Periostin Promotes Invasion and Anchorage-Independent Growth in the Metastatic Process of Head and Neck Cancer

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Abstract

Head and neck squamous cell carcinoma (HNSCC) is one of the most common types of human cancer. Typically, HNSCC cells show persistent invasion that frequently leads to local recurrence and distant lymphatic metastasis. However, molecular mechanisms associated with the invasion and metastasis of HNSCC remain poorly understood. Here, we identified periostin as an invasion-promoting factor in HNSCC by comparing the gene expression profiles between parent HNSCC cells and a highly invasive clone. Indeed, periostin overexpression promoted invasion and anchorage-independent growth both in vitro and in vivo in HNSCC cells. Moreover, periostin-overexpressing cells spontaneously metastasized to cervical lymph nodes and to the lung through their aggressive invasiveness in an orthotopic mouse model of HNSCC. Interestingly, periostin was highly expressed in HNSCCs in comparison with normal tissues, and the level of periostin expression was well correlated with the invasiveness of HNSCC cases. In summary, these findings suggest that periostin plays an important role in the invasion and anchorage-independent growth of HNSCC. (Cancer Res 2006; 66(14): 6928-35)

Introduction

Head and neck squamous cell carcinoma (HNSCC) is one of the most common types of human cancer, with an annual incidence of >500,000 cases worldwide (1). HNSCC is associated with severe disease- and treatment-related morbidity and has a 5-year survival rate of ~50%; this rate has not improved in more than two decades (2). Review of the literature indicates that the most important factor for the high mortality rate is the advanced stage of the disease at the time of diagnosis and treatment. In the prognosis of HNSCC, the extent of lymph node metastasis is a major determinant. Like most epithelial cancers, HNSCC develops through the accumulation of multiple genetic and epigenetic alterations in a multistep process. Recent molecular studies have advanced our understanding of the disease and provided a rationale to develop novel strategies for early detection, classification, prevention, and treatment. Attempts to identify the genes involved in the metastasis are pivotal for the early prediction of HNSCC behavior. However, the identity and time of onset of the alterations that endow cancer cells with these metastatic functions are largely unknown. The process of metastasis consists of sequential and selective steps including proliferation, induction of angiogenesis, detachment, motility, invasion into circulation, aggregation and survival in the circulation, cell arrest in distant capillary beds, and extravasation into organ parenchyma (3). The development of metastasis depends on the interplay between host factors and intrinsic characteristics of cancer cells, and the metastatic lesion represents the end point of many destructive events that only a few cells can survive (4). Moreover, neoplasms contain a variety of subpopulations of cells with differing metastatic potential, and the possible existence of highly metastatic clones may exist within a primary tumor (4). Indeed, we previously isolated highly invasive clones from parent HNSCC cell lines by using an in vitro invasion assay method (5).

Here, we compared the transcriptional profile of parent cells and a highly invasive clone by microarray analysis in order to identify genes that differ in their expression. We identified periostin [osteoblast-specific factor 2 (fasciclin I-like)] as the gene demonstrating the highest fold change expression in the invasive clone. Periostin is a secreted protein, which was originally identified from osteoblasts (6, 7). In the present study, we showed that periostin was involved in invasion and anchorage-independent growth in HNSCC.

Materials and Methods

Gene array analysis. The Amersham CodeLink system using the UniSet Human I Expression Bioarray, containing 10,458 gene probes, was used to compare the transcription profiles between parent cells and highly invasive clones. This array contains a broad range of genes derived from publicly available, well-annotated mRNA sequences. The CodeLink array is unique in being capable of detecting minimal differences in gene expression, as low as 1.3-fold with 95% confidence, because of the novel three-dimensional aqueous gel matrix, which the empirically tested 30-mer oligonucleotides are deposited on (8). This substantially reduces background, enhances sensitivity, and allows for the detection and quantification of subtle regulatory relationships among genes in parent HNSCC cells and highly invasive clones. Total RNA was isolated from cultures of confluent cells using the RNeasy Mini Kit (Qiagen, Chatsworth, CA) according to the manufacturer's instructions. Preparations were quantified and their purity was determined by standard spectrophotometric methods. Data were expressed as the average differences between the perfect match and mismatch probes for the periostin gene (Supplemental Data 1).

