Transduction of a Mesenchyme-specific Gene Periostin into 293T Cells Induces Cell Invasive Activity through Epithelial-Mesenchymal Transformation*

Wei Yan and Rong Shao

From the Pioneer Valley Life Sciences Institute, Baystate Medical Center, University of Massachusetts Amherst, Springfield, Massachusetts 01107 and Department of Veterinary and Animal Sciences, University of Massachusetts, Amherst, Massachusetts 01003

Tumor metastasis is a multistep pathological process involved in the final phase of tumor development. During this process, epithelium-derived tumor cells undergo fibroblast-like transformation, referred to as epithelial-mesenchymal transition (EMT), which contributes to aggressive behavior of tumors. We identify periostin, a mesenchyme-specific gene product, as a contributor to EMT and metastatic potential. Stable expression of a periostin transgene in tumorigenic but non-metastatic 293T cells caused cells to undergo fibroblast-like transformation accompanied by increased expressions of vimentin, epidermal growth factor receptor (EGFR), and matrix metalloproteinase-9. The cells expressing ectopic periostin increased cell migration, invasion, and adhesion by 2–9-fold. Invasive characteristics required signaling through integrin αvβ3 and EGFR. In addition, periostin-engineered 293T cells formed metastases in immuno-deficient mice following either cardiac inoculation or injection into mammary fat pad. These data demonstrate an active role for periostin in EMT and metastasis that requires cross-talk between integrin and EGFR signaling pathways.

Progression of a solid tumor to an invasive tumor is a major prerequisite for metastasis and involves changes in both cell morphology and motility (1, 2). Although genetic alterations in cancerous cells may vary in different types of metastatic cancers, they share the outcome that cancerous cells are disseminated to multiple distant organs (3, 4). Typically, these aggressive cells detach from the site of origin, move across tissue boundaries to penetrate into lymphatic and blood vessels, and eventually extravasate from these vessels and colonize new sites. Phenotypic conversion of tumor cells from epithelial to mesenchymal phenotype, termed epithelial-mesenchymal transition (EMT),2 is commonly associated with acquisition of metastatic potential (5–7). During this transition, tumor cells usually lose epithelial features, including apical-basal polarity, expression on cytokeratin filaments, and membrane-associated adherens or desmosomes junctions. Concurrently, they gain mesenchymal properties, including expression of vimentin filaments, spindle-like morphology, and increased motility allowing cells to invade new sites (8–10).

The molecular basis underlying the EMT process involves multiple changes in expression, distribution, and/or function of proteins that include vimentin, integrins, matrix metalloproteinases (MMP), and cadherins (6, 11–13). Acquired expression of vimentin by carcinoma cells, which replaces the cytoskeleton network, often symbolizes mesenchyme-like cell transformation (6, 11). Loss of E-cadherin or gain of N-cadherin on tumor cell surface is frequently observed in malignant carcinomas and also correlated with enhanced aggressiveness and dedifferentiation (14). Elevated MMP-9 expression and activity allow tumor cells to degrade and penetrate extracellular matrix (15, 16). Integrins, heterodimeric transmembrane receptor complexes, interact with specific regions of extracellular matrix protein and transmit "outside-in" signals to the cells, leading to an array of intracellular signaling events participating in cell proliferation, adhesion, and motility. For example, increased expression of integrin αvβ3 in solid tumor and melanomas was correlated with tumor malignancy, and its function was found to enhance tumor cell growth and invasion (17, 18). Likewise, the increased activity of integrin αvβ3 enhanced motility of ovarian cancer cells (19, 20).

Because integrins perform important functions in tumor progression, intensive research efforts have focused on the cross-talk between integrins and membrane tyrosine kinase receptors (21–23). Growth factors that activate receptor tyrosine kinases can alter integrin-mediated activities such as cell adhesion, spreading, and migration via alterations in integrin activation and localization. For instance, breast cancer cells expressing integrin αvβ3 depend on insulin-like growth factor stimulation for integrin αvβ3-mediated cell migration (24). Conversely, signals from integrins regulate full activation of growth factor signaling. EGFR is activated and required to cooperate with integrin αvβ3 for vitronectin-induced carcinoma cell migration (25). The selective coordination of inputs from different growth factor receptors and integrins largely depends on the cell types that inherently express distinct membrane receptors.

