Gastrin-releasing peptide mediates its morphogenic properties in human colon cancer by upregulating intracellular adhesion protein-1 (ICAM-1) via focal adhesion kinase

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Taglia L, Matusiak D, Matkowskyj KA, Benya RV. Gastrin-releasing peptide mediates its morphogenic properties in human colon cancer by upregulating intracellular adhesion protein-1 (ICAM-1) via focal adhesion kinase. Am J Physiol Gastrointest Liver Physiol 292: G182–G190, 2007. First published August 17, 2006; doi:10.1152/ajpgi.00201.2006.—Gastrin-releasing peptide (GRP) and its receptor (GRPR) act as morphogens when expressed in colorectal cancer (CRC), promoting the assumption of a better differentiated phenotype by regulating cell motility in the context of remodeling and retarding tumor cell metastasis by enhancing cell-matrix attachment. Although we have shown that these processes are mediated by focal adhesion kinase (FAK), the downstream target(s) of GRP-induced FAK activation are not known. Since osteoblast differentiation is mediated by FAK-initiated upregulation of ICAM-1 (Nakayamada S, Okada Y, Saito K, Tamura M, Tanaka Y. J Biol Chem 278: 45368–45374, 2003), we determined whether GRP-induced activation of FAK alters ICAM-1 expression in CRC and, if so, determined the contribution of ICAM-1 to mediating GRP’s morphogenic properties. Caco-2 and HT-29 cells variably express GRP/GRPR. These cells only express ICAM-1 when GRPR are present. In human CRC, GRPR and ICAM-1 are only expressed by better differentiated tumor cells, with ICAM-1 located at the basolateral membrane. ICAM-1 expression was only observed subsequent to GRPR signaling via FAK. To study the effect of ICAM-1 expression on tumor cell motility, CRC cells expressing GRP, GRPR, and ICAM-1 were cultured in the presence and absence of GRPR antagonist or FAK signaling via FAK. To study the effect of ICAM-1 expression on tumor cell motility, CRC cells expressing GRP, GRPR, and ICAM-1 were cultured in the presence and absence of GRPR antagonist or monoclonal antibody to ICAM-1. CRC cells engaged in directed motility in the context of remodeling and were highly adherent to the extracellular matrix, only in the absence of antagonist or ICAM-1 antibody. These data indicate that GRP upregulation of ICAM-1 via FAK promotes tumor cell motility and attachment to the extracellular matrix.

Stimulation of the GRPR regulates the normal function of many tissues, including those in the central nervous (1, 6, 40), immune (12), and pulmonary systems (54). However, the most important property of bombesin/GRP may well be its ability to act as a morphogen in colorectal cancer (CRC).

Although epithelial cells lining the adult gastrointestinal tract do not normally express GRP/GRPR, when aberrantly expressed in CRC these proteins promote the assumption of a better differentiated phenotype by regulating cell motility in the context of remodeling (7, 8) and retard tumor cell metastasis by enhancing cell attachment to the extracellular matrix (19). Our recent investigations suggested that GRP/GRPR mediate their morphogenic properties via focal adhesion kinase (FAK) phosphorylation (19, 36). Yet since FAK can act as a signaling intermediary, this observation did not fully explain how FAK activation in and of itself might mediate GRP/GRPR’s morphogenic properties.

A recent study of osteoblast-to-osteoclast differentiation indicated that this process critically included FAK-mediated upregulation of intracellular adhesion molecule-1 (ICAM-1) (42). Although this study examined FAK-initiated ICAM-1 upregulation in the context of extracellular matrix-integrin interactions, FAK has been shown to upregulate ICAM-1 independently of integrins in other cells, such as those within the synovium, by acting as a signaling intermediary (28, 43). Hence the purpose of this study was to determine whether GRP/GRPR upregulates ICAM-1 via FAK and, if so, to determine the contribution of this protein to mediating GRP/GRPR’s morphogenic properties.

In this study we show that ICAM-1 mediates GRP’s promotion of tumor cell motility in the context of remodeling and attachment to the extracellular matrix. This is particularly important since, although it is known that ICAM-1 can be aberrantly expressed in CRC and suppress cancer progression, the mechanism has been believed to be due to this molecule’s activation of the host immune surveillance system (2, 13, 16, 25, 26, 58, 62). In contrast, we herein demonstrate that ICAM-1 mediates many if not all of GRP/GRPR’s morphogenic effects, providing evidence for a hitherto unappreciated mechanism whereby ICAM-1 expression might promote CRC differentiation and retard metastasis. Thus these data not only identify the mechanism of GRP/GRPR action in CRC but also support adding ICAM-1 to the small but growing list of CRC morphogens.