Total RNA from 41 primary HNSCC and 13 normal tissues was labeled according to the manufacturer's instructions. The code-link arrays were hybridized to Affymetrix U133A Gene Chips as previously reported (9). To explore the genes that are coordinately expressed with periostin in HNSCC tumors, we did a similarity search using a Pearson correlation metric. The GeneChip Operating System (GCOS) software, version 1.2 (Affymetrix), was used to identify genes with expression levels that correlated significantly with periostin expression. Genes were considered to be differentially expressed if their expression levels differed by a factor of 2 or more, with a correlation coefficient of 0.7 or greater.

Supplementary materials. See http://cancerres.aacrjournals.org for supplementary materials.

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metric as implemented in tGeneData Analyst Pro 1.0 software (Supplemental Data 2).

**Cell culture.** MSCC-1 and MSCC-Inv1 cells were previously established in our laboratory (5, 10). These cells were maintained in keratinocyte/serum-free medium (In vitrogen, San Diego, CA) under the following conditions: 5% CO\textsubscript{2} in air at 37°C. Other HNSCC cell lines HSC2, HSC3, and Ca9-22 were provided by the Japanese Collection of Research Bioresources Cell Bank. They were maintained in RPMI 1640 (Nissui Pharmaceutical Co., Tokyo, Japan) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Invitrogen) and 100 units/mL of penicillin-streptomycin (Invitrogen) under the following conditions: 5% CO\textsubscript{2} in air at 37°C. For growth assay, cells were plated onto 24-well plates (Falcon, Becton Dickinson, Franklin Lakes, NJ), and trypsinized cells were counted by Coulter Z1 Cell Counter (Fullerton, CA).

Reverse transcription-PCR. Total RNA was isolated from cultures of confluent cells using the RNAeasy mini kit (Qiagen). Preparations were quantified and their purity was determined by standard spectrophotometric methods. cDNA was synthesized from 1 μg of total RNA according to the ReverTra Dash (Toyobo Biochemicals, Tokyo, Japan). Primer sequences were designed and their purity was determined by standard spectrophotometric methods. The primer pairs used were as follows: (forward) and 5'-acttcctcacgggtgtgtc-3' (reverse: product size, 239 bp); human glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 5'-tgccacctctgttgct-3' (forward) and 5'-accagccattcgtgcatctac-3' (reverse: product size, 450 bp). Aliquots of total cDNA were amplified with 1.25 units of rTaq-DNA polymerase (Qiagen), and amplifications were done in a PC701 thermal cycler (Astec, Fukuoka, Japan) for 30 to 35 cycles after an initial 30-second denaturation at 94°C, annealed for 30 seconds at 60°C, and extended for 1 minute at 72°C in all primers. The amplification reaction products were resolved on 1.5% agarose/TAE gels (Nacalai Tesque, Inc., Kyoto, Japan), electrophoresed at 100 mV, and visualized by ethidium-bromide staining.

Western blot analysis. Western blotting was carried out as we described previously (11). A polyclonal anti-pectin antibody was generated by immunizing the rabbits with specific peptides (GEPEFRLIKEGETC) for peristin and purified through an affinity column. An anti-His polyclonal antibody (Cell Signaling Technology, Inc., Beverly, MA) and \(\beta\)-actin monoclonal antibody (Sigma, St. Louis, MO) were also used. Thirty micrograms of protein were subjected to 10% PAGE, followed by electroblotting onto a nitrocellulose filter. For detection of the immunocomplex, the enhanced chemiluminescence Western blotting detection system (Amersham, Piscataway, NJ) was used.

**Generation of peristin-overexpressing HNSCC cells.** A peristin expression plasmid, pcDNA3.1, encoding a hexahistidine-tagged peristin cDNA, was kindly provided by Dr. X-F. Wang (Duke University, Durham, NC). The peristin/pDNA3.1 plasmid or the vector alone was introduced into HSC2 and HSC3 cells, and the stable clones were obtained by G418 selection (500 μg/mL; Life Technologies) in the culture medium. Cell transfections were done using FuGENE 6 (Roche, Castle Hill, Australia) according to the manufacturer's instruction. Four peristinst-expressing clones, and one control clone of each cell, were chosen for the subsequent experiments.

