperg (University of British Columbia, Vancouver, Canada) and maintained for no longer then 10 passages in a 1:1 mixture of media 199 (Sigma) and MCD8105 (Sigma), 5% fetal bovine serum, and 50 μg/ml gentamicin (Sigma).

**Three-dimensional Culture Model of Ovarian Cancer Metastasis**—To mimic initial stages of submesothelial matrix invasion by ovarian cancer cells, cells in suspension were plated atop of a three-dimensional collagen I gel. Three-dimensional collagen I gels were prepared by mixing rat tail type I collagen to the final concentration of 0.8 mg/ml with serum-free medium and solidified by polymerization for 30 min at 37 °C. Control cells were plated on thin layer collagen I (two-dimensional collagen I) prepared by coating tissue culture plates by passive adsorption with a dilute (10 μg/ml) solution of rat tail collagen I overnight. Prior to inducing matrix contact, cells were subjected to 20–24 h serum starvation, suspended with trypsin, neutralized with soybean trypsin inhibitor, resuspended in serum-free media, and subsequently plated atop three-dimensional or two-dimensional type I collagen surfaces for the indicated time points. Before collecting cells, media was removed by aspiration, plates were placed on ice and treated with 0.1 mg/ml collagenase solution in phosphate-buffered saline to remove collagen followed by scraping from the plate. The remaining collagen gel was sheared by pipeting and further disrupted by brief incubation (up to 5 min at 37 °C) with the above collagenase solution. Cells were collected by centrifugation for 5 min at 2000 rpm at 4 °C, washed twice with phosphate-buffered saline, and centrifuged as above to collect.

**Inhibitors**—The kinase inhibitors PP2, PP3, PD98059, SB203580, wortmannin, and LY294002 were used at concentrations 10, 10, 50, 25, 0.1, and 25 μM, respectively, for all experiments. As determined by trypan blue (Sigma) staining, cells were viable at these conditions. Where applicable, inhibitors were also co-polymerized into three-dimensional collagen gels. Prior to the experiment, starved cells were pretreated with the inhibitors for 1 h.

**Gelatin Zymography**—Gelatinase activities in conditioned media were determined by SDS-PAGE zymography as previously described (17). Gels were prepared with 9% acrylamide and 0.1% gelatin, samples were electrophoresed without reduction (18). SDS was removed from the gel through a 30-min incubation in 2.5% Triton X-100. To initiate gelatinase activity, gels were incubated in 100 ml of 20 mM glycine, 10 mM CaCl₂,
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and 1 μM ZnCl2, pH 8.3, at 37 °C for 24–48 h prior to staining for protein. Developed gels were dried and scanned using Epson Perfection 1640SU and Adobe Photoshop 7.0 software.

Western Blotting—Cells incubated under various conditions were collected as described above and lysed with buffer (TBST) containing 50 mM Tris, 150 mM NaCl, 1 mM CaCl2, 1 mM MgCl2, 1% Nonidet P-40, protease inhibitor mixture (Roche). Cell lysates (20 μg) were electrophoresed on 9% SDS-polyacrylamide gels under reducing conditions (18), electroblotted to a polyvinylidene difluoride membrane (19), blocked with 5% skim milk in TBST (25 mmol/liter of Tris, pH 7.5, 150 mmol/liter of NaCl, 0.1% Tween 20) for 1 h at room temperature (20 °C). Membranes were incubated for 1–2 h at room temperature with antibodies derived against proteins of interest. The antibodies were used at the following dilutions: 1:1000 for anti-human hinge region MT1-MMP polyclonal antibody in 3% bovine serum albumin in TBST; 1:500 for anti-human MT1-MMP monoclonal antibody in 5% skim milk in TBST, 1:1000 for anti-phospho-SRC (Tyr-416) polyclonal antibody in 3% bovine serum albumin in TBST, 1:500 for anti-total SRC polyclonal antibody in 5% skim milk in TBST, 1:1000 for anti-β-tubulin monoclonal antibody in 5% skim milk in TBST. Immunoreactive bands were visualized with an anti-(rabbit IgG)-peroxidase or anti-(mouse IgG)-peroxidase (1:1000 in 5% skim milk in TBST) and enhanced chemiluminescence using LAS3000 (Fuji-film) and LAS3000 ImageReader software. Band intensities were determined using LAS3000 ImageGauge software according to the manufacturer’s instructions.

mRNA Extraction and cDNA Synthesis—Total mRNA was purified from 1 to 2 × 106 cells using the Aurum Total RNA Mini Kit (Bio-Rad). cDNA was synthesized from 10 μg of total RNA using the iScript cDNA Synthesis Kit (Bio-Rad). mRNA purification and cDNA synthesis experiments were repeated three times.