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Tables of contents

METHODS

Materials. Anhydrous ethanol (100%, 95%) and xylene were purchased from Pharmco Products (Brookfield, CT). Wash buffer, target retrieval solution, protein block serum, antibody diluent, LSAB2 system-horseradish peroxidase (HRP), liquid DAB substrate-chromogen system, EnVision+ HRP (DAB)-rabbit system, and automated hematoxylin were all from DAKO (Carpenteria, CA). Actin, phosphorylated tyrosine 397 (p-Y397) of FAK and ICAM-1 antibodies, as well as goat anti-rabbit IgG-HRP, were from Santa Cruz Biotechnologies (Santa Cruz, CA). Alexa 488 and Alexa 568 conjugated IgG were purchased from Molecular Probes (Eugene, OR). Auto/Iodine, Redsol, and PVDF membranes were from Fisher Scientific (Pittsburgh, PA). Transwell permeable supports were from Corning (Acton, MA). Mammalian protease inhibitor cocktail was purchased from Sigma (St. Louis, MO). ECL plus Western blotting detection system was from Amersham (Piscataway, NJ). The 30% acrylamide/bis solution and Precision Plus protein standards were from Bio-Rad Laboratories (Richmond, CA). BCA protein assay kit was from Pierce (Rockford, IL).

As previously described (7), GRP expression was detected by use of a polyclonal rabbit anti-human peptide antibody directed to amino acids 14 to 27 from Abcam, whereas GRPR expression was detected by using a polyclonal rabbit anti-peptide antibody directed to the distal third intracellular loop of the mouse and human GRP-R (CVEGNIHVKKQIESRKK). The GRPR-specific antagonist d-Phe8(bombesin) methyl ester was kindly provided by Dr. David Coy (Tulane University, New Orleans, LA).

Tumor selection and assessment of differentiation. Human CRC were randomly selected from the Chicago Veterans Administration Medical Center Gastrointestinal Tissue Bank. This resource contains paraffin blocks of all CRC removed at this institution since its opening in 1956. For this study, 10 CRC resected between 1995 and 2005 were randomly selected; all tissues studied were different from, and have not been previously evaluated as a part of, prior evaluations performed by our group (7, 21, 23, 34, 36, 38, 39). The 10 CRC contained 61 separate regions of distinct differentiation, approximately equally distributed between those that were well, moderately, or poorly differentiated (Table 1). For each region of distinct differentiation, between 50 and 2,000 cells were analyzed, with the mean number of cells evaluated per region of defined differentiation for 75 and 580 cells.

Nonconfocal photomicrographs were obtained using a SPOT RT digital scanning camera from Diagnostic Instruments (Sterling Heights, MI) attached to a Nikon E600 microscope system. Tumor differentiation was performed using a three-grade classification system as previously described (8). Well-differentiated tumors were defined by the presence of well-formed glands containing malignant columnar cells displaying small regular nuclei. The complete absence of gland formation, or the presence of bizarrely shaped glands, identified poorly differentiated tumors. Moderately differentiated tumors possessed well-formed glands, but the cells were less columnar or frankly cuboidal, with reduced cell polarity and more dysplastic nuclei than those observed in well-differentiated tumors.

Quantitative immunohistochemistry: immunoperoxidase. A modified indirect immunoperoxidase technique was performed when DAB was used in avidin-biotin complex immunohistochemistry and was performed to permit quantification (described under Quantitative immunohistochemistry). Cells were seeded on uncoated, multiwell Permanox slides in complete medium. To achieve confluence by day 3 postplating, 400,000 cells were plated per 4.5-cm2 well of a two-well slide. Cells were starved for 24 h before immunohistochemistry. Cells were fixed in situ in 3.7% formaldehyde in PBS for 30 min at 37°C. Slides were processed using the Envision Plus system (DAKO). Briefly, fixing solution was removed and cells were washed with TBS supplemented with 0.05% Tween 20. Endogenous peroxidase activity was blocked by using 0.03% hydrogen peroxide for 5 min at room temperature followed by washing the cells and then incubating with primary antibody for 1 h at room temperature. Cells were probed with a polymer-labeled anti-rabbit secondary antibody for 30 min, followed by DAB+ for 3 min. Slides were counterstained with Gill’s hematoxylin for 2 min.