**In vitro invasion assay.** In vitro invasion assay was done as described previously (5). Briefly, invasion was measured by using a 24-well cell culture insert with 8 mm pores (Falcon, Becton Dickinson). The filter was coated with 20 μg of EHS extract (Iwaki Garasu, Tokyo, Japan), which was reconstituted basement membrane substance. The lower compartment contained 0.5 mL of serum-free medium. After trypsinization, 1.5 × 10\textsuperscript{5} cells were resuspended in 100 μL of serum-free medium and placed in the upper compartment of the cell culture insert for 6 to 24 hours. For invasiveness after peristin small interfering RNA (siRNA) treatment, we used 50 μg of EHS extract in these experiments because MSCC-Inv1 cells have a high invasive activity. After incubation, we collected the penetrating cells onto the lower side of the filter to isolate highly invasive clones by the method of Kalebic et al. with minor modification (12). To examine the invasiveness, cells formalin-fixed and stained with H&E. The invasiveness of the cells was determined by counting the penetrating cells onto the lower side of the filter through the pores under a microscope at ×100 magnification. Assays were done thrice, and three fields were randomly selected and counted for each assay.

**Generation of recombinant peristin.** Full-length human peristin cDNA was subcloned into pIZ/V5-His vector (Invitrogen), pIZ/V5-His vector containing peristin was transfected into High-Five insect cells by using Cellfectin reagent (Invitrogen). Stable clones were obtained by zeocin selection in the culture medium. A Ni-nitrilotriacetic acid column was used to purify recombinant peristin according to the manufacturer's instructions (Invitrogen).

**Silencing by siRNA.** Stealth siRNA (Oligo ID, HSSI16398, Invitrogen) is a 25-bp duplex oligoribonucleotide with a sense strand corresponding to nucleotides 62 to 86 of the reported human peristin mRNA sequence. The sense sequence of HSSI16398 is 5'-CCCUAAUACGCCAACAAAUCAUAAU-3'. MSCC-Inv1 cells were transfected with 150 pmol of siRNA in 1 mL of OPTI-MEM according to the manufacturer's instructions. Following siRNA treatment (48 hours), MSCC-Inv1 cells were used for in vitro invasion assay.

**Figure 1.** Peristin is identified as an invasion-related gene in HNSCC. A, schematic representation of the isolation process of highly invasive clone. Highly invasive clone (MSCC-Inv1) was isolated from parent cells (MSCC-1) by in vitro invasion assay. The transcriptional profile of MSCC-1 cells and MSCC-Inv1 cells were compared by microarray analysis. Highly expressed genes in the highly invasive clone are listed. Among these genes, the most overexpressed gene in the highly invasive clone is peristin (in boldface). B, confirmation of higher expression of peristin mRNA in the highly invasive clone by RT-PCR. Amplification was done for 30 cycles. C, confirmation of higher expression of peristin protein in the highly invasive clone by Western blot analysis. \(\beta\)-Actin expression was used as a loading control. D, production of peristin by MSCC-1 and MSCC-Inv1 cells. The production of peristin was measured by Western blot analysis with conditioned culture media.
as described above. A scrambled sequence that does not show significant homology to rat, mouse, or human gene sequences was used as a control.

**Cell adhesion assay.** Flat-bottomed 96-well ELISA plates (Costar, Cambridge, MA) were incubated overnight at 4°C with 10 μg/mL of periostin protein in the presence of anti-αvβ3 or anti-αvβ5 integrin antibody (10 μg/mL) and blocked for 1 hour at room temperature with PBS containing 2% bovine serum albumin. Cells were suspended in medium at a density of 3 × 10^3 cells/mL, and 0.1 mL of the cell suspension was added to each well of the coated plates. After incubation for 1 hour at 37°C, unattached cells were removed by rinsing with PBS. Attached cells were then trypsinized and counted by Cell Counter.

**Soft agar colony formation assay.** Assays of colony formation in soft agar were done using standard methods. Briefly, 4 mL underlayers consisting of 0.5% agar medium with 20% FBS were prepared in 60 mm dishes. Periostin-overexpressing cells and control cells were trypsinized, centrifuged, and resuspended in 0.2% agar medium with 20% FBS. Cells (1 × 10^5) were then plated onto the previously prepared underlayers. The cells were kept wet by adding a small amount of RPMI 1640 with 10% FBS. The cells were incubated at 37°C in a humidified 5% CO2 atmosphere for 3 weeks. Afterwards, colonies were photographed and counted.

