이학 석사 학위논문

A mutual activation loop between breast cancer cells and myeloid-derived suppressor cells facilitates spontaneous metastasis through IL-6 trans-signaling in a murine model

마우스 골수기원억제세포와 유방암 세포간의 IL-6 트랜스 신호전달 상호활성화고리를 통한 자발적인 전이촉진에 관한 연구

2013년 02월

서울대학교 대학원
협동과정 종양생물학 전공
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A mutual activation loop between breast cancer cells and myeloid-derived suppressor cells facilitates spontaneous metastasis through IL-6 trans-signaling in a murine model

By

Ok-Young Lee

A Thesis submitted to the Interdisciplinary Graduate Program in Partial Fullfillment of the Requirements for the Degree of Master of Science in Cancer Biology at the Seoul National University

December 2012

Approved by thesis committee:

Professor _____ Chairman

Professor _____ Vice chairman

Professor _____

마우스 골수기원억제세포와 유방암 세포간의 IL-6 트랜스 신호전달 상호활성화고리를 통한 자발적인 전이촉진에 관한 연구

지도교수 이 동 섭

이 논문을 이학 석사 학위논문으로 제출함

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이 옥 영

이옥영의 이학 석사 학위논문을 인준함

위 원 장 이 선우 (인)

부위원장 이 동섭 (인)

위 원 김 항 래 (인)

ABSTRACT

A mutual activation loop between breast cancer cells and myeloid-derived suppressor cells facilitates spontaneous metastasis through IL-6 trans-signaling in a murine model

Ok-Young Lee
Major in Cancer Biology
Department of Cancer Biology
The Graduate School
Seoul National University

Tumor cell interactions with the microenvironment, especially those of bone-marrow-derived myeloid cells, are important in various aspects of tumor metastasis. Myeloid-derived suppressor cells (MDSCs) have been suggested to constitute tumor-favoring microenvironments. I evaluated whether MDSCs potentiated by cancer cells directly increased breast cancer aggressiveness, leading to spontaneous distant metastasis of cancer cells.Usingamurine breast cancer cell model, I showed that murine breast cancer cells with high IL-6 expression recruited more MDSCs, and that the metastasizing capacity of cancer cells paralleled MDSC recruitment in tumor-bearing mice. Metastasizing, but not non-metastasizing, tumor-derived factors induced MDSCs to increase IL-6 production and full activation of recruited MDSCs occurred in the primary tumor site and metastatic organ in

the vicinity of metastasizing cancer cells, but not in lymphoid organs. In addition, tumor-expanded MDSCs expressed Adam-family proteases, which facilitated shedding of IL-6 receptor, thereby contributing to breast cancer cell invasiveness and distant metastasis through IL-6 trans-signaling. The critical role of IL-6 trans-signaling was confirmed in both the afferent and efferent pathways of metastasis. Collectively, my findings reveal that breast cancer cells and MDSCs form a synergistic mutual feedback loop and that thus-potentiated MDSCs directly affect breast cancer cell aggressiveness, leading to spontaneous metastasis.

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Keywords : Myeloid-derived suppressor cells (MDSCs), Breast cancer cells, IL-6 trans-signaling, Tumor metastasis

Student Number : 2011-21912

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INTRODUCTION

Breast cancer is the leading cause of cancer-associated death in women worldwide (1). Despite recent improvements in early detection and effective adjuvant chemotherapies, about one-third of patients with early disease will relapse with distant metastasis (2). Metastasis of breast cancer remains a largely incurable disease and is the major cause of mortality among breast cancer patients (3). Cancer metastasis is a complex process comprising dissociation of cancer cells from the bulk tumor, invasion of the neighboring tissue, intravasation, transport through the vascular system, extravasation, engraftment of disseminated cells, and finally outgrowth of micrometastases (4). In previous study, orthotopically grafted human breast cancer cells expressing high levels of IL-6, but not those with low levels of IL-6, spontaneously metastasized to the lung and liver in immunocompromised NOD/scid/γ_c-deficient (NOG) mice (5). IL-6 signaling in cancer cells themselves imbued them with cancer stem cell properties and epithelial-to-mesenchymal transition (EMT) phenotypes, which facilitate cancer cell invasion into the surrounding tissue and blood vessels, and cause distant metastasis (5,6). In addition, IL-6 is known to be an important mediator of the expansion and recruitment of myeloid-derived suppressor cells (MDSCs) (7,8).