Quantitative immunohistochemistry. Chromogen abundance was quantified by determining the cumulative signal strength of the digital image file of the relevant region or cell of interest (32–34, 37). For all specimens, control tissues were processed identically and at the same time except that primary antibody was not applied. Control tissues were within 4 μm of the tested tissue and treated with secondary antibody, DAB+ and counterstained with hematoxylin for precisely the same time as the experimental tissue. Thus all differences between the experimental tissue and the control tissue are ultimately due to DAB identification of the relevant protein. The amount of chromogen per pixel was determined by subtracting the mathematical energy (EM) of the control slide (i.e., not exposed to primary antibody) from that in the homologous region of the experimental slide (i.e., exposed to primary antibody). Chromogen quantity (EM) is expressed as energy units per pixel (eu/pix). Maximal and half-maximal EM values were determined after curve fitting the data by least-squares nonlinear regression.

Cell lines and cell culture. To study the effects of FAK, we used specially modified 293 cells that, as we have previously described, expressed the dominant negative regulator for FAK-related nonkinase (FRNK) (19). Briefly, these cells were modified to express FRNK...
under control of a doxycycline-inducible promoter (19). Although these cells do not express FRNK under basal conditions, FRNK is rapidly upregulated after exposure to 2 μg/ml doxycycline (Clonetech) for at least 18 h (19). Cells were cultured in Opti-MEM containing HEPES buffer, sodium bicarbonate, and l-glutamine supplemented with 10% tetracycline-free fetal bovine serum, 600 mg/ml G-418, and 200 mg/ml hygromycin B; 293 cells were maintained at 37°C in a 5% CO₂ atmosphere.

In contrast, Caco-2 and HT-29 cells were cultured in d-MEM/F12 containing glucose and l-glutamine supplemented with 10% fetal bovine serum. Cells were maintained at 37°C in a 5% CO₂ atmosphere in all instances.

Western analysis. Cells were grown to at least 60% confluence and then incubated in serum-free medium for 24 h. Thereafter, cell monolayers were rinsed in PBS and lysed in ice-cold RIPA (50 mM HEPES, pH 7.4; 150 mM NaCl; 1% Triton X-10; 0.1% SDS; 0.5% sodium deoxycholate; 1 mM sodium orthovanadate; 5 mM EDTA; 5 mM sodium fluoride) containing a 1:20 dilution of mammalian protease inhibitor cocktail (Sigma). In all instances, 200 μg of total cell protein were loaded into a preparative well and electrophoresed across a 10% polyacrylamide gel under denaturing and reducing conditions. Membranes were incubated with primary antibody for 2 h at the following concentrations: actin 1:100, GRPR 1:500, Y397 1:100, and ICAM-1 1:500. This was followed by two 10-min washes with TBST with immunoreactive bands visualized using a HRP-conjugated goat anti-rabbit IgG and the ECL Plus detection system.

Cell-matrix attachment. Cell adhesion to the extracellular matrix was determined as previously described (30, 56, 59). Briefly, cells were plated at a density of 50,000 cells per well in a 12-well plate. Cells were cultured for 24 h before being washed with PBS and treated for 3 h in serum-free medium alone, or in the presence of 1 μM d-Phε⁶ bombesin (methyl ester), ICAM-1 monoclonal antibody at a concentration of 1:50, or goat anti-mouse antibody at a concentration of 1:100 (used as a control). Cells were then exposed to 500 μl of 0.25% trypsin/1 mM EDTA for 2 min at the indicated time points, and total number of detached cells was quantified by use of a Beckman-Coulter Z2 counter (Miami, FL).

Motility. Cells were plated and cultured until 2 days postconfluent, washed, and then cultured in serum-free medium for an additional 18 h. A linear gap in the cell monolayer was generated by use of a sterile scalpel blade. Cells were then mounted in a custom-designed Leica inverted tissue culture microscope such that cells were consistently maintained at 37°C. Cell images were acquired every 5 min with a Hamamatsu 1.3 mega-pixel digital camera (Hamamatsu City, Japan). Evaluation of cell behavior was performed using Open Lab (Improvision, Coventry, UK).

