Angiogenesis, Metastasis, and the Cellular Microenvironment

Fibroblast Growth Factor Receptor Mediates Fibroblast-Dependent Growth in EMMPRIN-Depleted Head and Neck Cancer Tumor Cells

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Abstract

Head and neck squamous cell carcinoma tumors (HNSCC) contain a dense fibrous stroma which is known to promote tumor growth, although the mechanism of stroma-mediated growth remains unclear. As dysplastic mucosal epithelium progresses to cancer, there is incremental overexpression of extracellular matrix metalloprotease inducer (EMMPRIN) which is associated with tumor growth and metastasis. Here, we present evidence that gain of EMMPRIN expression allows tumor growth to be less dependent on fibroblasts by modulating fibroblast growth factor receptor-2 (FGFR2) signaling. We show that silencing EMMPRIN in FaDu and SCC-5 HNSCC cell lines inhibits cell growth, but when EMMPRIN-silenced tumor cells were cocultured with fibroblasts or inoculated with fibroblasts into severe combined immunodeficient mice, the growth inhibition by silencing EMMPRIN was blunted by the presence of fibroblasts. Coculture experiments showed fibroblast-dependent tumor cell growth occurred via a paracrine signaling. Analysis of tumor gene expression revealed expression of FGFR2 was inversely related to EMMPRIN expression. To determine the role of FGFR2 signaling in EMMPRIN-silenced tumor cells, ligands and inhibitors of FGFR2 were assessed. Both FGF1 and FGF2 enhanced tumor growth in EMMPRIN-silenced cells compared with control vector-transfected cells, whereas inhibition of FGFR2 with blocking antibody or with a synthetic inhibitor (PD173074) inhibited tumor cell growth in fibroblast coculture, suggesting the importance of FGFR2 signaling in fibroblast-mediated tumor growth. Analysis of xenografted tumors revealed that EMMPRIN-silenced tumors had a larger stromal compartment compared with control. Taken together, these results suggest that EMMPRIN acquired during tumor progression promotes fibroblast-independent tumor growth.

Introduction

Head and neck squamous cell carcinoma (HNSCC) develops from dysplastic mucosal epithelium and typically progresses to invasive islands of tumor cells. Transition to a highly invasive phenotype is thought to include a myriad of alterations, including the upregulation of extracellular matrix metalloprotease inducer (EMMPRIN, also known as CD147), on the cell surface which promotes tumor-stromal signaling (1–3). EMMPRIN is a cell surface glycoprotein (4) that is overexpressed in malignant neoplasms with significant dysplastic reactions, including bladder (5), lung (6), breast (7), and HNSCC (8, 9) but not in normal tissues (10). During the development of mucosal squamous cell carcinoma, EMMPRIN expression gradually increases as cells develop from dysplastic lesions to carcinoma in situ to invasive cancer (8). These factors suggest that gain of EMMPRIN during carcinogenesis contributes to the malignant phenotype.

Elevated EMMPRIN expression levels correlate with tumor proliferation, angiogenesis, metastasis, and invasion (11–13). Conversely, inhibition of EMMPRIN through genetic alterations or targeted inhibition in vivo results in inhibition of tumor growth (14, 15). In fact, anti-EMMPRIN antibody has been studied as a potential therapeutic agent alone and in combination with conventional therapies in HNSCC (16, 17). Although the mechanism by which EMMPRIN promotes tumor growth is not fully understood, it has been shown that EMMPRIN expression on the tumor cell surface stimulates surrounding fibroblasts and endothelial cells to secrete matrix metalloproteinases...
critical ligand systems (22, 23). Consistent with other embryologically
receptors (FGFR) and were originally discovered regulating
tumor metastasis, invasion, and angiogenesis.

Malignant transformation, FGF signaling has been found
to promote angiogenesis and mediate tumor and stroma
communication during tumor progression via a paracrine
feedback pathway (24–27). Although little is reported about
FGFR in head and neck cancer, increased expression of the
FGF2 receptor has been associated with disease progression
(28–30).