**Xenograft assays in nude mice.** To examine whether periostin expression in HNSCC cells affects anchorage-independent growth in vivo, periostin-overexpressing HSC2 cells (1 × 10^5 in 500 μL of HBSS) were injected s.c. into multiple sites in athymic (nude) mice. The control groups were injected with the same number of vector-transfected HSC2 cells. Experimental protocols were approved by the Committee of Research Facilities for Laboratory Animal Science, Hiroshima University. The animals were monitored for tumor formation every 3 days and sacrificed 2 weeks later. Tumor length (L) and width (W) were measured at the end of the experiment, and tumor volume was calculated by the formula: (L × W^2)/2.

**Orthotopic implantation.** Periostin-overexpressing and control HSC2 cells (5 × 10^5 in 50 μL of HBSS) were injected into the tongue of male athymic (nude) mice. The animals were monitored for tumor formation every 3 days and sacrificed 2 weeks later. Tongue tumors, cervical lymph nodes, and lungs were removed, and fixed in 4% formalin. Specimens were embedded in paraffin, cut into 4-μm-thick sections and stained with H&E. They were histologically evaluated for diagnosis, regional lymph node metastasis, and pulmonary metastasis.

**Tissue samples.** Tissue samples of HNSCC were retrieved from the Surgical Pathology Registry of Hiroshima University Hospital from 1998 to 2004, after approval by the Ethical Committee of our institutions. Buffered formalin-fixed and paraffin-embedded tissues (10%) were used for immunohistochemical examination. The histological grade and stage of tumor were classified according to the criteria of the Japan Society for Head and Neck Cancer. Fresh samples were taken from the neoplastic tissues and nonneoplastic tissues for reverse transcription-PCR (RT-PCR) analysis.

**Immunohistochemical staining.** Immunohistochemical detection of periostin in HNSCC cases was done on 4.5 μm sections mounted on silicon-coated glass slides, using a streptavidin-biotin peroxidase technique as described previously (11). The expression of periostin was graded as ++ (>30% of tumor cells showed strong or diffuse immunopositivity), + (10-30% of tumor cells showed moderate or patchy immunopositivity), and − (<10% of the tumor cells showed weak or focal immunopositivity or no staining). Three pathologists (Y. Kudo, I. Ogawa, and T. Takata) made all the assessments.

**Results**

Identification of periostin as an overexpressed gene in a highly invasive HNSCC cell line. We previously established a HNSCC cell line, MSCC-1, from lymph node metastasis (10). Moreover, we isolated a highly invasive clone, MSCC-Inv1, from MSCC-1 cells by using an in vitro invasion assay device (5). In the present study, we compared the transcriptional profile of parent...
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Periostin-enhanced anchorage-independent growth of HNSCC cells both in vitro and in vivo. Similar to periostin-transfected HSC2 cells, treatment with recombinant periostin protein also enhanced the invasiveness of HSC2 cells in a concentration-dependent manner (Fig. 3A). Periostin contains the four internal repeats of fascin I (FAS1) domain that represents an ancient cell adhesion domain common to plants and animals (13). In mammals, there are four proteins containing FAS1 domains, specifically, two secretory proteins, periostin and $\beta$ig-h3, and two membrane proteins, FEEL-1 and FEEL-2. FAS1 of $\beta$ig-h3 bears motifs interacting with integrins $\alpha$3$\beta$1 and $\alpha$v$\gamma$5 (14, 15), and mediates endothelial cell adhesion and migration via integrin $\alpha$v$\gamma$3 (16). Similarly to $\beta$ig-h3, recombinant periostin supports the adhesion of ovarian epithelial cells that could be inhibited by monoclonal antibodies against $\alpha$v$\gamma$3 or $\alpha$v$\gamma$5 integrin, but not by anti-$\beta$1 integrin antibody (17). We also found that treatment of specific anti-$\alpha$v$\gamma$3 and anti-$\alpha$v$\gamma$5 integrin antibodies inhibited the adhesion of HSC2 cells to the culture wells precoated with periostin, indicating that interference with the function of integrins has an effect on the ability of periostin to mediate the cell adhesion of HNSCC cells (Fig. 3B).