Cell motility was quantified as the percentage of the original wound size, or gap, remaining over time. Three separate values were obtained for each image, and each experiment was repeated a minimum of three separate times. Images were converted into QuickTime files at native size (640 × 480) using the “Video” codec, edited using Final Cut Pro 4.0 (Apple), and exported for viewing using the “JPEG A” codec at reduced size (320 × 240) at 10 frames/s. The resultant videos can be viewed at: http://www.uic.edu/com/dm/gastro/labvideos.html.

Statistical analyses. All evaluations were performed using Excel (Microsoft, Redmond, WA), with data reported as means ± SE. Data were evaluated by paired or unpaired t-test or by ANOVA, as appropriate, using built-in statistical functions provided by Excel.

RESULTS

**GRP/GRPR-ICAM-1 expression and location in human CRC.** We previously showed that Caco-2 and HT-29 cells variably express GRP/GRPR as a function of confluence (20). We determined the location of GRPR and ICAM-1 expression in preconfluent Caco-2 (Fig. 1) and HT-29 [data not shown (NS)] cells by confocal microscopy. GRPR were expressed...
primarily if not exclusively in the apical membrane of these cells (Fig. 1A). In contrast, ICAM-1 was present diffusely in the cytoplasm of Caco2 (Fig. 1B) and HT-29 cells, as well as showed evidence of being coexpressed with GRPR at the level of the apical membrane (Fig. 1C). To evaluate for expression at the level of the basolateral membrane (BLM), we used a specific antibody for FAK. Confocal images confirmed that ICAM-1 was also present in the region of the BLM (Fig. 1D).

Lastly we used an antibody for actin to demonstrate that ICAM-1 was not expressed in regions mediating cell-cell contact (Fig. 1E).

We next determined whether ICAM-1 expression was likewise associated with the amount of GRP/GRPR present in resected human CRC. To do this we evaluated the expression of GRP/GRPR and ICAM-1 in 10 human CRC randomly selected from our Gastrointestinal Tissue Bank (7). In all instances, consecutive 4-μm-thick sections were evaluated for GRP, GRPR, or ICAM-1 expression, allowing for expression to be quantified [by quantitative immunohistochemistry (Q-IHC) (32–34, 37)] in the same cells within any particular cancer. Similar to what we have previously shown (7), GRP/GRPR were not expressed by nonmalignant epithelial cells lining the colon (data NS). Similarly, no evidence of ICAM-1 expression could be detected in nonmalignant epithelial cells lining the colon (Fig. 2, A and B). In contrast, and similar to what we have previously reported (7), GRPR expression was a function of tumor cell differentiation, with over 270 ± 7 eu/pix detected in well-differentiated CRC cells and 159 ± 11 eu/pix in moderately differentiated cells, but only 35 ± 3 eu/pix in poorly differentiated cells (Fig. 3). Likewise, ICAM-1 expression in CRC was also a function of CRC cell differentiation, such that high levels of expression were noted in well differentiated tumor cells (237 ± 7 eu/pix; Fig. 2, C and D; Fig. 3) and low levels in poorly differentiated cells (39 ± 6 eu/pix; Fig. 2, E and F; Fig. 3). Overall, ICAM-1 expression tightly correlated with the coexpression of GRP/GRPR in any particular cell (Fig. 3; r² = 0.91). Importantly, and in contrast to what was observed for Caco-2 and HT-29 cells, most ICAM-1 expression (when present) was evident at the level of the BLM (Fig. 2D).

We next investigated the kinetics of ICAM-1 expression (Fig. 4). Cells were plated to confluence, washed, cultured in serum-free medium for 24 h, and then wounded with a sterile razor blade. At the indicated time point, protein expression was quantified by DAB-based immunohistochemistry as previously described (32–34, 37). GRPR were rapidly upregulated postwounding, with maximal expression noted at time (t) = 6.8 ± 1.3 h and half-maximal expression at 2.8 ± 1.6 h. In contrast, ICAM-1 expression lagged slightly, albeit significantly, such that maximal expression was noted at t = 7.0 ± 1.7 h and half-maximal expression at 4.0 ± 0.6 h (P < 0.001 for both conditions, paired t-test; Fig. 4). Similar results were obtained for HT-29 cells evaluated similarly (data NS). Finally, cells were coincubated with a GRPR antagonist d-Phe⁶(bombesin) methyl ester (11, 61), and ICAM-1 expression was assessed postwounding. Incubating wounded cells with this antagonist completely eliminated ICAM-1 expression (Fig. 4).