Integrins also play important roles in the activation of endothelial cells as well as tumor cells (26, 27). Engagement of acti-

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1 To whom correspondence should be addressed. Tel.: 413-794-9568; Fax: 413-794-0857; E-mail: rong.shao@bhs.org.

2 The abbreviations used are: EMT, epithelial-mesenchymal transition; MMP, matrix metalloproteinase; siRNA, short interference RNA; EGF, epidermal growth factor; EGFR, EGF receptor.
activated vascular endothelial growth factor (VEGF) receptor 2 (Flk-1/KDR) with integrin \( \alpha_v \beta_3 \) in endothelial cells is required for cell migration and adhesion in response to VEGF, whereas integrin \( \alpha_v \beta_5 \) is required for cancer cell adhesion and motility (20, 28, 29). Therefore, the convergence and cascades of signaling from integrins and tyrosine kinase receptors appear to be essential in both endothelial cells and tumor cells for the induction of tumor angiogenesis, invasion, and metastasis.

The secretory protein periostin, a mesenchyme-specific gene, is normally expressed in osteoblasts. Gillan et al. (30) have found that periostin promotes adhesion and potentiates cancer cell motility through binding to integrins \( \alpha_v \beta_3 \) and \( \alpha_v \beta_5 \). More importantly, clinical studies of periostin expression in human cancers have demonstrated that increased expression of periostin is correlated with tumor angiogenesis and metastasis (31–33). In the present study, we tested the hypothesis that periostin acquired by tumorigenic cells may facilitate EMT and induce metastatic behavior. In our studies the epithelium-derived, tumorigenic 293T cells were used to determine the effects of periostin both in vitro and in vivo. We found that 293T cells overproducing periostin induced EMT and metastasis and that EMT required the coordination of integrin \( \alpha_v \beta_5 \) and EGFR signals.

**EXPERIMENTAL PROCEDURES**

**Generation of Periostin-producing Cells**—Full-length human periostin cDNA (MluI-XhoI) was subcloned into a retroviral pCMV-neo-vector. 293T retroviral packaging cells were transfected with the periostin construct or vector control in the presence of pCL 10A1 vector using FuGENE 6 as the delivery vehicle. 48 h after transfection, the supernatant was harvested and filtered through a 0.45-μm pore size filter, and the virus-containing medium was used to infect parental 293T cells. Selection with 800 μg/ml of G418 was started 48 h after infection, and the drug-resistant cell populations were used for subsequent studies.

**Periostin siRNA Gene Knock Down**—Specific oligos (21 bp) targeting the periostin gene were selected, and an oligo template (64 oligo nucleotides) containing the 21-bp oligos was subcloned into a retroviral pSUPER-puro-vector. Retroviral medium was generated as described above, and consequently the medium infected 293T cells expressing periostin to establish a stable line containing periostin siRNA.