Quantitative Real Time PCR—Real time PCR was carried out with the LightCycler RT-PCR System (Bio-Rad) according to the manufacturer’s instructions. SYBR Green was used for quantitative PCR as a double-stranded DNA-specific fluorophore. The PCR was conducted by initial denaturation for 10 min at 95 °C followed by 40 cycles of 94 °C for 15 s, 60 °C for 30 s using the iTaq SYBR Green Supermix (Bio-Rad). To determine the specificity of the PCR primers, melting curves were collected by heating the products at 95 °C, then cooling down to 65 °C, and then slowly melting at 0.5 °C/s up to 95 °C. Primer sequences for EGR1 mRNA detection were according to the previously published sequences (20). Sequences of other primers used for the real-time RT-PCR were constructed according to the requirements for primers used for real time RT-PCR (supplemental Table 1). Efficiency of amplification was determined using the standard curves method. Relative quantification of gene expression between experimental (three-dimensional collagen I) and control (two-dimensional collagen I) samples was measured by normalization against endogenous RPL-19 using the ΔCt method (21). Prior to using RPL-19 as a control, it has been established that its expression correlated well with the total RNA concentration and did not change with the time and treatment used in our studies. -Fold changes were quantified as 2^(-ΔΔCt, sample-ΔCt, control) as described previously (21).

Transient Transfections—Transient transfections were performed using lipofection method with ExGene (Fermentas) as a vehicle. EGR1 and control siRNA (Santa Cruz Biotechnology) were transiently transfected into DOV13 cells according to the manufacturer’s instructions.

Cellular Invasion Assay—Invasion assays were performed using Transwell chambers (0.8 μm, BD Biosciences) as described before (14). In brief, Transwell inserts were coated on the bottom with a thin layer of human collagen (Sigma) as a chemoattractant for 1 h at 37 °C. The inner well of the filters contained 20 μg of human collagen that was allowed to air dry overnight. Cells (70,000) were plated in the collagen-coated inserts, and allowed to invade the collagen gel for 18–24 h at 37 °C. Filters were collected and the cells adhering to the lower surface were fixed and stained using the Diff-Quik staining kit (Dade Behring) according to the manufacturer’s instructions. Cells in several random fields were counted and averaged. Invading cells were expressed as a percent of total cells added to the invasion chamber.

RESULTS

Analysis of MT1-MMP Expression in Human Ovarian Carcinoma—The normal ovarian surface epithelium does not express MMPs and MT1-MMP is not detected in benign tumors; however, MT1-MMP has been detected in malignant ovarian tumors (22–24). To evaluate MT1-MMP expression in a large patient cohort, samples from 149 patients were examined for MT1-MMP immunoreactivity. Of these samples, 77 (52%) were serous carcinoma, 45 (30%) were endometrioid carcinoma, 18 (12%) were clear cell carcinoma, and 9 (6%) were mucinous carcinoma. The vast majority of ovarian tumors (78%) displayed positive MT1-MMP immunoreactivity (Table 1, Fig. 1). Staining, with the exception of 17 serous carcinomas, 11 endometrioid carcinomas, and one mucinous carcinoma, was typically diffuse (expression in greater than 75% of tumor cells). MT1-MMP expression was high (3+ or 2+) in 78% of patients. Representative examples of serous, endometrioid, clear cell, and mucinous ovarian tumors with intense MT1-MMP immunoreactivity are shown in Fig. 1. It is interesting to note that in clear cell carcinoma, the most invasive histotype, MT1-MMP, is expressed at high levels in 94% of cases (Table 1). No significant MT1-MMP staining was observed in the stromal compartment. Strong MT1-MMP positivity (3+ or 2+) was not differentially distributed according to Fédération Internationale des Gynécologues et Obstétristes stage.