Role of FAK in GRPR-initiated ICAM-1 expression. We previously showed that GRPR-induced phosphorylation of FAK at Y397 (19, 36) critically mediated many of this protein’s morphogenic properties. Because FAK is known to upregulate ICAM-1 (28, 42, 43), whereas GRP is known to upregulate FAK, we determined whether activated GRPR upregulated ICAM-1 expression in Caco-2 (data NS) and HT-29 cells (Fig. 5). Preconfluent cells were cultured in serum-free medium for 24 h in the absence (Fig. 5, left) or presence (Fig. 5, right) of the GRPR-specific antagonist d-Phe⁶(bombesin) methyl ester. Preconfluent GRP-secreting, GRPR-expressing cells showed evidence of FAK activation by virtue of tyrosine 397 being phosphorylated, consistent with what we have previously published, and ICAM-1 being expressed. In contrast, abrogating GRPR signaling using d-Phe⁶(bombesin) methyl ester eliminated tyrosine 397 phosphorylation as well as ICAM-1 expression.

To confirm the role of FAK in regulating ICAM-1 expression, we studied GRP/GRPR-expressing 293 cells that had been modified to express FRNK, the dominant negative inhibitor of FAK, under control of a doxycycline-sensitive inducible promoter (19). Under basal conditions, GRP autocrine activation of its cognate receptor results in Y397 phosphorylation of FAK and ICAM-1 expression (Fig. 6, left). When the same cells are now exposed to doxycycline, thereby activating FRNK, no change in GRP expression is noted, but both FAK phosphorylation at Y397 and ICAM-1 expression are completely ablated (Fig. 6, right). Thus GRP-induced upregulation of ICAM-1 occurs via FAK.

Contribution of ICAM-1 to mediating cell-ECM attachment. We have previously shown that GRP activation enhances cell attachment to the extracellular matrix, thereby retarding metastasis, and increases cell motility in the context of remodeling, promoting the assumption of a better differentiated phenotype (19). To assess cell attachment, we plated Caco-2 cells at subconfluent densities for 24 h to promote GRP/GRPR expression. Cells were then washed in serum-free PBS in the absence or presence of exogenous GRP, the antagonist d-Phe⁶(bombesin) methyl ester, control antibody, or monoclonal antibody directed to ICAM-1 for 3 h. Cells were then washed again followed by exposure to 0.25% trypsin for the indicated time, and detached cells were counted. When exposed to trypsin in serum-free medium for 10 min all cells were detached, whereas exposure for 1 min resulted in ~50% of attached cells detaching. We therefore used 1 min exposure to trypsin as our baseline condition so that alterations in avidity of cell attachment in either direction could be assessed.

Exposure to exogenous GRP (1 μM) for 3 h markedly increased Caco-2 cell attachment to the ECM by 62.3 ± 2.1% (P < 0.0001, paired t-test) (Fig. 7). In contrast, exposure to the GRP-specific antagonist t-Phe⁶(bombesin) methyl ester decreased Caco-2 cell attachment by 53.7 ± 3.9% compared with cells exposed to medium and autocrine GRP alone (P < 0.0001, paired t-test). Although ICAM-1 antagonists do not exist, we explored whether the commercially available antibody used in our immunohistochemical studies could act to inhibit ICAM-1 action, similar in concept to what we have previously done to inhibit the action of the galanin-1 receptor (35). Thus we exposed cells to monoclonal antibody directed to ICAM-1 for 3 h. In so doing we noted a similar 48.9 ± 3.3% decrease in cell attachment to the ECM compared with cells exposed to serum-free medium alone (P < 0.0001, paired t-test) (Fig. 7). In contrast, rabbit anti-human IgG antibody (1:100) used as a control had no impact the avidity of Caco-2 cell attachment to the ECM (data NS).
We next evaluated motility in the context of remodeling by culturing Caco-2 cells to confluence, wounding them with a sterile razor blade, and tracking cell position at the indicated time points. Similar to what we have previously described (20), Caco-2 cells migrated rapidly into the gap created by the razor blade, and once the gap was closed no further cell movement could be detected, consistent with the motility occurring to retain tissue architecture and not representative of greater malignancy (Fig. 8) (see video at http://www.uic.edu/com/dom/gastro/Caco2.videos.html). When cultured in the presence of the antagonist D-Phe6(bombesin) methyl ester, and similar to what we have previously reported (20), cells initially migrated into the gap, consistent with the fact that GRPR are not originally present, with migration attenuating from 3 h onward, consistent with increasing receptor expression (Fig. 8). Lastly, we used ICAM-1 antibody to probe the contribution of this protein to mediating GRP-