**Gelatin Zymography**—Cell-conditioned serum-free medium was collected for zymographic analysis. Gelatinolytic activities were assessed under non-reducing conditions using a 6% SDS-polyacrylamide gel with 1 mg/ml of gelatin. After being washed with 2.5% Triton X-100 twice for 30 min, the gel was incubated in zymography buffer containing 150 mM NaCl, 5 mM CaCl\(_2\), 50 mM Tris-HCl, pH 7.5, for overnight at 37 °C. The gel was then stained with Coomassie Brilliant Blue and destained with a solution of 20% methanol and 10% acetic acid.
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**FIGURE 3.** Periostin increases EGFR expression, and tyrosine-phosphorylated EGFR is detectable in the presence of periostin and EGF. A, 293T cells mainly express integrin subunits αv, β3, and periostin induces EGFR expression. Cell lysates from control or periostin-producing cells were used to determine integrin subunits αv, β3, and periostin and EGFR expression by immunoblotting. B, integrin αvβ3 is associated with EGFR in the periostin-producing cells. Cell lysates from control or periostin-producing cells were immunoprecipitated with anti-integrin αv or β3 antibody followed by immunoblotting with anti-EGFR antibody. No specific interaction between integrin β3 and EGFR was found in either control or periostin-expressing cells (data not shown). C, integrin αvβ3 is associated with tyrosine-phosphorylated EGFR in the presence of periostin and EGF. Left, following starvation by the removal of serum for 24 h, control or periostin-producing cells were stimulated with EGF (100 ng/ml) for 5–10 min and cell lysates were immunoprecipitated with anti-integrin β3 or β5 antibody. The immunocomplex was then blotted with antibody PY-20, an antibody against phosphotyrosine protein. Right, periostin-producing 293T cells were pretreated with serum-free medium in the absence or presence of αv, β3 antibody (10 μg/ml) or tyrophostin 25 (50 μM) for 1 h, followed by stimulation with EGF (100 ng/ml). The lysates were immunoprecipitated with anti-integrin β3 antibody prior to immunoblotting with PY-20. D, reciprocal immunoprecipitation and immunoblot confirming the interaction between integrin αv, β3, and tyrosine-phosphorylated EGFR. Periostin-producing cells were stimulated with EGF (100 ng/ml) for 5–10 min and immunoprecipitated with anti-EGFR or p-EGFR (Tyr-1086) antibody, followed by immunoblotting with anti-integrin β3 or β5 antibody. No specific bands were observed in the control cells with either anti-integrin β3 or β5 antibody (data not shown).

stained with 0.2% Coomassie Brilliant Blue for 4 h and destained with ethanol/acetic acid/water for 2 h.

**Recombinant Periostin**—Periostin was isolated and purified from baculoviral system as described previously (31).

**Migration Assay**—Cells (2 × 10⁵) were preincubated with serum-free medium for 24 h and transferred onto transwells (24-well plates) precoated with fibronectin (50 μg/ml). The lower chamber of transwells included EGF, periostin, tyrophostin 25, or anti-integrin αvβ3, αvβ5, antibody. After 4 h of incubation, top cells on the transwell membrane were removed using Q-tips. The cells trapped by membrane were fixed and stained with hematoxylin. Average cell numbers from five different areas in each example were counted.

**Invasion Assay**—Cells (2 × 10⁵) were preincubated with serum-free medium for 24 h and transferred onto transwells (24-well plates) preloaded with 50 μl of matrigel (1 μg/ml). After 18 h of incubation, the gel including non-migrated cells was removed and cells invading into the membrane were fixed and stained.

**Adhesion Assay**—Cells (5 × 10⁵) were preincubated with serum-free medium for 24 h and transferred to 48-well plates precoated with periostin or vitronectin (10 μg/ml). After 1 h of incubation, cells were gently washed and attached cells were fixed, stained, and counted.

**Immunocytochemistry**—Cells were transferred onto glass slides to culture for 2 days. After being fixed with 4% paraformaldehyde, cells were blocked with phosphate-buffered saline containing 5% goat serum, 1% bovine serum albumin, and 0.05% Nonidet P-40 followed by incubation with anti-vimentin antibody for 1 h. Fluorescein-conjugated secondary antibody was then added for 1 h, and fluorescence was examined under a microscope.