HNSCC tumors contain tumor cells along with dense
fibroblasts, which are known to promote tumor growth
(31). However, the mechanism of fibroblast-mediated
growth remains unclear. Although EMMPRIN is known
to induce fibroblast expression of MMPs and various
angiogenic stimuli (18–21), the effect of EMMPRIN on
fibroblast-enhanced tumor growth has not been character-
ized. In this study, we found that downregulation of tumor
cell–derived EMMPRIN inhibits cell proliferation as well as
promotes fibroblast-dependent tumor growth. Our results
suggest that EMMPRIN plays a role in fibroblast-depen-
dent tumor growth by modulating FGF–FGFR signaling.
Gain of EMMPRIN expression during tumor progression
not only corresponds to promotion of tumor growth but also
allows the tumor growth to be less dependent on
fibroblasts.

Materials and Methods

Cell culture and reagents

FaDu (ATCC) and SCC-5 (University of Michigan) were
grown in Dulbecco’s modified Eagle’s medium (DMEM)
supplemented with 10% FBS, penicillin (100 U/mL), and
streptomycin (100 μg/mL). Cells were incubated at 37°C in
a humidified atmosphere containing 5% CO₂. To obtain
human normal dermal fibroblasts (NDF), normal skin
specimens were minced, washed in 70% ethanol followed
by PBS and then dried on 6-well culture plates in triplicate
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confluence. Culture medium was replaced with prewarmed
PBS containing 10 μmol/L CFSE. After incubation at 37°C
for 15 minutes, the cells were washed with medium 3 times
and cultured for additional 24 hours. For tumor and fib-
roblast coculture, 6.6 × 10³ cells were seeded into 24-well plates. After 96 hours, the cells were trypsinized and analyzed by using flow cytometer.

Tumor cell proliferation assay

Tumor cell proliferation. FaDu, SCC-5, or the cells
with EMMPRIN silenced were seeded into 24-well plate at
density of 10⁴ cells per well; after 72 hours culture, the cells
were trypsonized. The number analyses were done a minimum of
3 times to confirm findings, and representative data shown.

EMMPRIN Modulates Fibroblast-Dependent Tumor Growth via FGFR

(MMP; refs. 18, 19) and VEGF (20, 21). The release of
these cytokines within the tumor microenvironment favors
tumor metastasis, invasion, and angiogenesis.

Fibroblast growth factors (FGF) signal through FGF
receptors (FGFR) and were originally discovered regulating
fundamental developmental pathways of multiple organ
systems (22, 23). Consistent with other embryologically
critical ligand–receptor pathways that are resurrected in
malignant transformation, FGF signaling has been found
to promote angiogenesis and mediate tumor and stroma
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3 times to confirm findings, and representative data shown.
Western blot analysis

Cells were grown to 70% to 80% confluence, washed twice with cold PBS, and lysed in lysis buffer [50 mmol/L Tris-HCl (pH 7.5), 150 mmol/L NaCl, 1% (v/v) NP40, 0.5% (w/v) sodium deoxycholate, 1 mmol/L EDTA, 0.1% SDS], and a protease inhibitor cocktail tablet (Roche Applied Science) was added. The cleared lysates were collected by centrifugation at 12,000 \( \times C \) for 20 minutes at 4°C. The protein concentrations were measured by BCA protein assay (Thermo Scientific). Lysates with 10 \( \mu \)g of total protein were resolved by SDS-PAGE and transferred to polyvinylidene difluoride membranes. The membranes were incubated with the primary antibody. After washing and incubating with HRP-conjugated secondary antibodies, the membranes were washed again and detected by the Amersham ECL Western blotting detection system (GE healthcare). The membranes were reprobed with HRP-conjugated mouse monoclonal anti-human \( \beta \)-actin to ensure equal protein loading.