**Fig. 2.** Expression of ICAM-1 in normal colonic epithelium and in human colon cancer as a function of tumor cell differentiation. A, C, and E: differential interference contrast (DIC) images of normal adult colon (A) as well as well-differentiated (C) and a poorly differentiated (E) tumors. B, D, F: confocal images of normal colonic epithelium (B) stained for actin (green) and ICAM-1 (red) as well as well-differentiated (D) and poorly differentiated (F) tumor cells. Boxes in C and E indicate region of DIC image magnified in the adjacent confocal image. White arrow in D identifies the margin of the basement membrane.

**Contribution of ICAM-1 to mediating cell motility in the context of remodeling.** We next evaluated motility in the context of remodeling by culturing Caco-2 cells to confluence, wounding them with a sterile razor blade, and tracking cell position at the indicated time points. Similar to what we have previously described (20), Caco-2 cells migrated rapidly into the gap created by the razor blade, and once the gap was closed no further cell movement could be detected, consistent with the motility occurring to retain tissue architecture and not representative of greater malignancy (Fig. 8) (see video at http://www.uic.edu/com/dom/gastro/Caco2.videos.html). When cultured in the presence of the antagonist D-Phe6(bombesin) methyl ester, and similar to what we have previously reported (20), cells initially migrated into the gap, consistent with the fact that GRPR are not originally present, with migration attenuating from 3 h onward, consistent with increasing receptor expression (Fig. 8). Lastly, we used ICAM-1 antibody to probe the contribution of this protein to mediating GRP-
initiated cell motility. Whereas rabbit anti-human IgG antibody (1:100) used as a control had no impact on cell motility (Fig. 8), ICAM-1 antibody used at the same concentration attenuated Caco-2 cell motility similarly as GRPR antagonist D-Phe6(bombesin) methyl ester (Fig. 8) (see videos of both conditions at http://www.uic.edu/com/dom/gastro/labvideos.html). Specifically, ICAM-1 antibody had no effect on attenuating cell migration into the gap immediately after wounding, consistent with there being no ICAM-1 expressed. After the first 3 h, however, there was progressive attenuation in Caco-2 cell motility, with the attenuation paralleling the increase in ICAM-1 expression (Fig. 4). Thus these data strongly suggest that ICAM-1 mediates GRP’s morphogenic actions of enhancing cell attachment and promoting directed cell motility.

**DISCUSSION**

Epithelial cells lining the adult colon do not normally express ICAM-1 but can be expressed subsequent to malignant transformation (13, 26, 57). When present, significant evidence exists showing that ICAM-1 expression decreases CRC metastasis. Lymph node or liver metastases are decreased in patients whose primary tumor expresses ICAM-1 compared with those whose tumor does not (31), whereas lower numbers of ICAM-1 positive cells are observed in primary tumors that metastasize (62). Direct evidence for ICAM-1 expression decreasing CRC metastasis comes from a recent study that, using a nude mouse model, noted decreased liver metastases in the human CRC cell line LM-H3 ectopically expressing ICAM-1 compared with cells not expressing this protein (55). However,
Whereas GRPR acting as morphogens and regulating tumor cell differentiation.

The differentiation of solid tumors correlates with patient survival for all tumors studied (5, 9, 27, 41, 45–47, 51, 52, 60), the regulation of ICAM-1 expression in CRC is not known, whereas the mechanism whereby ICAM-1 is believed to decrease metastasis is less than convincing.