**Immunoprecipitation and Western Blot**—Cells were lysed with lysis buffer, pH 7.4, containing 0.25 mM HEPES, 14.9 mM NaCl, 10 mM NaF, 2 mM MgCl₂, 0.5% Nonidet P-40, 0.1 mM phenylmethylsulfonyl fluoride, 20 μM pepstatin A, and 20 μM leupeptin. The lysates were centrifuged at 1,000 × g for 10 min, and the resulting supernatant was collected for immunoblotting. For immunoprecipitation, cells from 60-mm plates were lysed with 0.5 ml of cell lysis buffer containing 10 mM Tris, pH 7.4, 1% Triton, 0.5% Nonidet P-40, 150 mM NaCl, 20 mM NaF, 0.2 mM Na₂VO₃, 1 mM EDTA, 1 mM EGTA, and 0.2 mM phenylmethylsulfonyl fluoride. The samples were incubated with anti-integrin β3 or β5 antibody at 4°C for overnight followed by incubation with protein A-Sepharose beads at 4°C for 2 h. The immunocomplex was extensively washed, and the samples (30 μg) were subjected to immunoblotting. The primary antibodies against vimentin, actin, cytokeratin (Sigma), PY-20 (ICN, Aurora, OH), EGFR, Tyr1086 EGFR (Cell Signaling, Beverly, MA), MMP-2, MMP-9 (Oncogene, Boston, MA), and integrins αv, β3, β5, and αvβ3 and αvβ5 (Chemicon, Temecula, CA) were used to examine protein expression. Specific signals were detected using an ECL kit (Pierce).

**Induction of Tumor Xenografts and Metastasis in Mice**—4-week-old female SCID-Beige/NOD mice (Charles River, Wilm-
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We employed tumorigenic 293T cells, a typical non-metastatic, epithelium-derived tumor line, to investigate the effects of periostin on metastasis. First, we tested whether periostin is capable of promoting EMT, a process tightly associated with cell malignant behavior. We used a retroviral infection system to introduce the periostin cDNA into 293T cells and to stably produce secreted periostin. Transduction of periostin gene resulted in extensive cell spreading and elongation, displaying a spindle-like morphology, whereas this alteration was not observed in control cells (Fig. 1A, top panel). 293T cells ectopically expressing periostin dramatically increased vimentin expression, a hallmark for mesenchymal cells, by 7–8-fold (Fig. 1, A and B). The level of vimentin in vector control cells and parental 293T cells was barely detectable. The elevation of vimentin expression was validated by immunocytochemistry staining. Fibronectin expression was also induced by the periostin transgene in the cells. Expression of the epithelial marker cytokeratin was not significantly altered in periostin-producing 293T cells. The active form of MMP-9 was strikingly increased in periostin-producing cells and accumulated in the medium ~5–8-fold higher than the level produced from control cells, accompanied by high proteolytic activity in the cells engineered with periostin (Fig. 1C). In contrast, neither the active form of MMP-2 (Fig. 1B) nor MMP-3 (not shown) was changed in the conditioned medium. Expression of E- or N-cadherin was also unaltered (data not shown). In aggregate, the data demonstrate that periostin expressed by 293T cells drives the cells to undergo EMT.

To explore membrane receptors that may mediate periostin-induced cell EMT, we examined its interaction with integrins on the cell surface. As periostin was shown to be a ligand of integrins αvβ3 and αvβ5 on ovarian cancer cells (30), its interactions with integrins on 293T cells were examined (Fig. 2). Periostin-coated plates enhanced cell adhesion by 3.5-fold compared with controls. This interaction was abolished by incubation with anti-integrin αvβ3 antibody. This effect was specific for integrin αvβ3, as anti-integrin αvβ5 antibody failed to block periostin-induced cell adhesion.

293T cells predominantly expressed subunits integrin αv and β3, but not β5, implicating that a heterodimer is mainly composed of the integrin αvβ3 form in the cells (Fig. 3A). A similar expression pattern was observed in both control cells and periostin-expressing cells. To determine whether the cross-talk between integrins and growth factor receptors is required for...
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A

Tumor volume (mm³)

Control
Periostin

Time (days)

B

Survival (%)

Control
Periostin

Time (days)