### TABLE 1

| Histotype       | 3+ | 2+ | 1+ | 0  |
|-----------------|----|----|----|----|
| Serous (77)     | 8  | 24 | 24 | 21 |
| (10%)           | (31%) | (31%) | (28%) |
| Endometrioid (45) | 4  | 19 | 10 | 12 |
| (9%)           | (42%) | (22%) | (27%) |
| Clear cell (18) | 3  | 14 | 0  | 1  |
| (17%)          | (78%) | (0%) | (5%) |
| Mucinous (9)   | 2  | 3  | 2  | 2  |
| (22%)          | (34%) | (22%) | (22%) |
β1 Integrin-mediated MT1-MMP Expression on Three-dimensional Type I Collagen—Ovarian carcinomas metastasize intra-peritoneally through implantation into the collagen-rich submesothelium, providing a mechanism for anchoring of secondary lesions to enable proliferation at multiple sites in the peritoneal cavity. We have previously demonstrated preferential adhesion of primary and established ovarian cancer cells to interstitial collagen relative to other matrix proteins including fibronectin, laminin, type IV collagen, and vitronectin (16, 25). Modeling matrix-induced integrin aggregation using microbead-immobilized β1 integrin antibodies demonstrated that integrin clustering enhanced MT1-MMP expression and this stimulation of MT1-MMP collagenolytic activity was a rate-limiting step for invasion of three-dimensional collagen gels (14). To evaluate further the molecular mechanisms that regulate MT1-MMP expression, a model of intraperitoneal metastasis comprised of tumor cells on three-dimensional collagen gels was utilized. Cells cultured on three-dimensional collagen type I gels adopt a distinct elongated morphology relative to cells cultured on thin layer (two-dimensional) collagen (Fig. 2A). These morphological changes are abrogated by pretreatment with β1 integrin function blocking antibodies (Fig. 2A, right panel), supporting the role of β1 integrins in ovarian cancer cell interaction with three-dimensional collagen gels. Cells generate internal tension via cytoskeletal modulations, balanced in part by adhesion to the ECM, and ample evidence suggests that integrin-mediated ECM adhesion is inherently a mechanosensory process (26–28). It has been proposed that it is the ability of the ECM to resist tension and promote cell distortion that controls cellular behavior (29). To test this hypothesis, ovarian cancer cells were cultured in a three-dimensional peptide hydrogel (PuraMatrix Peptide Hydrogel, BD Biosciences) to modify cell shape in the absence of collagen-induced integrin clustering. Control experiments included three-dimensional collagen gels and thin layer type I collagen.

Induction of MT1-MMP activity was evaluated by monitoring MT1-MMP-catalyzed activation of pro-MMP2 via gelatin zymography (13, 14). Consistent with previous reports (13, 14), thin layer collagen culture did not induce MT1-MMP activity and corresponding changes in pro-MMP2 activation (Fig. 2B). Similarly, altering cell shape by three-dimensional culture in PuraMatrix Peptide Hydrogel in the absence of collagen contact was not sufficient to induce MT1-MMP activity (Fig. 2C).
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In contrast, cells cultured in three-dimensional type I collagen gels exhibited a time-dependent increase in MT1-MMP activity (Fig. 2, D and E). This is supported by Western blot analysis of MT1-MMP protein levels, showing a similar time-dependent increase in MT1-MMP protein (Fig. 2, F and G). Levels of pro-MMP2 were not reproducibly altered by any of the above culture conditions.