MDSCs are a heterogeneous population of cells comprising immature cells of monocyte or granulocyte lineage. They expand dramatically under conditions such as trauma, tumor growth, and various chronic inflammatory disorders, including infection, sepsis, and immunization (7,8). Originally

described as suppressive myeloid cells, thus-expanded MDSCs negatively regulate immune responses through multiple contact-dependent and -independent pathways (8,9). Nitrosylation of T cell receptors (TCRs) and CD8 molecules leads to defective cytotoxic T cell (CTL) responses, rendering the cells unresponsive to antigen-specific stimulation (10). Shortage of l-arginine due to arginase I activity in MDSCs inhibits T cell proliferation by several mechanisms (11). Nitrous oxide (NO) and TGF-β produced by MDSCs induced further immunosuppressive microenvironments favoring tumor growth (7-9). In addition to the abovementioned immunosuppressive functions, MDSCs actively formulate microenvironments favoring the generation and survival of cancer cells in association with chronic inflammation. Induced expression of IL-1β in gastric epithelial cells induces the recruitment of MDSCs and leads to gastric inflammation and cancer, while activation of NF-κB in MDSCs is strongly associated with carcinogenesis (12). MDSCs have been suggested to facilitate cancer cell metastasis through their immunosuppressive activities (8,13,14). Recently, cancer-derived remote signals were shown to induce the accumulation of myeloid cells including MDSC populations in putative metastatic sites before migrating cancer cells arrived, forming a 'pre-metastatic niche', which aided extravasation of migrating cancer cells and facilitated new blood vessel formation (15-17). Accumulating evidence shows that tumor-derived factors and tumor-cell-signaling mediators, such as Hsp72 and S1pr1, activate MDSCs to potentiate their immunosuppressive functions or increase the recruitment and colonization of these cells into pre-metastatic tissues (18,19). However, the evidence for the direct roles of cancer cell-exposed MDSCs in

enhancing cancer cell aggressiveness, leading to spontaneous metastasis of these cells, from their invasion into the surrounding tissue and vascular system to their colonization of the target organ, and the underlying mechanisms is either missing or merely circumstantial.

I questioned whether MDSCs activated by cancer cells directly increase breast cancer aggressiveness leading to spontaneous distant metastasis. To adequately evaluate the mutual interaction of breast cancer cells and inflammatory cells including MDSCs, I utilized murine models in which breast cancer cells were orthotopically grafted into immunocompetent syngeneic mice (20). I found that murine breast cancer cells with high IL-6 expression recruited more MDSCs, and that the metastasizing capacity of cancer cells paralleled MDSC recruitment in tumor-bearing mice. Depletion and addition of MDSCs from tumor-bearing mice, respectively, reduced and increased the distant metastasis of breast cancer cells. Metastasizing, but not non-metastasizing, cancer cells activated MDSCs, increasing their expression and secretion of both IL-6 and soluble IL-6Rα, and facilitated breast cancer cell invasiveness and distant metastasis through IL-6 trans-signaling, acting both in afferent and efferent metastatic pathways. Thus, I provide evidence that breast cancer cells and MDSCs formed a synergistic mutual feedback loop and that thus-potentiated MDSCs directly affect breast cancer cell aggressiveness, leading to spontaneous metastasis.

MATERIALS AND METHODS

Cell lines. The mouse breast carcinoma cell lines 4T1 (ATCC CRL-2539) and EMT6 (ATCC CRL-2755) were purchased from the American Type Culture Collection (Manassas, VA, USA) and maintained in RPMI 1640 (WelGENE, Daegu, South Korea) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (GIBCO, Grand Island, NY, USA) and 1% antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin) at 37°C in a humidified 5% CO_2 atmosphere. IL-6-expressing EMT6 cells (EMT6_IL-6cells) were established by transfection with the pcDNA3.1_IL-6 constructusing PromoFectin (PromoKine, Heidelberg, Germany), according to the manufacturer's instructions. Control cells were transfected with the pcDNA3.1 vector only. Stably transfected clones were established by selection with G418 (Sigma-Aldrich, St.Louis, MO, USA) at a concentration of 500μg/ml for 3 weeks. IL-6-expressing EMT6 (EMT6_IL-6) clones were selected by enzyme-linked immunosorbent assay (ELISA). Stat3-knock down 4T1cells (4T1_shSTAT3 cells) were established using the lentiviral vectors containing the short hairpin RNA (SantaCruzBiotechnology, SantaCruz, CA, USA). Cells were infected with the shSTAT3 virus and cultured in the presence of puromycin, and Stat3-knockdown 4T1 (4T1_shStat3) clones were selected by ELISA and Western blotting, respectively.