It has been suggested that alterations in cell-cell adhesion molecules, integrins, or integrin-associated signaling molecules in cancer cells may be involved in anchorage-independent growth (18). Therefore, we hypothesize that periostin may affect the anchorage-independent growth of HNSCC through interaction with integrins. To test this hypothesis, we examined the anchorage-independent growth of periostin-overexpressing cells in vitro by using soft agar colony formation assay. In the following analysis, we used clone no. 2 of HSC2 cells, which showed higher invasiveness. The periostin-overexpressing cells formed considerably larger colonies in soft agar in comparison with control cells (Fig. 3C). In addition, the number of colonies with periostin-overexpressing cells was higher than those with control cells (Fig. 3C). To determine anchorage-independent growth in vivo,
periostin-overexpressing HNSCC cells were injected s.c. into nude mice. After 28 days, the growth characteristics of the resulting tumors were analyzed. Interestingly, transplantation of the periostin-overexpressing cells showed comparatively larger tumor volumes than that of the control cells (Fig. 3D, a-c). The volume of tumors derived from periostin-overexpressing cells was ~16-fold higher than that of tumors from control cells (Fig. 3D, d). Tumor volume was 187 ± 57.1 and 11.4 ± 10.4 mm³ in the periostin overexpressed and control cells, respectively.

**Periostin-overexpressing cells frequently metastasize to lymph nodes and lung through their aggressive invasiveness.** As shown in the above experiments, periostin enhanced invasion and anchorage-independent growth of HNSCC cells. To further evaluate if periostin overexpression affects metastasis in vivo, we orthotopically implanted the periostin-overexpressing cells into the tongues of nude mice (Fig. 4A). An orthotopic implant technique has been previously used to examine the lymphatic metastatic activity of human HNSCC derived from different patients (19, 20). After 2 weeks of implantation, mice were sacrificed. Tumors were observed in the tongues of mice implanted with both periostin-overexpressing cells and control cells (Fig. 4B, a and C, a). Strikingly, in mice implanted with periostin-overexpressing cells, the tumors were larger than in mice implanted with control cells. Histologically, periostin-overexpressing tumors showed a poorly differentiated phenotype characterized by a diffuse and trabecular growth pattern without keratinization. In contrast, control tumors showed characteristics consistent with a well-differentiated phenotype with tumor islands with keratin pearl formation (Fig. 4B, b and C, b).

Moreover, periostin-overexpressing tumors showed remarkable invasiveness, including destruction of mandibular bone and lymphocytic infiltration (Fig. 4C, a and c). Interestingly, the periostin-overexpressing cells spontaneously metastasized to cervical lymph nodes (Fig. 4C, d) and lung (Fig. 4C, e). Overall, 6 of 11 mice implanted with periostin-overexpressing cells showed metastasis to regional lymph nodes and/or lung, but no metastasis was observed in mice implanted with control cells (0 of 10; Fig. 4D). These findings suggest that periostin overexpression may be involved in metastasis through aggressive invasiveness.

**Overexpression of periostin is frequently found in HNSCC cases and is associated with the invasiveness of HNSCC.** To determine if the up-regulation of periostin is a common feature of HNSCC in human subjects, we did RT-PCR analysis on three normal oral mucosae and nine HNSCC samples. As shown in Fig. 5A, a, the expression levels of periostin mRNA in cancer tissues was higher than in normal tissues. The average of periostin expression levels (periostin/GAPDH signal intensity ratio) was 3-fold higher in tumors than in normal tissues (Fig. 5A, b).