C

Periostin
Control

Lung

Liver

D

Periostin
Actin

siRNA control
Periostin siRNA

Cell invasion

siRNA control
Periostin siRNA

Lung
periostin activity, we first examined EGFR expression in 293T cells. As shown in Fig. 3A, EGFR expression was augmented in the cells expressing periostin ∼2-fold higher than that in control. We examined the physical interaction between integrins and EGFR by immunoprecipitation and immunoblotting after the cells were exposed to EGF. Following stimulation of the cells with EGF, integrin β3-associated proteins were co-immunoprecipitated. EGFR was associated with integrin β3 in the periostin-expressing cells but not the cells with vector alone (Fig. 3B). Activated EGFR was examined by immunoblotting with an antibody against tyrosine-phosphorylated molecules. Once cells were exposed to EGF, tyrosine-phosphorylated EGFR was detected in integrin β3-associated complex from periostin-engineered 293T cells but not control cells (Fig. 3C). No tyrosine-phosphorylated EGFR was observed in integrin β3-associated complex from either control or periostin-engineered cells. This specific association between integrin β3 and activated EGFR was inhibited by the preincubation of periostin-producing cells with anti-integrin αvβ3 antibody or tyrphostin 25, an EGFR kinase inhibitor (Fig. 3C). The specific interaction was also confirmed by the reciprocal immunoprecipitation and immunoblot. There was no detectable interaction between EGFR and integrin β3 whereas phosphorylated EGFR showed the association with integrin β3 (Fig. 3D). The data suggest that integrin αvβ3 and EGFR form a complex but require both periostin and EGFR as ligands.

Next, to test whether periostin expression can induce aggressive activities in the absence of exogenous EGF, we monitored functional alterations in cell migration and invasion. 293T cells expressing periostin exhibited a significant increase in cell motility (Fig. 4A). More than two times as many periostin-producing cells migrated into the membrane relative to control cells. When either anti-integrin αvβ3 antibody or tyrphostin 25 was added, the increased motility in periostin-producing cells was blocked, whereas treatment with anti-integrin αvβ3 antibody failed to inhibit the cell migration. We also monitored cell invasive behavior using a modified migration assay in which a layer of matrigel was preloaded on transwells. As shown in Fig. 4A, ectopic expression of periostin in the cells resulted in a 9-fold increase in cell invasive activity relative to the control cells. Treatment of these aggressive cells with either anti-integrin αvβ3 antibody or tyrphostin 25 substantially impaired cell invasion as the activity was reduced to the basal level. As expected, inclusion of anti-integrin αvβ3 antibody did not block invasion. Likewise, periostin enhanced cell adhesive activity, but it was abrogated by anti-integrin αvβ3 antibody or tyrphostin 25 (data not shown). To determine the regulation of intracellular molecules that may play an important role in cell aggressive behavior, we examined MMP-9 gene expression.

Consistent with the previous result, periostin-expressing cells contained a higher level of MMP-9 than in control cells. However, the increased MMP-9 level in periostin-producing 293T cells was fully inhibited to the control level by an addition of either anti-integrin αvβ3 antibody or tyrphostin 25, whereas blockage of integrin αvβ3 failed to attenuate MMP-9 production (Fig. 4B). The data suggest that co-activation of integrin αvβ3 and EGFR is required for periostin-induced MMP-9 production and cell invasive function. The effectiveness of inhibition by anti-integrin αvβ3 antibody on periostin-induced cell functions demonstrates that periostin produced from the cells acts through its secreted protein that interacts with integrin αvβ3 but not via intracellular pro-periostin.

The in vitro data that periostin induces EMT and invasive activity encouraged us to test whether periostin promotes tumor invasion and metastasis in vivo. For this, we injected control and periostin-producing 293T cells into mouse mammary fat pad tissue and monitored growth of tumors at the injection site as well as in distant organs. The mice receiving periostin-producing 293T cells grew local tumors significantly faster than did the mice receiving control cells during the 35-day observation (Fig. 5A). During the next 2 weeks, the mice injected with periostin-producing cells began to die, resulting in a rapid decrease in survival rate. But no death occurred in the control group within the same time period of observation (Fig. 5B). Strikingly, we found that the tumors bearing periostin-producing cells metastasized to the lung and liver (Fig. 5C and Table 1), in which the rate of tumor formation was 83 and 17%, respectively. No tumor metastasis was identified in either organ in mice injected with control cells. New blood vessels adjacent to the tumors developed in some lung metastatic cases, indicating that periostin promotes tumor angiogenesis in the secondary solid tumor formation during metastasis. To confirm that periostin has ability to promote tumor cell invasion and metastasis, we utilized the siRNA gene knockdown approach in periostin-expressing 293T cells. As shown in Fig. 5D, the periostin activity was dramatically reduced, and tumor invasion as well as the ability to form distant metastases in the lung and liver were inhibited at a level comparable with the control group.

