Upregulated IL-19 in Breast Cancer Promotes Tumor Progression and Affects Clinical Outcome

Chung-Hsi Hsing¹,²,⁴, Hung-Chi Cheng⁵, Yu-Hsiang Hsu⁵, Chien-Hui Chan⁵, Ching-Hua Yeh⁶, Chien-Feng Li³, and Ming-Shi Chang⁵

Abstract

Purpose: Interleukin (IL)-19 was expressed in invasive ductal carcinoma (IDC) of the breast tissue but not in healthy breast tissue. We explored the effects of IL-19 on the pathogenesis of breast cancer and its clinical outcome.

Experimental Design: Tumor expression of IL-19 was assessed by immunohistochemistry and/or real-time quantitative PCR between two groups of patients with breast IDC (n = 60 and 143, respectively) with available clinical and survival data. We examined the effects of IL-19 on cytokine and chemokine production as well as proliferation and migration in breast cancer cells. Mice were injected with IL-19–overexpressing or vector control 67NR cells and the tumor growth and lung metastatic micrometastases were measured.

Results: Of the IDC specimens, high IL-19 expression was associated with advanced tumor stage, high tumor metastasis, and worse survival. In vitro, IL-19 induced transcripts of IL-1β, IL-6, TGF-β, matrix metalloproteinase (MMP)2, MMP9, and CXCR4 in 4T1 breast cancer cells; induced fibronectin expression and assembly; and promoted cancer cell proliferation and migration, which were inhibited by anti-IL-19 monoclonal antibody (mAb). Endogenous fibronectin expression and cancer cell migration were lower in IL-19 knockdown 4T1 cells. In 4T1 cells, hypoxia induced IL-19 and CXCR4 expression, which was inhibited by anti-IL-19 mAb. IL-19 overexpression in noninvasive 67NR cancer cells increased cell proliferation and migration. In vivo, mice injected with IL-19–overexpressing 67NR cell clones showed larger tumors and more metastatic micrometastases in the lung.

Conclusions: High IL-19 expression in breast cancer tissue is associated with a poor clinical outcome. IL-19 is pivotal in the pathogenesis of breast cancer. Clin Cancer Res; 18(3); 713–25. ©2011 AACR.

Introduction

Breast cancer continues to be one of the leading causes of cancer-related mortality in women. Evidence suggests that inflammation is associated with a poor prognosis in breast cancer (1–2). Inflammatory mediators, including cytokines and chemokines, in tumor microenvironments affect the progression of breast cancer (3–6). Some cytokines, such as interleukin (IL)-1, IL-6, IL-11, and TGF-β, stimulate cancer cell proliferation and invasion (3). Cytokine receptor activation and intracellular signaling, by NF-kB, for example, promote tumor progression (7). On the other hand, IFNs, IL-2, and IL-12, all of which induce hormone sensitivity or stimulate cellular immunity, have been used for antitumor treatment of advanced breast cancer (8, 9). Exploring which cytokines are involved in the pathogenesis of breast cancer thus may help improve therapeutic strategies.

IL-19 is a cytokine in the IL-10 family with multiple roles in immune regulation and disease (10–15). One important cellular source of IL-19 is monocytes, in which lipopolysaccharide (LPS), granulocyte macrophage colony-stimulating factor (GM-CSF), IL-6, and TNF-α upregulate IL-19 expression (16, 17). IL-19 binds to IL-20R1/IL-20R2, a heteroreceptor complex that activates STAT3 and induces cell proliferation (15, 18, 19). IL-19 induces apoptosis in lung epithelial cells, stimulates liver cells to produce reactive oxygen species (ROS), promotes neutrophil chemotaxis (19), and is involved in inflammatory diseases such as rheumatoid arthritis (20, 21). Acutely induced IL-19 in systemic inflammation may promote lung and other tissue injury in mice undergoing endotoxic shock (22). IL-19 alters the balance of Th1 and Th2 cells in favor of Th2 cells.
Evidence suggests that inflammation is associated with a poor prognosis in breast cancer. Inflammatory mediators, including cytokines and chemokines, in tumor microenvironments affect the progression of breast cancer. Interleukin (IL)-19 expression in breast cancer tissue is not only associated with higher mitotic rate and advanced tumor stage and metastasis but also with predicted worse disease-specific survival and metastasis-free survival with more than 3-fold increased risk. The IL-19 in breast cancer cells has an autocrine effect and provides a microenvironment for tumor progression. IL-19 not only directly promotes the proliferation and migration of cancer cells but also was indirectly involved in tumor progression mediated through matrix metalloproteinase (MMP)2, MMP9, IL-6, TGF-β, CXCR4, and fibronectin. Therefore, antagonizing IL-19 could have therapeutic potentials in breast cancer.