ICAM-1 is believed to decrease tumor cell metastasis by promoting heterotypic cell-cell contact with inflammatory cells (2, 13, 16, 25, 26, 58, 62). Since ICAM-1 expression in CRC correlates with the number of inflammatory cells within a particular tumor (26), and given that ICAM-1 promotes the attachment of such cells to human CRC cell lines (26), this protein has been proposed to play a role in promoting lymphocyte-mediated tumor killing (26, 58). Indeed, even the study whereby ICAM-1 was ectopically expressed in LM-H3 cells thereby decreasing metastasis suggested that this occurred as a result of enhanced binding of peripheral blood mononuclear cells to the tumor cells and subsequent tumor cell lysis (55). Yet the data presented herein suggest an alternative mechanism, namely that ICAM-1 enhances tumor cell attachment to the extracellular matrix. Along with contributing to tumor cell architecture by promoting motility in the context of remodeling, ICAM-1 appears to be acting as a morphogen, mediating the actions of GRP upon autocrine activation of its cognate receptor.

GRP and its receptor are typically studied in the context of their ability to act as mitogens when aberrantly expressed in cancer, including those derived from the colon (17, 18, 44, 49, 50). Yet we have shown that, when aberrantly expressed in CRC, GRP’s mitogenic properties are subordinate to its morphogenic properties (reviewed in Ref. 24). Like ICAM-1, GRP/GRPR are not expressed by epithelial cells lining the normal adult human colon, but when aberrantly expressed in CRC they are present in better differentiated cancer cells but not poorly differentiated cells or metastases (7). Our study of GRPR−/− mice provided direct evidence for GRP/GRPR acting as morphogens and regulating tumor cell differentiation. Whereas GRPR−/− mice develop CRC that become progressively more poorly differentiated, those developing in wild-type GRPR-expressing mice are well differentiated (8). Since the differentiation of solid tumors correlates with patient survival for all tumors studied (5, 9, 27, 41, 45–47, 51, 52, 60), including those developing in the colon (22, 63), morphogens not only regulate tumor appearance but also regulate behaviors altering the propensity of a tumor cell to metastasize.

We previously identified biophysical properties that are surrogate markers for tumor cell differentiation and metastasis, including tumor cell migration in the context of remodeling and cell attachment to the extracellular matrix (19). Although FAK phosphorylation has been shown to mediate GRP-stimulated cell proliferation (48), we have also shown that FAK phosphorylation at tyrosine 397 regulates tumor cell motility in the context of remodeling and attachment to the extracellular matrix (19, 36). However, since FAK can serve as a signaling molecule, these observations begged the question as to what downstream proteins might exist mediating GRP’s morphogenic effects. The recent studies showing that osteoblast to osteoclast differentiation critically involved ICAM-1 upregulation via a FAK-dependent pathway, along with our prior studies indicating that GRP/GRPR regulate CRC differentiation via FAK, suggested that this downstream protein might be ICAM-1.

In this paper we show for the first time that autocrine activation of the GRPR causes ICAM-1 to be rapidly expressed via a FAK-dependent signaling cascade. We also show that ICAM-1 is primarily expressed at the level of the BLM, where it mediates GRP’s morphogenic properties. We specifically show that ICAM-1 mediates tumor cell attachment to the extracellular matrix. In so doing ICAM-1 would be expected to prevent cells from detaching from the primary tumor mass and thus attenuate or eliminate metastasis. Thus this represents a process that is completely independent of this protein’s previous documented ability to improve patient survival by promoting the binding of inflammatory cells to CRCs (2, 13, 16, 25, 26, 58, 62).

Equally important, we also show for the first time that ICAM-1, by mediating tumor cell motility in the context of remodeling, acts as a motogen. The qualification that this
motility occurs only in the context of remodeling is important. Cells of greater malignancy show no evidence of contact inhibition and would in our wounding assay show evidence of intercalating between one another after the gap was filled. In contrast, we herein demonstrate that after the wound is filled cell motility ceases. Thus this directed motility is similar in principle to what is observed with nonmalignant cells as they maintain the architecture characteristic of the organ system whence they reside or is required as a part of organogenesis. Hence our bioassay quantifying directed cell motility measures a core property of morphogens in malignancy. As such, our data not only identify the mechanism of GRP/GRPR action as a morphogen when aberrantly expressed in CRC but also indicate that ICAM-1 itself is a morphogen in at least this type of tumor.

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