**TABLE 1**

| Metastatic organs | Lung Rate | Liver Rate |
|-------------------|-----------|------------|
| Control           | 6/6 (100%)| 0/5 (0%)   |
| Periostin         | 1/6 (17%) | 1/6 (17%)  |
| siRNA control     | 0/6 (0%)  | 0/5 (0%)   |
| siRNA             | 0/5 (0%)  | 0/5 (0%)   |

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**FIGURE 5.** Periostin promotes tumor growth and metastasis, but periostin gene knockdown leads to inhibition of metastasis. A, time course for tumor growth. Control vector or periostin-producing 293T cells (1 × 10⁶ cells) were introduced into immunodeficient SCID/Beige mice through mammary fat pad tissue injection. Tumor sizes were measured weekly. After day 35 of observation, tumor sizes reached maximum. *, p < 0.05 compared with respective control groups (n = 6). B, decreased survival rate in mice carrying periostin tumors. Survival of mice bearing control and periostin-producing tumors was observed daily for 50 days following injection. C, periostin-producing tumors metastasize to the lung and liver. Following sacrifice of moribund mice during day 40–50, the lung and liver were fixed in formalin, embedded in paraffin, and processed for hematoxylin and eosin staining. Arrows indicate tumor mass, and arrowheads indicate blood vessel formation (×40). D, periostin gene knockdown inhibits tumor invasive behavior. Periostin-expressing 293T cells were engineered with control vector or vector plus siRNA oligos that specifically target the periostin gene. Inhibition of periostin was evaluated by immunoblotting as shown in the top panel. Accordingly, blockage of cell invasive function was determined by the invasion assay (middle panel). In addition, control or periostin siRNA cells were injected into the mice as described in panel A. Lung metastases were found in mice injected with siRNA control cells but not in mice injected with YKL-40 siRNA cells (n = 5).
gene in siRNA cells was suppressed to ~30% of the level in periostin-producing cells. This decreased expression of periostin was sufficient to abrogate cell invasion in vitro and metastasis in vivo (Fig. 5D and Table 1), although the local tumor growth was similar between the siRNA vector control and periostin siRNA tumor (data not shown).

To further evaluate the metastatic activity, we injected control or periostin-producing 293T cells directly into the blood system via left ventricle inoculation. In agreement with the above results, the survival rate in the mice bearing periostin-producing cells was significantly lower than that of the mice carrying control cells (Fig. 6A). A variety of visible large secondary tumors in the liver, lung, and abdomen were identified in two of six mice receiving periostin-producing cells (Fig. 6B). Moreover, an extensive arbor of ramified blood vessels was also found in those tumors, consistent with enhanced tumor angiogenesis. No tumors were detected in organs from the control mice. The data strengthen our hypothesis that expression of periostin by tumor cells prompts tumor invasion and metastasis.

DISCUSSION
A wealth of evidence has demonstrated that tumors of epithelial origin usually undergo phenotypic transformation dur-
ing tumor metastasis (34, 35). A variety of classic molecules involved in cell motility, cell-cell contacts, and cell-extracellular matrix interaction have been well characterized in metastasis such as vimentin, E/N-cadherin expression, and MMP production. In addition, a number of growth factors including TGF-β, FGF1, EGF, and SF/HGF can induce tumor cell EMT process under culture conditions (36). In the present study, we have observed the direct effects of periostin on aggressive cell behavior by expressing periostin in 293T cells. We found that exogenous expression of periostin in 293T cells promoted the cells to undergo EMT. They gained vimentin expression, a hallmark of EMT, and transformed to fibroblast-like phenotype including spreading of cells accompanied with increased MMP-9 production. Consequently, these cells increased cell adhesion, migration, and invasion in vitro and metastatic potential in vivo.