The chronic expression of IL-19 is correlated with Th2 cytokine production in patients with uremia and is associated with the pathogenesis of asthma and psoriasis (24–27). IL-19 also can induce the angiogenic potential of endothelial cells (28).

We previously showed that IL-19 was expressed in some malignant tumors, especially oral squamous cell carcinoma, renal cell carcinoma, invasive ductal carcinoma (IDC) of the breast, and hepatocellular carcinoma (29). We also showed that IL-19 was expressed more in breast cancer tissue than in healthy breast tissue, which suggests that IL-19 may be involved in the pathogenesis of breast cancer. Little is known about the function of IL-19 in breast cancer. Therefore, we examined the correlation between IL-19 expression in breast tumors and clinical outcome. We overexpressed or deleted IL-19 in cancer cells to investigate the effects of IL-19 on the tumorigenesis and metastasis of breast cancer in vitro and in vivo.

Materials and Methods

Patients and tissue specimens

This retrospective study was done in accordance with the guidelines of the Institutional Review Board of Chi-Mei Medical Center, Tainan, Taiwan (IRB9705–003). Primary localized IDC tissue samples of 60 patients obtained from the Chi-Mei Medical Center Tumor and Serum Bank between January 1999 and December 2001 were used for immunostaining and real-time quantitative PCR (RT-qPCR) analysis. Healthy tissue samples were from nonpathologic areas distant from tumors in surgical specimens (confirmed by histology examination). Nontumor tissue samples with signs of inflammation were excluded (30). The IDC samples for qPCR analysis included more than 70% tumor cells qualified by pathologist. The clinicopathologic variables evaluated are listed in Table 1. Another group of breast IDC samples of 143 patients obtained from the Pathology Archive of Chi-Mei Medical Center between January 1999 and December 2003 were used for immunostaining. The clinicopathologic variables evaluated are listed in the Supplementary Table S1.

Cell lines

Human (MCF-7 and Hs578T) and mouse (67NR and 4T1) breast cancer cell lines were obtained from the American Type Culture Collection and maintained in Dulbecco’s Modified Eagle’s Medium (DMEM) with 10% FBS (Life Technologies, Inc.), 2 mmol/L of l-glutamine (Life Technologies), 100 μg/mL of streptomycin, and 100 units/mL of penicillin in a humidified 5% CO2 atmosphere at 37°C.

Expressing and purifying IL-19 recombinant protein

A cDNA clone coded for the human (h) IL-19 sequence from leucine to leucine (amino acids 25–176) was inserted into the expression vector of Pichia pastoris (pPICZ-a; Invitrogen). We used affinity chromatography to express and purify hIL-19 from the culture medium of the yeast cells (24).

Generating mouse monoclonal antibody against hIL-19

Mouse monoclonal antibody (mAb) against hIL-19 (1BB1) was generated following the standard protocols (24, 31). We previously reported that 1BB1 neutralized hIL-19. To determine the specificity of IL-19 mAb (1BB1), the human IL-10 family including IL-10, IL-19, IL-20, IL-22, IL-24, and IL-26 were coated on the plate with various concentrations and analyzed for their binding with 1BB1 (1 μg/mL) using direct ELISA (Supplementary Fig. S1; refs. 22, 29).