Next, we examined the expression of periostin in 12 normal oral mucosae and 102 HNSCC cases by immunohistochemistry. HNSCC cells showed high expression of periostin in comparison with normal oral mucosae (Fig. 5B, a and b; Table 1). In 102 HNSCC cases, 43 (42.2%) cases showed high expression of periostin. Then, we investigated the relationship between periostin expression and invasiveness in 102 HNSCC cases. For evaluation of invasiveness of HNSCC, we used the grading of mode of invasion, grades 1 to 4 as first described by Jacobsson et al. (21). We defined two groups, low

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**Figure 4.** Periostin promotes metastasis of HNSCC mediated by aggressive invasiveness in vivo. A, schema of orthotopic implantation of HNSCC cells with or without periostin overexpression. Periostin-overexpressing and control cells (5 × 10⁵ cells) were orthotopically implanted into the tongue of nude mice. After 2 weeks, tongue tumors, cervical lymph nodes, and lungs were dissected. B, representative H&E-stained images of histopathologic sections from mice injected with control cells: (a) histology of tumor mass in the tongue (original magnification, ×5); (b) high-power magnification of (a). Tumor mass is enclosed with dotted line (original magnification, ×25). C, representative H&E-stained images of histopathologic sections from mice injected with periostin-overexpressing cells: (a) histology of tumor mass in the tongue (original magnification, ×5); (b) high-power magnification of (a); (c) histology of invading tumor in lymphatics; (d) histology of lymph node metastasis; (e) histology of lung metastasis (original magnification, ×25). Arrow, invaded tumor (c) and metastasized tumor (d and e). D, summary of the metastasis of periostin-overexpressing and control cell–injected mice.
(grades 1 and 2) and high (grades 3 and 4). Interestingly, higher expression of peristin was significantly associated with the grading of mode of invasion (P < 0.05; Fig. 5B, c and d; Table 1). In particular, cancer cells at the invasive front expressed peristin at higher levels (Fig. 5B, e). We also examined the association between peristin expression and metastasis in 62 HNSCC cases with available clinical information. HNSCC cases with metastasis (56.2%) showed high expression of peristin (Table 1).

To further evaluate the expression of peristin in patients with HNSCC, we compared the expression in a previously published microarray data set of 41 HNSCC patients and 13 normal controls (9). Similar to our data, peristin was expressed at higher levels in HNSCC tissues, in comparison with normal oral mucosal tissues (Fig. 5C). Moreover, HNSCC cases with angiolympathic invasion showed higher expression of peristin (Fig. 5C). To further explore the genes that are coordinately expressed with peristin in HNSCC tumors, we did a similarity search using a Pearson correlation metric as implemented in tGeneData Analyst Pro 1.0 software. The expression of selected genes demonstrating the highest coordinate expression with peristin in normal oral mucosal tissue and HNSCC tissues was then visualized by hierarchical clustering (Fig. 5D). FAR, SULF1, COL5A2, COL3A1, COL10A1, COL4A1, FN1, and INHBA were well correlated with peristin expression (Fig. 5D; Supplemental Data 2).

**Discussion**

It is believed that neoplasms contain a variety of subpopulations of cells with differing metastatic potentials, and the presence of highly metastatic clones may exist within a primary tumor (4). Recent data shows that reinjection of metastatic cell populations could lead to enrichment in the metastatic phenotype by work on mice model of experimental metastasis using cancer cell lines (22–25). Furthermore, metastasis-related genes were identified by comparing the gene expression profiles between parent and metastatic cell populations using microarray analysis (22–25). We previously established HNSCC cell lines from metastatic cervical lymph nodes of HNSCC, and then isolated highly invasive clones from this cell line by *in vitro* invasion assay (5, 10). By using these cell lines, we showed that the methylation of E-cadherin and degradation of β-catenin were involved in the invasion of HNSCC through the loss of cell-cell adhesion (5). Thus, using a previous *in vitro* invasion assay method, we could obtain the highly invasive phenotype by the isolation of cell populations. Therefore, we thought that comparing the gene expression profile of the parent and highly invasive clone could be a good approach to identify genes that influence the invasion of HNSCC. Here, we identified several genes which encode secretory or cell surface proteins implicated in invasion, cell adhesion, angiogenesis, and growth factor as candidate genes for the invasion of HNSCC by comparing...
the gene expression profiles between parent HNSCC cells and a highly invasive clone using microarray analysis. Among these genes, periostin was found to be the most highly expressed gene in invasive HNSCC cells. As expected, overexpression of periostin dramatically promoted the invasion of HNSCC cells in vitro. Moreover, the invasive phenotype was abolished by periostin siRNA treatment. These observations strongly indicate that periostin plays an important role in the invasion of HNSCC. In addition, we found that periostin overexpression did not promote proliferation, but instead promoted soft agar and in vivo growth as tumor xenografts, demonstrating that periostin enhances the anchorage-independent growth of HNSCC cells.