Assessing effect of FGFR2 inhibitors on fibroblast-mediated tumor cell growth

Tumor cells were cultured with or without CFSE-labeled NDF cells as described above. Anti-FGFR2 antibody (20 \( \mu \)g/mL, Abcam, clone MM0278-6L19), its isotype mouse IgG1 (BioLegend), or 100 nmol/L PD173074 (Sigma-Aldrich) were used in tumor cell culture with or without NDF. After 96 hours culture, the cells were trypsinized and analyzed by using flow cytometer.

Assessment of effect of FGF1 and FGF2 on tumor proliferation

A total of 2 \( \times 10^3 \) tumor cells were seeded into 48-well tissue culture plate; 5 ng/mL FGF1 (Sigma-Aldrich) or 5 ng/mL FGF2 (Sigma-Aldrich) along with 10 \( \mu \)g/mL heparin (Sigma-Aldrich) were used in cell culture. The cells were cultured for 96 hours and then subjected to cell number analysis.
Examination of FGF1 and FGF2 expression in NDF and tumor cells

A total of $2 \times 10^5$ cells were cultured in 6-well plate for overnight. Then, the cells were cultured in serum-free medium for 48 hours. FGF1 or FGF2 concentration in the medium was measured by using human FGF acidic and human FGF basic kits (R&D System).

Assessment of tumor cell growth in vivo

Mice with severe combined immune deficiency (SCID, 8 week-old females) were obtained from NCI-Frederick and maintained in accordance with Institutional Animal Care and Use Committee (IACUC) guidelines of University of Alabama at Birmingham. Animals were inoculated s.c. on the right flank with $2 \times 10^6$ tumor cells or
1.5 × 10⁶ tumor cells plus 0.5 × 10⁶ NDF cells in 0.2-mL serum-free DMEM. Animals were followed for 4 weeks after the tumor cell inoculation. Tumor measurement were converted to a calculated tumor weight (in milligram) by using the formula [width (mm)² × length (mm)]/2 (32).

Assessing tumor cells and host stromal cells in tumor tissue by flow cytometry and hematoxylin and eosin stain
Fresh tumors were cut into a small piece and incubated with PBS with 2 mg/mL collagenase (Fisher Scientific) at 37°C for 2 hours to disaggregate cells. Dead cells and debris were removed by density centrifugation (800 × g, 15 minutes) by using Ficoll-Paque Plus. Interface cells were resuspended in Hanks’ buffer (containing 3% FBS), then incubated with antibodies to mouse CD16/CD32 (eliminating nonspecific binding), mouse H2k[d]-PE, and human EMMPRIN-FITC; propidium iodide (PI)-negative cells were electronically gated and then analyzed with respect to mouse H2k[d] and human EMMPRIN surface staining. The rest of tumor was fixed with formalin, sub- stained with hematoxylin and eosin (H&E) as previously described (9). Tumors from each animal (n = 7) underwent flow analysis and multiple sections were analyzed with similar results and representative data shown.

Assessment of effect of tumor cells with altered EMMPRIN on fibroblast migration
Cloning cylinders were put in 12-well tissue culture plate with 100 μL of type I collagen (4 mg/mL) in each well. The plate was then left in tissue culture hood with cover opened until collagen solution was dried and sealed the cylinder to bottom of the plate. A total of 4 × 10⁶ tumor cells with different level of EMMPRIN were placed inside of cylinders and 1 × 10⁵ fibroblasts were cultured outside. After 24 hours culture, both tumor cells and fibroblast grow to confluence and cylinders were then removed. A consistent gap appeared between tumor cells and fibroblasts. The cells were kept cultured for additional 24 hours. Alteration of gap represents the relative migration rate of cells. Tumor cell migration was also assessed as control.