Immunohistochemistry

Paraffin-embedded tissue samples were used for immunohistochemical (IHC) staining with purified 1BB1 (diluted 1:50) at 4°C overnight (26, 29). Incubating paraffin tissue sections with mouse IgG1 isotype (clone 11711; R&D Systems) instead of primary antibody was the negative control. Two investigators trained in breast pathology and blinded to the sample sources analyzed the histology and the IL-19 expression levels of at least 5 sections from each patient. The scoring of IHC stains in each specimen was determined using a histologic (H) score (32). The IL-19 immunostaining was labeled low-grade (H < 200) or high-grade (H ≥ 200).

Knockdown of IL-19 gene expression

A short hairpin (sh) mIL-19 RNA constructed with the pGIPZ lentiviral vector was purchased from Thermo Fisher Scientific. We silenced mIL-19 gene expression in 4T1 cells using a reagent (Lipofectamine 2000; Invitrogen) and the silencing vector (60 pmol/L) and then puromycin, according to the manufacturer’s protocols. A pGIPZ nonsilencing vector was used as a negative control.
IL-19 overexpression in breast cancer cell lines

Human and mouse IL-19 coding sequences were constructed in PcDNA3.1 and PCEP4 vectors, respectively. MCF-7 and 67NR cells were transfected with the above vector constructs or vector alone using Lipofectamine 2000 and then neomycin or hygromycin, according to the manufacturer’s protocols.

Animal experiments

Female BALB/c mice (age: 6–8 weeks) were used in all experiments. The mice were housed at 20°C in individually ventilated sterile cages. All cage accessories were sterilized and autoclaved before they were placed in the cage. The left mammary fat pad of each mouse was injected with 67NR-IL-19 or 67NR vector (2 × 10^6 cells), as indicated. The growth of the mammary tumor was evaluated by measuring its size every day. Fifteen days after the tumor cells had been injected, the tumors were surgically removed and measured. Thirty days after the removal of tumors, the mice were killed and the lungs were then embedded in paraffin and 3 nonsequential serial sections per animal were obtained. The presence of metastases was analyzed by light microscopy with hematoxylin/eosin staining. Total number of metastatic micronodules per lung section was counted and averaged among the animals.

Table 1. Associations between IL-19 protein and mRNA expressions in 60 breast IDC tumors with other important clinicopathologic variables

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<th>IL-19-H^b ((n = 38))</th>
<th>(P)</th>
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^aIL-19-L, low-grade immunostaining, H score < 200.

^bIL-19-H, high-grade immunostaining, H score ≥ 200.

^cData were normalized to β-actin and relative to healthy breast tissue.

^dExpressed as mean ± SD.

^eStatistically significant.

^fHER scoring was done using standard HercepTest guidelines.

Immunocytochemistry and immunofluorescence

Anti-hIL-19 (1BB1), anti-hIL-20R1, rat anti-mIL-19, anti-mIL-20R1 mAb (from R&D Systems), and anti-hIL-20R2 and anti-mIL-20R2 mAb (from Abcam) were used to conduct immunocytochemical staining. The immunocytochemical staining was conducted as described previously (33). Immunocytochemical and immunofluorescence staining of fibronectin were conducted using anti-fibronectin mAb (R&D Systems) following the manufacturer’s instructions.

Western blotting

Western blotting was done using antibodies specific for phospho-STAT3, phospho-JNK, phospho-Erk, phospho-Akt, and phospho-NF-κB (Cell Signaling Technology, Inc.) or mIL-19, fibronectin, and CXCR4 (R&D Systems) or HIF-1α and β-actin (Santa Cruz Biotechnology) following the manufacturer’s instructions.

Reverse transcriptase PCR

Total RNA was extracted using a reagent (RNA-Be; Tel-Test Inc.) and then the total RNA underwent reverse transcription according to the manufacturer’s instructions. IL-19 and CXCR4 were amplified using PCR with gene-specific primers (Supplementary Table S2). PCR products were visualized on 2% agarose gels containing ethidium bromide.
bromide. β-Actin amplification was used as an internal control.