Cadherins, the cell-cell adhesion molecules, have been shown to exert important functions in the regulation of cell-cell communication. E-cadherin usually maintains epithelial properties, whereas N-cadherin mainly contributes to the mesenchymal transformation. In addition, recent evidence has demonstrated that N-cadherin up-regulates MMP-9 expression in tumor progression (37–39). Thus, either gain of N-cadherin or loss of E-cadherin expression is frequently associated with tumor metastasis (14, 40, 41). In the present study, no alteration was detected in either E-cadherin or N-cadherin expression after introduction of periostin into the cells. Although it is possible that N- and E-cadherin may participate in the actions of periostin, they were not rate-limiting for the responses in gene expression, migration, or invasion.

Given that periostin directly interacts with integrin αβ5 and the activation of basal EGFR level is necessary for aggressiveness in response to periostin, we have revealed a model of the cross-talk for periostin that requires coordination of integrin αβ5 and EGFR pathways. Periostin induced features of metastasis including cell adhesion, migration, and invasion. Tyrosine-phosphorylated EGFR was found to physically associate with integrin αβ5 exclusively in the cells when they were exposed to both receptor stimuli, suggesting that signal transduction is enhanced once both receptor ligands are present. The cytoplasmic tail of integrin β subunit participates in the physical association with the cytoplasmic domain of growth factor receptors favoring the coordination of both membrane receptor-mediated signaling pathways (42–44). A similar active role of the specific interaction between integrins and tyrosine kinase receptors has been established in a variety of cell types (45). For example, in endothelial cells, the interaction between integrin αβ3 and vascular endothelial growth factor receptor 2 is essential for vascular endothelial growth factor-induced angiogenesis (46, 47). Likewise, activation of EGFR is required for integrin αβ5-directed motility in FG human pancreatic carcinoma cells, whereas the interaction between integrin αβ3 and EGFR mediates cell survival and proliferation in smooth muscle cells (25, 44). Our results, in context with other reports, have underscored the paradigm that the coordination of inputs from integrins and growth factor receptors is essential for periostin-induced cell invasive activity.

Clinical studies found higher serum levels of periostin in cancer patients with malignant diseases compared with patients with local cancers (32, 33, 48). The high concentration of periostin in the blood was directly correlated with poor prognosis and short survival in multiple cancers, including breast, lung, head, and neck cancers. Therefore, periostin may be a valuable biomarker for tumor metastasis. The elevated production of periostin in metastatic diseases may directly cause cancerous cells to induce malignant transformation of mesenchymal phenotype.

To this end, our data have identified important functional and molecular mechanisms for periostin in tumor metastasis. Periostin triggers the co-activation of integrin αβ3 and EGFR signaling and induces expression of multiple genes such as MMP-9, vimentin, and fibronectin, leading to EMT and metastasis (Fig. 7). Elevated serum levels of periostin in cancer patients provide a promising tool for early detection of cancer and for monitoring cancer patients. More importantly, the results underscore possible applications of EGFR kinase inhibitors for control of metastasis in multiple human cancers in which both EGFR and periostin are overproduced (33, 49, 50).