Animals. BALB/c mice were purchased from the Jackson Laboratory. Experiments involving mice were approved by the Institutional Animal Care

and Use Committee of Seoul National University (authorization no. SNU05050203).

Tumor models. 4T1 (CRL-2539) and EMT6 (CRL-2755) were purchased from the ATCC. 4T1 and EMT6 cells (1×10^5/mouse) were injected into the mammary fatpads of BALB/c mice. To deplete MDSCs, mice were intraperitoneally injected with 100μg of anti-Gr-1 antibody (RB6-8C5, eBioscience) or control Rat IgG1 twice a week, starting 3 days after 4T1 cell injection. To block IL-6 trans-signaling in the afferent pathways of metastasis, 4T1 cells were injected into the mammary fat pads and gp130-Fc (R&D Systems) was administered continuously using an osmotic mini-pump (5 or 10 μg for 14 days; Alzet). To block IL-6 trans-signaling in the efferent pathways of metastasis, 4T1 cells were injected intravenously into BALB/c mice and mice were intravenously injected with gp130-Fc (2.5 μg/mouse) 4 days after cell injection.

MDSC isolation. To isolate splenic MDSCs, splenocytes were prepared and labeled with anti-CD11b and anti-Gr-1 mAbs. $CD11b^+Gr\text{-}1^+$MDSCs were purified using a FACS Aria cell sorter (BD Biosciences). The purity after sorting was greater than 95%.

Tumor and MDSC conditioned medium preparation. EMT6 cells (1×10^4) and 4T1 cells (1×10^4) were incubated for 72h on a 24-well plate and the culture supernatants were collected. To obtain MDSC CM, FACS-sorted splenic MDSCs (4×10^5) were cultured for 24h. 4T1/MDSC-CM and

EMT6/MDSC-CM were prepared by cultivating MDSCs (4×10^5) in 50% (v/v) 4T1-CM or EMT6-CM for 24h. A volume of 4T1/MDSC-CM containing 1ng of IL-6, or the same volume of EMT6/MDSC-CM or MDSC-CM, was added to the 4T1 and EMT6 cell cultures. To some cultures, the following signaling inhibitors were added ; Stat3 inhibitor peptide (1μM; Millipore), PI3K inhibitor (LY294002, 10μM; Calbiochem), NF-κB inhibitor (Bay-117082, 10μM; Calbiochem), JNK inhibitor (SP600125, 10μM; Calbiochem), p38 MAPK inhibitor (SB403250, 10μM; Cell signaling), and ERK inhibitor (PD98059, 10μM; Calbiochem).

Immunofluorescence microscopy. Tissues were fixed in 4% PFA and embedded in paraffin. Sections were stained with H&E for histopathological analysis. To investigate IL-6, IL-6Rα, and Adam17 expression levels in MDSCs, sections were stained with anti-Gr-1 mAb and other appropriate antibodies. The following primary antibodies were used: anti-mouse IL-6 (Abcam, Cambridge, MA, USA), anti-mouse IL-6Rα (Santa Cruz Biotechnology), anti-mouse Adam17 (Abcam), and anti-mouse Gr-1 (eBioscience). The following secondary antibodies were used: Alexa 488-conjugated anti-rabbit IgG (Invitrogen) and Alexa 594-conjugated anti-rat IgG (Invitrogen). Image acquisition and processing was performing using a confocal fluorescence microscope (Olympus, Center Valley, PA) and an FV10-ASW 2.0 Viewer (Olympus).