Real-time PCR analysis
RT-qPCR was done using the LightCycler-Fast Start DNA Master SYBR Green I kit (Roche) according to the manufacturer’s instructions (29). IL-1β, IL-6, IL-19, TGF-β, matrix metalloproteinase (MMP)2, MMP9, and CXCR4 were amplified using PCR with gene-specific primers (Supplementary Table S2).

Flow cytometry
For cell-cycle analysis, synchronized cells were cultured without FBS for 16 hours. The cells were treated with 10(ng/mL) for 12 hours and then fixed in 95% ice-cold ethanol and stored at −20°C overnight. After PBS washing, the cells were stained for DNA in a solution containing 0.5 mL of RNase (Sigma-Aldrich; 1 mg/mL) and 0.5 mL of propidium iodide (500 μg/mL) for 30 minutes in the dark and then were flow cytometrically analyzed (FACScan; Becton Dickinson). The fraction of cells present in each cell-cycle phase (G1, S, and G2–M) was determined using a software (WinMDI; The Scripps Research Institute, La Jolla, CA) analysis of DNA histograms.

Cell proliferation assay
To determine the effect of IL-19 on cell proliferation, MCF-7 and 4T1 cells were exposed to various concentrations of hIL-19 or mIL-19, as indicated, for 24 hours. Cell proliferation was assessed by bromodeoxyuridine (BrdUrd) incorporation using a BrdUrd ELISA colorimetric assay (Roche). To determine the proliferation of IL-19 knockdown cells and IL-19-overexpressing cells, the cells were initially plated at a density of 2 × 105 per 60-mm dish. After the cells had been incubated, they were counted using a hemocytometer and then plotted.

Cell migration assay
Cell migration was measured using a modified Boyden chamber with a polycarbonate filter with 8-μm pores (Nucleopore, Inc; ref. 22). The cells were treated with hIL-19 or mIL-19 (400 ng/mL), as indicated, and run over for 6 hours.

Wound-healing assay
A “wound-healing” assay was used to study alterations in cell motility and migration (34). Microphotographs were taken at 0 and 24 or 36 hours, as indicated. The percentage of wound healing was quantitatively analyzed by measuring the distances across the wound (N = 20) at the indicated times and then dividing that by the distance measured at 0 hours for each cell line.

Follow-up and statistical analysis
Statistical analysis was done using SPSS 14.0 (SPSS Inc.). Associations and comparisons of IL-19 expression with various parameters were evaluated using Student t tests or Spearman correlation analysis. In this cohort, 60 patients had available follow-up data in August 2009 (median, 82.5 months; mean, 59.6 months; range, 1–127 months); 143 patients from another archive had available follow-up data in August 2010 (median, 103.6 months; mean, 89.2 months; range, 5.6–143.6 months). The endpoint analyzed was the disease-specific survival (DSS) and metastasis-free survival (MFS) of patients with low- and high-grade IL-19 expression, assessed using Kaplan–Meier methods and compared using a log-rank test. A χ2 test, Fisher exact test, Student t test, or Kruskal–Wallis one-way ANOVA test, as indicated, and then Dunn test were used. Significance was set at P < 0.05.