Results
EMMPRIN regulates fibroblast-dependent tumor cell growth
To examine effect of EMMPRIN on fibroblast-dependent tumor cell growth, tumor cell EMMPRIN was genetically manipulated by lentiviral particle delivery system. Because HNSCC express high level of EMMPRIN, further exogenous overexpression was not done and only the impact of EMMPRIN downregulation was assessed. HNSCC cells stably silenced for EMMPRIN expression (SiE1 and SiE2), as well as cells with vector as control (VC)-transfected cells were generated from SCC-5 and FaDu cell lines. The EMMPRIN expression of these cells was verified by flow cytometry and Western blot (Fig. 1A and B). Loss of EMMPRIN expression resulted in decreased tumor cell proliferation (Fig. 1C). It is well established that tumor cells obtain a significant growth advantage from interaction with fibroblasts (33). To determine the role of EMMPRIN in fibroblast-dependent tumor cell growth, we conducted a coculture assay in which HNSCC cells silenced for EMMPRIN expression were mixed with CFSE-labeled fibroblasts. As labeled fibroblasts emitted fluorescence, tumor cells and fibroblasts were able to be separated by flow cytometry and counted (Fig. 2A). Although either EMMPRIN-silenced or control vector–transfected tumor cells showed an increased growth in response to fibroblasts, proliferation of SiE1 or SiE2 cells was more dramatic in the presence of fibroblasts (Fig. 2B). Coculture of tumor cells with normal human dermal fibroblasts significantly accelerated growth of tumor cells with less EMMPRIN in vitro. To assess this phenomenon in vivo, EMMPRIN-silenced tumor cells with and without fibroblasts were inoculated into the flank SCID mice. NDFs (tumor cell: fibroblast ratio, 3:1) accelerated growth in the EMMPRIN-silenced cell lines (SiE1) to a far greater extent than control vector–transfected cells (Fig. 2C). Similar results were also obtained from cells with SiE2...
These findings suggest that silencing EMMPRIN promotes tumor growth sensitive to the presence of fibroblasts; gain of EMMPRIN accelerates tumor growth and allows the growth to be less dependent on stromal fibroblasts.

Fibroblast-enhanced tumor growth is mediated by paracrine signaling

To characterize the interaction between tumor cells and surrounding fibroblasts, tumor cell proliferation was measured in transwell chambers (Fig. 3). Tumor cell proliferation was similar regardless of whether fibroblasts were in direct contact with tumor cells [NDF(c)] or whether cells were separated within the chambers of the transwell system [NDF(s)], indicating that a soluble factor secreted from fibroblasts plays a role in enhancing tumor growth; the event was fulfilled by paracrine rather than by juxtacrine communication.

Downregulation of EMMPRIN augments FGFR2 expression and FGFR2 participates in fibroblast-mediated tumor growth

To determine the possible molecular mechanisms by which fibroblasts mediate tumor cell growth in this context, gene expression profile of EMMPRIN-silenced cells was compared with control vector–transfected cells by gene microarray analysis (Supplementary Fig. S1A). FGFR2 gene expression was elevated in EMMPRIN-silenced cells. To confirm this, FGFR2 expression was assessed by Western blot assay (Fig. 4A) and showed that silencing EMMPRIN enhances FGFR2 expression. Importantly, FGF signaling through FGFRs has been reported to mediate tumor and stroma communication during tumor progression via a paracrine loop (34). To determine the significance of FGFR2 expression in fibroblast-mediated HNSCC tumor growth, ligands and inhibitors of FGFR2 were assessed. Supplement of FGF1 (5 ng/mL) or FGF2 (5 ng/mL), a ligand of FGFR2