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REFERENCES
1. Friedl, P., and Wolf, K. (2003) Nat. Rev. Cancer 3, 362–374
2. Yokota, J. (2000) Carcinogenesis 21, 497–503
3. Chambers, A. F., Groom, A. C., and MacDonald, I. C. (2002) Nat. Rev. Cancer 2, 563–572
4. Bogenrieder, T., and Herlyn, M. (2003) Oncogene 22, 6524–6536
5. Boyer, B., Valles, A. M., and Thiery, J. P. (1996) Acta Anat. 156, 227–239
6. Savagner, P. (2001) BioEssays 23, 912–923
7. Birchmeier, C., Birchmeier, W., and Brand-Saberi, B. (1996) Acta Anat. 156, 217–226
8. Putz, E., Witter, K., Offner, S., Stosiek, P., Zippelius, A., Johnson, J., Zahn,
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R., Riethmuller, G., and Pantel, K. (1999) Cancer Res. 59, 241–248
9. Cui, W., Fowlis, D. J., Bryson, S., Duffie, E., Ireland, H., Balmain, A., and Akhurst, R. I. (1996) Cell 86, 531–542
10. Janda, E., Lehmann, K., Killisch, I., Jechlinger, M., Herzig, M., Downward, J., Beug, H., and Gruenert, S. (2002) J. Cell Biol. 156, 299–313
11. Kiemer, A. K., Takeuchi, K., and Quinlan, M. P. (2001) Oncogene 20, 6679–6688
12. Pulyaeva, H., Bueno, J., Polette, M., Birembaut, P., Sato, H., Seiki, M., and Thompson, E. W. (1997) Clin. Exp. Metastasis 15, 111–120
13. Kim, J. B., Islam, S., Kim, Y. J., Prudoff, R. S., Sass, K. M., Wheelock, M. J., and Johnson, K. R. (2000) J. Cell Biol. 151, 1193–1206
14. Kupferman, M. E., Fini, M. E., Muller, W. J., Weber, R., Cheng, Y., and Muschel, R. J. (2000) Am. J. Pathol. 157, 1777–1783
15. Lakka, S. S., Gondi, C. S., Yanamandra, N., Olivero, W. C., Dinh, D. H., Gujrati, M., and Rao, J. S. (2004) Oncogene 23, 4681–4689
16. Kiemer, A. K., Takeuchi, K., and Quinlan, M. P. (2001) Cancer Res. 61, 4681–4689
17. Hood, J. D., Bednarski, M., Frausto, R., Guccione, S., Reisfeld, R. A., Xiang, R., and Cheresh, D. A. (2002) Science 296, 2404–2407
18. Kiemer, A. K., Takeuchi, K., and Quinlan, M. P. (2001) Cancer Res. 61, 5358–5364
19. Hood, J. D., Bednarski, M., Frausto, R., Guccione, S., Reisfeld, R. A., Xiang, R., and Cheresh, D. A. (2002) Nat. Rev. Cancer 2, 91–100
20. Mizejewski, G. J. (1999) Proc. Soc. Exp. Biol. Med. 222, 124–138
21. Eliceiri, B. P. (2001) Circ. Res. 89, 1104–1110
22. Schwartz, M. A. (1997) J. Cell Biol. 139, 575–578
23. Bill, H. M., Knudsen, B., Moores, S. L., Muthuswamy, S. K., Rao, V. R., Brugge, J. S., and Miranti, C. K. (2004) Mol. Cell. Biol. 24, 8586–8599
24. Doerr, M. E., and Jones, J. I. (1996) J. Biol. Chem. 271, 2443–2447
25. Klemke, R. L., Yebra, M., Bayna, E. M., and Cheresh, D. A. (1994) J. Cell Biol. 127, 859–866
26. Senger, D. R., Ledbetter, S. R., Claffey, K. P., Papadopoulos-Sergiou, A., Peruzzi, C. A., and Detmar, M. (1996) Am. J. Pathol. 149, 293–305
27. Soldi, R., Mitola, S., Strasly, M., Defilippi, P., Tarone, G., and Bussolino, F. (1999) EMBO J. 18, 882–893
28. Varner, J. A., and Cheresh, D. A. (1996) Curr. Opin. Cell Biol. 8, 724–730
29. Max, R., Gerritsen, R. R., Nooijen, P. T., Goodman, S. L., Sutter, A., Keilholz, U., Ruiter, D. J., and De Waal, R. M. (1997) Int. J. Cancer 71, 320–324