Results
IL-19 expression in tumor tissue was correlated with tumor metastasis and clinical outcome
Sixty IDC of the breast tissue samples were immunohistochemically stained with 1BB1. Staining intensity was high-grade in 38 samples [Fig. 1A (i)] and low-grade in 22 [Fig. 1A (ii)]. Healthy breast tissue samples were not stained [Fig. 1A (iii)]. RT-qPCR showed that transcript levels of IL-19 in healthy breast tissue, high-grade IL-19–stained tissue (IL-19-H), and low-grade IL-19–stained tissue (IL-19-L) were compatible with IHC staining results [Fig. 1A (iv) and Table 1]. IL-19 expression levels in breast tumors were related to human epidermal growth factor receptor (HER)-2 status (Table 1). High IL-19 expression was associated with advanced tumor stage and a high incidence of lymph node metastasis and distant metastasis on follow-ups (Table 1). There were no significant differences in serum levels of IL-19 between the IL-19-H and IL-19-L groups (data not shown). Mitotic figures are an important characteristic of malignant tumor progression. The number of mitotic figures was correlated with the IL-19 expression level in tumor tissue (Fig. 1B). DSS for IL-19-L patients was higher than for IL-19-H patients (Fig. 1C). To test using IL-19 expression as a prognosticator in breast IDC, we determined clinical outcome in the second archive including 143 IDC cases using IHC staining. Of the 143 breast IDC samples, 75 were IL-19-H and 68 were IL-19-L (Supplementary Table S1). A strong correlation was substantiated between IL-19 expression with primary tumor status (P = 0.03), nodal status (P = 0.001), advanced stage (P < 0.001), and HER-2 expression (P = 0.009; Supplementary Table S1). The findings of strong associations between IL-19 expression and several adverse clinicopathologic prognosticators suggested its crucial role in tumor progression of breast cancer. In survival analysis, IL-19 expression was significantly associated with inferior MFS (P < 0.0001; Fig. 1D; Supplementary Table S3). In multivariate analysis, IL-19 expression remained a prognosticator in MFS (P = 0.0004) with an HR of 3.322 (Supplementary Table S4).

IL-19 induced proliferation and migration in breast cancer cells
We analyzed the expression of IL-19 and its cognate receptors IL-20R1/IL-20R2 in human and mouse breast
cancer cell lines using RT-PCR and immunocytochemical staining. IL-19 mRNA expressed in Hs578T and 4T1 cells but little in MCF-7 and 67NR cells, and all 4 lines expressed IL-20R1/IL-20R2 mRNA (Fig. 2A). Immunocytochemical staining showed that IL-19, IL-20R1, and IL-20R2 were expressed in 4T1 (Fig. 2B) and Hs578T cells (Supplementary Fig. S2A). 67NR and MCF-7 cells were also stained positively for IL-20R1 and IL-20R2 (data not shown). We treated breast cancer cells with IL-19 to determine whether IL-19 directly activated intracellular signals. mIL-19 induced STAT as well as JNK, Erk, AKT, and NF-kB phosphorylation in 4T1 cells (Fig. 2C). hIL-19 also induced JNK, Erk, and AKT phosphorylation in Hs578T (Supplementary Fig. S2B) and MCF-7 cells (data not shown). We treated
MCF-7 and Hs578T cells with recombinant hIL-19 and then evaluated cell proliferation using a BrdUrd incorporation assay. hIL-19 promoted the proliferation of both types of cells and anti-hIL-19 mAb, 1BB1, inhibited that proliferation (Fig. 2D). Recombinant mIL-19 also induced cell proliferation in 67NR and 4T1 cells (Supplementary Fig. S3A). Propidium iodide staining with flow cytometry showed that hIL-19 increased the G2–M stage of the cell cycle of MCF-7 cells (Fig. 2E), which is consistent with increased cell proliferation. hIL-19 and mIL-19 also induced migration of Hs578T (Fig. 2F) and 4T1 cells (Supplementary Fig. S3B), respectively. Anti-hIL-19 and anti-mIL-19 mAb inhibited the migration of Hs578T and 4T1 cells, respectively. Thus, IL-19 activated intracellular signaling and promoted proliferation and migration in breast cancer cells.