MT1-MMP Induction in Three-dimensional Collagen I Is Transcriptionally Regulated by EGR1—MT1-MMP is subject to considerable post-translational control via protein trafficking, zymogen activation, and autolysis; however, mechanisms of transcriptional regulation have not been extensively evaluated. We previously reported that collagen-induced MT1-MMP activity was blocked, in part, by actinomycin D, an inhibitor of RNA synthesis (14). The promoter region of MT1-MMP has many putative transcription factor binding sites (30), several of which have been demonstrated to regulate MT1-MMP expression (31–34). Among those, EGR1 was shown to play a role in induction of MT1-MMP in murine endothelial cells in collagen culture (31). In addition to EGR1, several related transcription factors including EGR2, -3, and -4, and SP1 share a similar consensus sequence (35–38). To evaluate matrix-induced expression of EGR family transcription factors, cells were cultured on three-dimensional collagen gels or on thin layer collagen for various time points and differential expression of mRNAs encoding SP1, EGR1, -2, -3, and -4 was evaluated by real time RT-PCR. RPL-19 was used as a control housekeeping gene. Whereas a modest induction of EGR2 was apparent at 4 h, a robust induction of EGR1 was observed at early time points (Fig. 3A). The increase in EGR1 expression was not observed when cells were cultured three-dimensionally in PuraMatrix Peptide Hydrogel (data not shown). No significant changes in SP1 expression were obtained (Fig. 3A) and mRNAs encoding EGR3 and -4 were not detected (data not shown). A related temporal sequence evaluating MT1-MMP mRNA levels indicated that, following the increase in EGR1 expression at 2 h, MT1-MMP mRNA levels were significantly enhanced at the 4-h time point (Fig. 3B). In control experiments, levels of β1 integrin mRNA (Fig. 3B), as well as TIMP2, and MMP2 (data not shown) were not altered by collagen culture. As the real time RT-PCR data implicate EGR1 as a primary candidate for transcriptional regulation of MT1-MMP in three-dimensional collagen, EGR1 protein levels were evaluated by Western blotting. Expression of EGR1 on three-dimensional collagen peaked after 3 h of induction and was 2-fold higher than that on thin layer collagen (Fig. 4, A and B). Expression of EGR1 on thin layer collagen did not change significantly during the 24-h time course of the experiment (Fig. 4, A and C). In control experiments using immortalized normal ovarian epithelial cells (IOSE398), no significant change in mRNA expression for either EGR1 or MT1-MMP were observed in response to three-dimensional collagen culture (data not shown).

β1 Integrin Signaling through SRC Kinase Regulates EGR1 Expression in Three-dimensional Collagen—To further evaluate regulation of EGR1 expression, a panel of inhibitors was utilized to block the activity of kinases reported to participate in signaling events either upstream of EGR1 (31) or downstream
using an siRNA approach to silence in collagen-induced MT1-MMP expression was evaluated (Fig. 5, A).

Inhibition of Egr-1 Blocks Collagen-induced MT1-MMP Expression and Cellular Collagen Invasion

The role of EGR1 in collagen-induced MT1-MMP expression was evaluated using an siRNA approach to silence EGR1 gene expression.

Inhibition of Egr-1 Blocks Collagen-induced MT1-MMP Expression and Cellular Collagen Invasion—The role of EGR1 in collagen-induced MT1-MMP expression was evaluated using an siRNA approach to silence EGR1 gene expression.

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Cells transfected with EGR1 siRNA failed to up-regulate EGR1 protein in response to three-dimensional collagen culture, whereas a control siRNA had no effect on EGR1 induction (Fig. 6A). Moreover, cells with a silenced EGR1 gene did not exhibit enhanced MT1-MMP expression on three-dimensional collagen (Fig. 6B), supporting a key role for EGR1 in collagen-induced MT1-MMP expression. As invasion of three-dimensional collagen gels by ovarian cancer cells is MT1-MMP dependent (13, 14), the effect of EGR1 gene silencing on collagen invasion was evaluated. A significant decrease in collagen invasion was observed in cells transfected with EGR1-specific siRNA relative to cells transfected with a control siRNA (Fig. 6C), supporting the hypothesis that induction of MT1-MMP expression via β1 integrin signaling to EGR1 alters the invasive behavior of ovarian cancer cells.

DISCUSSION

The mesodermally derived ovarian surface epithelium displays both epithelial and mesenchymal characteristics, rare in epithelial cells in situ. Reversible modulation of ovarian epithelial cells to a fibroblastic form occurs during post-ovulatory repair of the epithelium. This unique phenotypic plasticity suggests that ovarian epithelium adapts to changes in the cellular microenvironment by transition between epithelial and fibro-
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blastic phenotypes, a characteristic usually limited to immature, regenerating, or neoplastic epithelia (8). Pre-neoplastic changes in the OSE are characterized by acquisition of a polarized glandular phenotype (8). Rather than migrating through the ovarian stroma, tumor nodules are exfoliated into the peritoneal cavity where they resist anoikis and generate diffuse intraperitoneal metastases with malignant ascites. In the majority of women with ovarian cancer, death is caused by widespread intraperitoneal metastases resulting in bowel obstruction. Numerous studies have indicated that interaction between tumor cells and stromal elements plays a key role in tumor progression and metastasis (8, 9). This is supported by data indicating that survival of ovarian cancer patients correlates with the outcome of debulking surgery (39), indicating that separation of epithelial ovarian cancer cells from the intraperitoneal microenvironment is a fundamental step to prevention of metastatic growth.