Figure 4. Effects of FGFR2-mediated signaling on fibroblast-dependent tumor cell proliferation. A, effects of EMMPRIN on FGFR2 expression. FGFR2 expressions in EMMPRIN-silenced cells and control cells were examined by Western blot; β-actin was used as loading control. B, effect of anti-FGFR2 antibody on fibroblast-mediated tumor cell growth. Anti-FGFR2 antibody (20 μg/mL) or its isotype mouse IgG1 were used in tumor cell culture with or without NDF. *, P < 0.05 (∗NDF versus −NDF); **, P > 0.05 (∗NDF versus −NDF). C, effect of FGFR1 or FGFR2 on tumor proliferation. FGFR1 (5 ng/mL) or FGFR2 (5 ng/mL) along with heparin (10 μg/mL) was used in cell culture. *, P < 0.05 (∗FGF versus −FGF); D, examination of FGFR1 and FGFR2 expression in NDF and tumor cells. The cells were cultured in serum-free medium for 48 hours. FGFR1 as well as FGFR2 concentrations in the medium were then measured by ELISA. E, confirmation of essential of FGF–FGFR signaling in fibroblast-mediated tumor cell growth. PD173074 (100 nmol/L), a specific inhibitor for FGF–FGFR signaling was added into SCC-5 cell culture or SCC-5 and NDF coculture. The cells were cultures for 96 hours and cell proliferation was checked by Accuri flow cytometry. *, P < 0.05 (∗NDF versus −NDF); **, P > 0.05 (∗NDF vs. −NDF). Columns, mean for triplicate; bars, SD. The data presented are representative of 3 independent experiments.
enhanced tumor growth in HNSCC cells (Fig. 4C); however, EMMPRIN-silenced cells display more aggressive acceleration of growth in response to both FGF1 and FGF2. Importantly, both FGF1 and FGF2 were detected in conditioned media of NDF culture (Fig. 4D). Addition of FGFR2-blocking antibody to cocultures of tumor cells and fibroblasts abrogated the increase seen in tumor cell proliferation (Fig. 4B). Although anti-FGFR2 antibody can block NDF-enhanced tumor cell growth, the incomplete abrogation of fibroblast-dependent growth suggests FGFR2-independent signaling pathways contribute or the blocking antibody did not completely saturate FGFR2 in the SiE2 cell line. Furthermore, PD173074 (35), a specific inhibitor for FGF–FGFR signaling (FGFR1, 2, and 3), similarly blocked the fibroblast-mediated proliferation in the coculture assay (Fig. 4E). Gene microarray data and Western Blot results showed that FGFR1 and FGFR3 expression levels are much lower than FGFR2 in HNSCC and EMMPRIN stimulation does not affect these receptor expression of FGFR1 and 3 (Supplementary Fig. S1). This suggests that PD 173074 suppresses the fibroblast-mediated tumor cell proliferation primarily through blocking FGFR2 function. These results suggest that FGFR2 mediates fibroblast-dependent cell proliferation and as EMMPRIN expression is silenced, tumor cell growth is more sensitive to fibroblast promotion via upregulated FGFR2 signaling.

**Figure 5.** Effects of EMMPRIN on tumor stroma compartment. A, silencing EMMPRIN reduced tumor growth in vivo. SCC-5 cells with vector or SiE1 were inoculated into flank of SCID mice. Tumor sizes were measured at indicated times. The data represent average sizes of tumors from 7 mice in each group. P, SiE1 versus VC. B, analysis of both host stromal and tumor components by flow cytometry. Xenografted SCC-5 tumors isolated after 4 weeks of growth were disaggregated by collagenase degradation of extracellular matrix. The cells were stained with mouse H2k[d]-PE and human EMMPRIN–FITC. Live cells (PI negative) are either stained by human EMMPRIN or mouse H2k[d] antibodies, clearly showing tumor and host populations. C, silencing EMMPRIN affects stromal compartment. In the top panel, cells isolated from xenografted tumor were analyzed by flow cytometry. V2-L and V2-R display mouse stroma and human SCC-5 population respectively. Bottom panel displays H&E stain of xenografted tumor slides. The islands with spindle cells represent stroma compartment. The data presented are representative of 7 samples from each group. D, statistic analysis of data from flow cytometry. Average percent of stromal cells were calculated from 7 mice of control or EMMPRIN-silenced xenografted tumors. Bars, SD; P, SiE1 versus control. E, confirmation EMMPRIN expression status in vivo. EMMPRIN expression was quantified by fluorescence intensity of tumor cells stained with anti-EMMPRIN–FITC. Average mean fluorescence intensity represents data from 7 mice of control or EMMPRIN-silenced xenografted tumors. EMMPRIN-silenced SCC-5 cells still kept lower EMMPRIN level than control in vivo. Bars, SD; P, SiE1 versus control.
EMMPRIN-silenced tumors are associated with a larger stromal compartment