ELISA. EMT6 and 4T1 cells were plated on a 24-well plate (1×10^4/well). The cells were permitted to grow for 24 or 48h. Supernatants were collected

and assayed for IL-6 and soluble IL-6Rα levels by ELISA. For IL-6 detection, anti-mouse IL-6 (eBioscience) was used as the capture antibody, biotinylated anti-mouse IL-6 (eBioscience) in 0.1% BSA in PBS/T as the detection antibody, and recombinant IL-6 (eBioscience) as the standard. To detect soluble IL-6Rα, I used anti-mouse IL-6Rα (R&D Systems) as the capture antibody, biotinylated anti-mouse IL-6Rα (R&D Systems) as the detection antibody, and recombinant IL-6Rα (R&D Systems) as the standard.

RNA analysis. Total RNA was isolated from EMT6 cells, 4T1 cells and sorted splenic MDSC using the RNeasy kit (QIAGEN; 74104). cDNA was generated from 1μg of total RNA by reverse transcriptase from Moloney Murine Leukemia Virus (M-MLV) (TAKARA, Shiga, Japan), and subjected to PCR. The following primer pairs were used for PCR: GAPDH, 5'-GTCA GTGGTGGACCTGACCT-3' and 5'-AGGGGTCTACATGGCAACTG-3'; IL-6, 5'-GACAAAGCCAGAGTCCTTCAGAG -3' and 5'-CTAGGTTTGCCGAGTA GATCTC-3'; IL-6Ra, 5'-GTTGCAAACAGTGTG GGAAG-3' and 5'-CCGTG AACTCCTTTGACCAT-3'; Adam10, 5'-TGTGGCTACAG TGACCAGTG-3' and 5'-TAAAGTTGGGCTTGGGATCA-3'; and Adam17, 5'-GAGGAGTG TGACCCGGGTA-3' and 5'-GGGGGCACTCACTGCTATT -3'. PCR products were analyzed by 1.5% agarose gel electrophoresis and subjected to densitometric analysis (Labworks 4.6, UVP BioImaging Systems) after ethidium bromide staining.

Western blot analysis. Cells were harvested in lysis solution containing 50mM Tris/HCl (pH7.6), 1% NP40, 150mM NaCl, 2mM EDTA, 100μM

PMSF, a protease inhibitor cocktail (Roche Applied Science, Basel, Switzerland), and a phosphatase inhibitor (Sigma-Aldrich). After incubation on ice for 30min, cellular debris was removed by centrifugation (10min,4°C). Proteins (10μg) were separated by SDS-PAGE and then transferred to a polyvinylidenedifluoride membrane. After blocking with 5% skim milk, the membranes were probed with an appropriate antibody. Blots were developed with an enhanced chemiluminescence Western blotting detection system (Amersham, GE Healthcare, Buckinghamshire , UK). The following antibodies were used: anti-β-actin (Sigma-Aldrich), anti-phospho-Stat3 (Tyr705) (Cell Signaling, Danvers, MA, USA), anti-Stat3 (Santa Cruz Biotechnology), anti-phospho-NF-κB p65 (Ser536) (Cell Signaling), anti-NF-κB p65 (Cell Signaling), anti-phospho-JNK (Santa Cruz Biotechnology), anti-phospho-ERK (Santa Cruz Biotechnology), and anti-phospho-p38 (Cell Signaling).

Invasion Assay. Matrigel matrix solution (200 μg/ml, Matrigel™ Basement Membrane Matrix, BD Bioscience) was applied to each Transwell (FALCON). 4T1 cells (5×10^4) were seeded into the upper chamber of the Transwell and then the lower chamber was filled with collagen matrix (5μ g/ml). Invasion assays were carried out for 18h. Non-invading cells on top of the matrix were removed by rubbing with amoistened cotton swab. Invaded cells on the lower surface of the Matrigel matrix were fixed with 4%PFA and stained with 0.2% crystal violet. Cells were counted using Image Jsoftware (version1.46).

Statistical analyses. IL-6 and IL-6Rα levels are expressed as means ± SD. The two-tailed Student's *t*-test was used to compare measurements. For *in vivo* experiments, all values represent the means ± standard errors of the mean (SEM) of each group. Two-way analysis of variance (ANOVA) and Bonferroni *post hoc* testing were used to compare the tumor volumes of the two groups. Values of $p < 0.05$ were deemed to indicate statistical significance. The SPSS software (ver.11.0; SPSSInc., Chicago, IL) was used for all statistical analyses.