Following attachment of disseminated ovarian tumor cells to the peritoneal mesothelium (40, 41), integrin-mediated cell-matrix interaction potentiates intraperitoneal adhesion. Ovarian cancer cells extend cytoplasmic processes through the junctional margins of neighboring mesothelial cells, inducing cellular retraction and exposure of the submesothelial extracellular matrix, followed by integrin-mediated adhesion to the newly exposed matrix (42–44). As metastasis is the end result of numerous intraperitoneal adhesive events, cellular integrins play a major role in regulation of ovarian cancer metastatic behavior. Ovarian cancer cells adhere preferentially to interstitial collagen types I and III using α2β1 and α3β1 integrins (13, 14). Affinity for interstitial collagens likely reflects the phenotypic plasticity of ovarian surface epithelium as well as the composition of the mesothelial ECM that contains predominantly interstitial types I and III collagen (10–12). These data suggest that β1 integrin-mediated adhesion to peritoneal collagens represents an important early event unique to ovarian cancer metastatic dissemination and, together with the current data, support a model wherein integrin signaling is an epigenetic effector of ovarian cancer progression (45).

Proteolytic activity is important at multiple stages in intraperitoneal metastasis, including disruption of cell-cell interactions, migration, and invasion through the mesothelial cell layer and the colonization of the submesothelial interstitial collagen-rich matrix. The transmembrane proteinase MT1-MMP is the major collagenase expressed by ovarian tumors. MT1-MMP is not detected in benign tumors (23), but is overexpressed in malignant ovarian tumors as evaluated by in situ hybridization and immunohistochemistry (Fig. 1 and Refs. 23 and 45–48) and expression of MT1-MMP in peritoneal metastatic lesions is correlated with poor survival (23, 45, 46). Using model systems that mimic peritoneal metastasis, we have previously demonstrated that aggregation of collagen-binding integrins, induced either by culturing ovarian carcinoma cells on a three-dimensional collagen lattice or using bead-immobilized integrin subunit-specific monoclonal antibodies, stimulates MT1-MMP expression and activity (13, 14), resulting in enhanced collagen invasion. Rat microvascular endothelial cells respond similarly to three-dimensional collagen and previous studies have demonstrated that both three-dimensional culture and cyclic mechanical strain up-regulate MT1-MMP via enhanced production of the transcription factor EGR1 (31, 49). These studies further demonstrated binding of EGR1 to the MT1-MMP promoter, resulting in increased transcriptional activity in promoter/reporter assays that was abrogated upon mutation of the Egr1 binding site. Interestingly, fibroblasts infected with Rous sarcoma virus were found to up-regulate EGR1 expression via the protein-tyrosine kinase activity of v-Src (50–52). In cancer cells, activation of c-SRC has been linked to proliferation, motility, and survival (53). It has been demonstrated that SRC is present in active form in cancers affecting many tissues, including pancreas (54), breast (55), lung (56), prostate (57), liver (58), ovary (59), colon (60), and rectum (61). Interestingly, activation of SRC in ovarian cancers is found specifically in late stage disease (59). Furthermore, it was shown that inhibition of SRC sensitizes drug-resistant ovarian cancer cells to pharmaceutical agents including paclitaxel and cisplatin (62, 63). c-SRC and SRC family kinases also participate in integrin-mediated transmission of mechanical signals from the extracellular microenvironment, either via focal adhesion kinase activation (64, 65) or by a focal adhesion kinase-independent direct interaction between SRC and the β integrin cytoplasmic tail that induces Src activation and stabilizes the activated kinase following integrin clustering (66). It was recently shown that SRC-induced podosomes in human umbilical vein endothelial cells display gelatinolytic activity due to MMP2, activated by MT1-MMP (67). In addition, when formation of podosomes was compromised by addition of PP2, an inhibitor of Src as well as podosome formation, MT1-MMP-driven matrix degradation was inhibited, suggesting that SRC kinase is an upstream regulator of MT1-MMP-dependent matrix degradation in podosomes (68). The current data provide support for a mechanistic link between collagen adhesion, integrin clustering, and integrin-mediated signaling via Src kinases to induce expression of EGR1, resulting in transcriptional activation of the MT1-MMP promoter and subsequent MT1-MMP-catalyzed intra-peritoneal invasion. This model highlights the role of unique interactions between ovarian carcinoma cells and interstitial collagens in the ovarian tumor microenvironment in inducing epigenetic changes that potentiate intraperitoneal metastatic progression.

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