If EMMPRIN expression compensates for fibroblast-modulated enhancement of tumor proliferation, one would hypothesize that this stromal-independent growth would result in a smaller stromal component in cells with high levels of EMMPRIN expression. To this end, SCC-5 tumor cells were inoculated s.c. on a flank of SCID mice, and tumor growth was monitored. The EMMPRIN-silenced cells grew at a slower rate (Fig. 5A). Xenografted tumors were harvested after 4 weeks of growth and assessed for mouse stromal elements as well as human tumor. Disaggregated tumor cells were stained with mouse H2k[d]-PE and human EMMPRIN–FITC antibodies. Tumor and stromal cell populations were separated by flow cytometry (Fig. 5B). Although the EMMPRIN-silenced tumors (SIE1) grew more slowly when measuring the entire tumor mass, they were associated with a significantly larger stromal compartment, as measured by flow cytometry and histologic analysis (Fig. 5C and D). Similar results were also obtained from cells with SIE2 (data not shown). Low EMMPRIN expression of silenced cells in vivo was confirmed (Fig. 5E). To determine whether enlarged stromal compartments in EMMPRIN-silenced tumors are the result of fibroblast migration, we evaluated the effects of EMMPRIN-silenced tumor cells on migration of human and mouse fibroblasts. Tumor cells and fibroblasts were cultured atop type I collagen in different compartments separated by a cloning cylinder. When the cells grow to confluence, the cylinder was removed and a consistent gap appeared between tumor cells and fibroblasts. After a 24-hour culture, cell migration was examined (Fig. 5F). There was no apparent different migration between EMMPRIN-silenced cells and control vector–transfected cells during experiment period (data not shown); alteration of gap reflects the relative migration of fibroblasts. Migration of either human fibroblast cells or mouse fibroblast cells across the collagen matrix gap was enhanced by EMMPRIN-silenced cells compared with control. The data suggest that EMMPRIN-silenced tumor cells accelerate fibroblast migration, which may account for construction of larger stromal compartments inside the tumor.

Discussion

EMMPRIN has been shown to mediate communication between malignant cells and the surrounding stromal compartment. Either cell surface or soluble EMMPRIN is able to stimulate production of MMP1, MMP2, MMP3, and MT1–MMP (19, 36, 37) as well as cytokines VEGF, interleukin (IL)-1β, IL-6, and IL-8 (16, 20, 21) from adjacent tumor cells as well as from surrounding fibroblast or endothelial cells. EMMPRIN-mediated secretion of these factors contributes to a microenvironment suitable for tumor cell growth. However, there is a limited understanding of how fibroblasts promote growth in HNSCC tumors. To this end, we evaluated the growth promoting effects of fibroblasts on tumor cells with variable EMMPRIN expression. In the absence of high EMMPRIN expression, tumor cell proliferation was very sensitive to fibroblasts, which we identified as mediated by FGFR2 signaling.

We and others have shown the importance of EMMPRIN in tumor cell proliferation in vitro and in vivo (12–15). The mechanism for EMMPRIN-promoted tumor cell proliferation remains to be elucidated. It is reported that EMMPRIN associated with hyaluronan and CD44 enhances lactate efflux, resulting in promoting cell proliferation (38). It is also likely that autocrine signaling is involved or alternatively, that there are fibroblasts mixed within tumor cells. Although tumor cell lines can occasionally be contaminated with fibroblasts, the FaDu and SCC-5 cell lines did not show morphologically and immunohistochemical staining for vimentin, suggesting that these cell lines did not include fibroblast cell types (Supplementary Fig. S2).