**IL-19 induced fibronectin expression and assembly**

Fibronectin expression and assembly, which are correlated with tumor progression and promote pulmonary metastasis in breast cancer cells (35), were higher in MCF-7, 4T1, and Hs578T cells (Fig. 3A–C) after hIL-19 treatment. Furthermore, we used shRNA to knockdown endogenous IL-19 in tumor cells and analyzed whether it affected fibronectin expression and assembly. IL-19-shRNA-4T1 (4T1 IL-19 knockdown) cells showed lower fibronectin expression (Fig. 3D) and assembly (Fig. 3E) than did control-shRNA-4T1 cells. Migration (Fig. 3F) and proliferation (Fig. 3G) in IL-19-shRNA-4T1 cells were also lower than in control-shRNA-4T1 cells. However, mIL-19 protein treatment increased migration in both control-shRNA-4T1 and IL-19-shRNA-4T1 cells.

**IL-19 upregulated CXCR4, MMP2, MMP9, TGF-β, IL-1β, and IL-6 expression in breast cancer cells**

Chemokine receptor CXCR4 is critical in breast cancer metastasis (36). To investigate whether IL-19 induces CXCR4 expression, 4T1 cells were treated with mIL-19 and then CXCR4 mRNA as well as protein levels were determined using RT-qPCR and Western blotting, respectively. mIL-19 induced CXCR4 mRNA [Fig. 4A (i)] and protein [Fig. 4A (ii)] expression in 4T1 cells, and hIL-19 induced CXCR4 expression in MCF-7 cells (data not shown).

Hypoxia in breast cancer affects tumor metastasis and is associated with a poor clinical outcome (37, 38). Hypoxia upregulates CXCR4 expression in breast cancer (39). We hypothesized that hypoxia-induced CXCR4 expression in breast cancer was mediated through IL-19 expression. To test this possibility, we treated MCF-7 cells with CoCl2 (300 ng/mL) to determine the mRNA levels of hIL-19 and CXCR4 using RT-qPCR. Both IL-19 and CXCR4 were upregulated in response to hypoxia treatment [Fig. 4B (i)]. We next treated MCF-7 cells with CoCl2 or CoCl2 combined with 1BB1 for 24 hours to determine whether 1BB1 affected hypoxia-induced CXCR4 expression. CoCl2 induced CXCR4 protein expression in MCF-7 cells, which was attenuated by 1BB1 treatment [Fig. 4B (ii)]. These data indicated that hypoxia-induced CXCR4 expression is mediated, at least in part, by IL-19. We also treated MCF-7 cells in a hypoxia chamber for 24 hours with anti-hIL-19...
mAb (1BB1) or control IgG. CXCR4 mRNA expression levels were significantly higher in hypoxia-treated cells than in control (normoxia) cells but significantly lower in 1BB1-treated cells than in PBS control (data not shown). This result is consistent with CoCl2 treatment. Both MMP2 and IL-6 are involved in the interaction between tumor and endothelial cells and important in metastasis (40–43). IL-1β, IL-6, and TGF-β are also involved in tumor progression (3). RT-qPCR showed that IL-1β, IL-6, TGF-β, MMP2, and MMP9 were upregulated in IL-19–treated 4T1 cells (Fig. 4C).

IL-19 overexpression promoted 67NR cell tumor growth and lung metastasis

67NR cells are a relatively noninvasive mouse breast cancer cell line with little endogenous IL-19 expression (Fig. 2A). To investigate the effect of IL-19 overexpression on 67NR cells and to determine whether IL-19 causes the proliferation and metastasis of these cells in vitro and in vivo, we constructed IL-19–overexpressing 67NR cell clones (67NR/IL-19). IL-19 overexpression in 67NR cells promoted proliferation and migration [Fig. 5A (i) and B (i), respectively]. Similar results were found in
IL-19–overexpressing MCF-7 cells [Fig. 5A (ii) and B (ii)]. We injected 67NR cells into the left mammary fat pads of BALB/c mice. The growth of tumors was larger in mice injected with 67NR/IL-19 cells than in those injected with control vector 67NR cells [Fig. 5C (i)]. We removed the tumors at 15 days after 67NR cells injection. The tumor sizes were 0.19 ± 0.02 g and 0.10 ± 0.02 g (P = 0.036) in 67NR/IL-19 and 67NR/vector cells injected mice, respectively [Fig. 5C (ii)]. IL-19 mRNA levels in 67NR/IL-19 tumors were higher than in control vector 67NR tumors [Fig. 5C (iii)]. IHC staining showed strong IL-19 and fibronectin stained in 67NR/IL-19 but not 67NR/vector tumors [Fig. 5C (iv)]. Thirty days after removal of tumors, the mice were sacrificed and lung metastasis was determined using histology analysis. More lung metastatic micronodules were observed in 67NR/IL-19–injected mice than in control mice (Fig. 5D).