Periostin contains an NH2-terminal secretory signal peptide, followed by a cysteine-rich domain, four internal homologous repeats, and a COOH-terminal hydrophilic domain. The four internal repeat regions of periostin share a homology with the axon guidance protein FAS1-containing sequences that allows binding of integrins and glycosaminoglycans in vivo (26). FAS1 domains of β1/β3, which share a significant structural homology with periostin, bear motifs interacting with integrin α3β1 and αvβ3 (14, 15), and mediate endothelial cell adhesion and migration via integrin αvβ3 (16). Similarly to β1/β3, we found that interference with the function of integrins by specific anti-αvβ3 and anti-αvβ5 integrin antibodies had an effect on the ability of periostin to mediate cell adhesion in HNSCC cells. Taken together, these data strongly suggest that the FAS1 domain of periostin binds to integrins. It is well known that integrin mediates cell–extracellular matrix interaction and that integrin-mediated adhesion regulates a variety of intracellular events (27). Therefore, we hypothesize that periostin-integrin interaction may inhibit the extracellular matrix–integrin interaction and trigger the intracellular signaling and activation of certain genes that are involved in invasion and anchorage-independent growth of HNSCC. This hypothesis is supported by a recent report that periostin activated the Akt/PKB pathway via the αvβ3 integrin to promote cellular survival in colon cancer (28). We suggest that overexpression of periostin may confer on HNSCC cells the ability to survive in the absence of anchorage by inhibiting anoikis-related apoptotic pathways, thus allowing periostin-overexpressing HSC2 cells to form colonies in soft agar and tumors in nude mice. However, in order to clarify the underlying mechanism of invasion and anchorage-independent growth by periostin, further studies are required.

As mentioned above, periostin promoted invasion and anchorage-independent growth in HNSCC cells. These striking phenotypes seem to be important for cancer metastasis. Interestingly, periostin overexpression dramatically induced metastasis to the lymph nodes and to the lung in an orthotopic implant model of HNSCC, demonstrating spontaneous metastasis from the tongue. This orthotopic implant model of HNSCC seems clinically relevant because its tumor progression–containing metastasis mimics the clinical scenario. Moreover, periostin-overexpressing tumors became apparent, forming a larger tumor mass and remarkable invasiveness including destruction of mandibular bone and lymphatic infiltration. Therefore, we suggest that aggressive invasiveness and anchorage-independent growth by periostin overexpression may consequently lead to metastasis. To confirm the role of periostin in metastasis as described above, we would like to examine the effect of periostin siRNA in vivo in the future.

Indeed, immunohistochemical analysis revealed that periostin expression was well associated with the pattern of invasion in HNSCC cases. Moreover, 56.2% of HNSCC cases with metastasis showed higher expression of periostin, but we could not find any statistical significance between periostin expression and metastasis. In the near future, we will examine the correlation between periostin expression and metastasis in a large number of HNSCC cases. Interestingly, Bao et al. also showed that a colon cancer cell line with low metastatic potential, engineered to overexpress periostin, displayed a striking phenotype of greatly accelerated tumor metastatic growth as xenografts in the animal model system of metastasis (28). Importantly, higher expression of periostin was frequently observed in HNSCC tissues compared with normal tissues. By evaluation of periostin expression in HNSCC patients, a strong correlation of periostin expression with FAP, SULF1, COL5A2, COL3A1, COL10A1, COL4A1, FN, and INHBA was observed. Although we have yet to confirm this correlation, this finding gives us the impression that periostin expression seems to be correlated with a stromal reaction likely related to invasion and metastasis. In addition, previous studies have shown that periostin expression is up-regulated in various types of tumor including HNSCC (29), colon (28, 30), breast (31), lung (32), and ovarian

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*The expression of periostin was graded as ++ (>30% of tumor cells showed strong or diffuse immunopositivity), + (10-30% of tumor cells showed moderate or patchy immunopositivity), and − (<10% of the tumor cells showed weak or focal immunopositivity or no staining).
Mao L, Hong WK, Papadimitrakopoulou VA. Focus on metastatic potential, as shown in this study, raises the possibility that it could be used as a molecular target in the antimetastatic therapy of patients with HNSCC.

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