Figure 5. (Continued) F, EMMPRIN-silenced tumor cells accelerated fibroblast migration. Tumor cells and fibroblasts were implanted in different chambers separated by a cloning cylinder. Consistent gap appeared between tumor cells and fibroblasts following removal of cylinders (left). Alteration of gap after 24 hours culture reflected fibroblast migration. The results presented are representative of 3 independent experiments.
In this study, we show that EMMPRIN expression promotes tumor cell growth in a fibroblast-independent manner. Our data illustrate the role of EMMPRIN in tumor progression. It is well known that EMMPRIN expression increases as epithelial cells transition from normal to dysplastic to cancer (8). We hypothesize that at an early stage of tumor progression, the EMMPRIN expression is low and the cells need support from surrounding fibroblasts for growth. The tumor cells display sensitivity to fibroblasts via upregulating FGFR2. As tumor progression occurs, higher levels of EMMPRIN allow tumor cells to be less dependent on stroma for their proliferation. However, tumor progression needs angiogenesis and metastasis. It is possible that increased EMMPRIN stimulates stromal fibroblasts to produce VEGF and MMP, which create a favorable microenvironment for this to occur. As EMMPRIN expression increases along with tumor progress, elevated EMMPRIN enhances tumor growth and allows the growth to be less dependent on stroma; and meanwhile, it stimulates fibroblast to produce MMP1, MMP2, MMP3, and MT1-MMP to facilitate tumor cell migration and invasion; allowing focus of crosstalk between tumor and stroma shifts from tumor growth to tumor metastasis.

Previous immunohistochemical studies of EMMPRIN expression in human tumors have shown a mosaic pattern of expression throughout the tumor (9). It is likely that tumor growth is both FGFR2 dependent and FGFR2 independent within the tumor mass depending on the expression of EMMPRIN within individual islands of tumor cells. EMMPRIN likely continues to stimulate surrounding tumor cells to generate the appropriate microenvironment. Taken together, the results suggest that FGFR2-FGFR2 signaling mediates fibroblast-enhanced tumor cell proliferation, and upregulation of EMMPRIN suppresses FGFR2 expression, leading to fibroblast-independent growth. Although we and others have shown that EMMPRIN promotes tumor growth, here we show in vivo and in vitro that EMMPRIN expression reduces the dependence of tumor cells on fibroblasts by modulating FGFR2 expression. In vivo experiments using knockdown EMMPRIN cells confirmed in vitro findings. Although it is possible that murine EMMPRIN could compensate in the in vivo experiments, because EMMPRIN is a membrane anchored protein with very low level of expression in fibroblasts, it is unlikely that murine EMMPRIN could compensate silenced human EMMPRIN.

It is well established that FGF–FGFR signaling is critical for tumor growth via working on tumor endothelial cells to promote tumor angiogenesis (24–27). The direct association of FGFR with tumor growth was also documented. Aberrant FGFR2 signaling, through overexpression of FGFR2 and/or its ligands, mutations, and receptor amplification, has been found in endometrial, ovarian, breast, lung, gastric, as well as head and neck cancers (39–44). Although FGF expression is known to be important in head and neck cancer (28–30, 44), the mechanism by which it influences tumor growth has not been characterized. Consistent with our findings, FGFR2 expression in advanced stage squamous cell carcinoma revealed that higher level of FGFR2 expression is associated with tumor dysplasia and early invasion, compared with later stages of oral carcinogenesis (28). This suggests that FGFR2 plays an important role in the initial stages of oral cancer development in which EMMPRIN expression is also low, because our data show that EMMPRIN level is inversely related with FGFR2 expression. FGFR2 has also been shown to be important in fibroblast modulation of the tumor microenvironment for esophageal carcinoma progression (45).

EMMPRIN has been identified as a therapeutic target in head and neck because it is highly expressed in malignant, but not normal tissues, and expression of EMMPRIN correlates with poor patient prognosis. Furthermore, preclinical data has shown that silencing EMMPRIN expression leads to reduced cell proliferation and tumor size. This study reveals that prior to upregulation of EMMPRIN, tumor cells are more dependent on fibroblast-initiated FGF2–FGFR2 signaling and enlarged stroma compartment. Hence, potential head and neck therapies involving EMMPRIN silencing must involve combination treatments to overcome FGFR2 signaling or tumor-associated fibroblast function.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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