Discussion

We showed that IL-19 is involved in the pathogenesis of breast cancer. IL-19 expression in breast cancer tissue is not
only associated with higher mitotic rate and advanced tumor stage and metastasis but also with predicted worse DSS and MFS with more than 3-fold increased risk. IL-19 directly affected breast cancer cell proliferation and migration and indirectly affected tumor progression by inducing the expression of cytokines and chemokines. Because no difference was observed in the serum level of IL-19 between patients with high and low IL-19 expression in tumors, it is possible that IL-19 acted primarily as a local mediator in the microenvironment that affects breast cancer cells. In

Figure 4. IL-19 promoted hypoxia-induced CXCR4 and upregulated MMP2, MMP9, TGF-β, IL-1β, and IL-6 in breast cancer cells. A, 4T1 cells were treated with mIL-19 (400 ng/mL) for the indicated times, and then CXCR4 mRNA and protein were analyzed using (i) RT-qPCR and (ii) Western blotting. * P < 0.05 versus untreated (UT) group. B, i, MCF-7 cells were treated with CoCl2 (300 ng/mL) for 6 hours, and then the mRNA levels of IL-19 and CXCR4 were analyzed using RT-PCR. ii, MCF-7 cells were treated with CoCl2 (300 ng/mL) or CoCl2 plus 1BB1 for 24 hours, and then HIF-1α and CXCR4 protein levels were analyzed using Western blotting. The expression of HIF-1α protein was a control to monitor the mimetic of hypoxia. C, 4T1 cells were treated with mIL-19 (400 ng/mL) for the indicated times, and the expression levels of IL-1β, IL-6, TGF-β, MMP2, and MMP9 were analyzed using RT-qPCR. * P < 0.05 versus untreated group. All data are means ± SEM of triplicate experiments.
addition, breast cancer cells also expressed IL-19 receptors, which indicated that IL-19 may act in an autocrine manner in breast tumors.

In this study, exogenous IL-19 significantly induced proliferation and migration, which were antagonized by anti-IL-19 mAb. However, the anti-IL-19 mAb did not inhibit the migration of Hs578T cells in PBS control group. We speculated that the concentration of endogenous IL-19 in cultured medium produced by untreated Hs578T cells might be too low to have detectable biologic function as exogenous IL-19 did. The little migration activity observed in untreated cells might be attributed to some factors other than IL-19 or it was a nonspecific migration activity.

Hypoxia, which is common in solid tumors, may induce tumor progression by increasing angiogenesis and the expression of chemokines and chemokine receptors, such as CXCR4, which are associated with tumor cell migration (44, 45). We showed that hypoxia induced IL-19 as well as CXCR4 expression in breast cancer cell lines. Our preliminary study also identified a functional hypoxia response element on IL-19 promoter (data not shown) that may explain the upregulation of IL-19 in breast tumor samples. Because 1BB1 reduced hypoxia-mediated CXCR4 expression, we conclude that hypoxia-induced CXCR4 is mediated, at least in part, by IL-19 expression. Therefore, IL-19 may be an upstream molecule that drives CXCR4 expression and tumor metastasis in hypoxia. HER-2 expression is also associated with breast cancer cell progression. HER-2 may induce CXCR4 expression, which is important in breast cancer metastasis (46). We found a significant correlation between IL-19 and HER-2 expression. Our in vitro experiments also showed that IL-19 induced CXCR4 expression in MCF-7, which suggested that an IL-19/CXCR4 pathway affects breast cancer metastasis. However, the association between IL-19 and HER-2 gene expression and their cross-talk on CXCR4 production await further investigation.

Patients with breast cancer frequently develop lung metastasis. Fibronectin assembly is critical for lung metastasis (35), presumably because it facilitates pulmonary vascular arrest via endothelial dipeptidyl peptidase IV (CD26; ref. 47). We showed that IL-19 induced fibronectin expression and assembly in breast cancer cells. Thus, our data further support the relevance of IL-19 expression to lung metastasis in breast cancer.

IL-19 not only directly affected cancer cells by promoting their proliferation and migration but also induced the expression of IL-1b, IL-6, TGF-β, MMP2, MMP9, CXCR4, and fibronectin, all of which are involved in cancer cell proliferation (IL-1b, IL-6, TGF-β), migration or metastasis (MMP2, MMP9, CXCR4, and fibronectin), or angiogenesis (MMP2 and MMP9; refs. 3, 41). A variety of direct cell–cell, cell–matrix, and paracrine interactions are involved in...
metastasis. MMP and IL-6 are also involved in endothelial cell injury when tumor cells cross the endothelial barrier (41). Cytokines are intercellular mediators that regulate survival, growth, differentiation, and the effector functions of cells (8, 48). They also represent a network with a large variety of different members that may promote tumor growth. Thus, we consider that IL-19 produced by breast cancer cells promotes tumor progression not only by its autocrine effect but also by providing a microenvironment for tumor growth and metastasis. Furthermore, cytokines are mediators of the effector response from innate and acquired cellular immunities (49, 50); they are probably involved in the mechanism for tumor cell evasion in the immunosurveillance system. This might also be one of the mechanisms by which overexpression of IL-19 in breast cancer cells promoted tumor growth and metastasis in vivo.

In this study, the ability of tumor growth and metastasis of 67NR cells was significantly promoted after the cells were transfected with IL-19 gene. We noted that the IL-19–transfected tumors were almost twice the size of the control ones and therefore this could easily be the reason for the increased metastases. More evidence of direct effect of IL-19 on tumor metastasis in vivo remains to be investigated. Nevertheless, IL-19 indeed has a tremendous contribution to make a noninvasive cell to have more invasive biologic behavior. These findings support the effects of IL-19 on breast cancer. Thus, antagonists against IL-19 might have therapeutic potential in breast cancer therapy. More informative in vivo models such as

Figure 5. (Continued) C, 67NR cells ($2 \times 10^5$) were injected into the left mammary fat pads of BALB/c mice and growth of tumor was determined (i). Fifteen days later, the tumors were removed and tumor size was measured (ii). IL-19 mRNA expression levels in the tumors were analyzed using RT-PCR (iii). Expression of mIL-19 and fibronectin in tumors were determined using IHC staining (iv). D, thirty days after surgical removal of tumor, the mice were killed, and histologic analysis showed that lung metastasis (arrows) was significantly higher in 67NR/IL-19 cells than in control cells (magnification, 100×). Data are means ± SEM (n = 6 mice in each group). *P < 0.05 versus control mice.
orthotopic implantation model and use of fluorescent protein to visualize cancer in real-time (51, 52) may confirm the therapeutic role of anti-IL-19 antibody in breast cancer treatment.

In summary, our findings provide evidence that IL-19 is an important mediator in breast cancer. IL-19 expression in breast cancer is correlated with lymph node metastasis and distant metastasis. The DSS and MFS in patients with low IL-19 expression were higher than in patients with high IL-19 expression. Our study showed that IL-19 not only promoted the proliferation and migration of cancer cells but also was involved in tumor progression mediated through MMP2, MMP9, IL-1β, IL-6, TGF-β, CXCR4, and fibronectin. We conclude that the IL-19 in breast cancer cells has an autocrine effect and provides a microenvironment for tumor progression. Therefore, antagonizing IL-19 could have therapeutic potentials in breast cancer.

References

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