RESULTS

Correlation of spontaneous distant metastasis of breast cancer cells with MDSC recruitment

To elucidate the relationship between MDSC recruitment and distant metastasis of cancer cells, I created a murine breast cancer model using 4T1 and EMT6 breast cancer cells, which exhibit differential IL-6 expression (Supplementary Fig. S1). 4T1 and EMT6 cells were orthotopically grafted into the mammary fat pads of syngeneic BALB/c mice. Primary tumor growth was slightly but significantly greater for EMT6 cells compared to 4T1 cells during the entire experimental period (Fig. 1A-i). At 25–27 days after grafting, 4T1 cancer cells showed extensive lung metastasis, while EMT6 cancer cells showed no distant metastasis in the lung, liver, bone, or brain (Fig. 1A-ii). IL-6-expressing 4T1 cell-bearing mice showed dramatic recruitment of $CD11b^{+}Gr\text{-}1^{+}$MDSCs in the spleen (alymphoid organ), metastasizing organs (liver and lung), and primary tumor mass; the total number of MDSCs recruited was 2–8 times higher in 4T1 cell-bearing mice than in EMT6 cell-bearing mice (Fig. 1B). To further evaluate the role of MDSCs in the distant metastasis, the 4T1 cell tumor-bearing mice were depleted of MDSCs. Depletion of MDSCs reduced 4T1 lung metastasis (P=0.0006) and primary tumor growth in the mammary fatpads (Fig. 1C and 1D). These results show that MDSCs expanded and recruited in the tumor-bearing mice are critically associated with the distant metastasis of cancer cells.

Induction of IL-6 expression facilitated MDSC recruitment and increased their metastatic capacity

Next, I evaluated whether IL-6-mediated MDSC recruitment promoted the metastasis of EMT6 cancer cells. I stably transfected EMT6 cells with a vector encoding murine IL-6 (EMT6_IL-6). EMT6_IL-6 cancer cells grafted into the mammary fat pads of syngeneic recipients recruited more MDSCs to the spleen, liver, lung, and primary tumor mass compared to the control empty vector-transfected EMT6 (EMT6_Con) cells (Fig. 2A). The percentages and numbers of MDSCs recruited to these sites were comparable in EMT6_IL-6-bearing mice and 4T1 cell-bearing mice (Fig. 1B). EMT6_IL-6 cells showed increased tumor growth compared to the control EMT6_Con cells (Fig. 2B). However, unexpectedly, distant lung metastasis was only slightly increased in EMT6_IL-6 cell-bearing mice (P=0.039) (Fig.2C). Thus, I concluded that IL-6 secreted from breast cancer cells is an important and sufficient factor for MDSC expansion and recruitment, but that additional factors are required to facilitate the recruited MDSC-mediated metastasis of cancer cells.

To reconstitute a microenvironment that more closely resembles that of 4T1 cell-bearing mice, I adoptively transferred splenic MDSCs from 4T1 cell-bearing mice into EMT6 cell-bearing mice. MDSC-transferred EMT6 cell-bearing mice showed similar primary tumor growth in the mammary fat pads, and only slightly increased lung metastasis, compared to vehicle-treated EMT6 cell-bearing mice (Fig. 2D). Thus, neither repeated transfer of splenic MDSCs from metastatic tumor-bearing mice nor over-expression of IL-6 was sufficient to confer on non-metastasizing EMT6 cancer cells a metastasizing

capacity comparable to that of 4T1 breast cancer cells. I assume that metastasizing cancer cells produce additional effects to potentiate the recruited MDSCs, there by leading to distant metastasis.

Metastasizing, but not non-metastasizing, breast cancer cells activated MDSCs

To evaluate whether metastasizing, but not non-metastasizing, cancer cells further activate recruited MDSCs, I collected splenic MDSCs from naïve and tumor-bearing mice and co-cultivated them with 4T1 and EMT6 cells. Splenic MDSCs co-cultured with 4T1 cells showed increased production of IL-6, irrespective of their source (naïve, EMT6 cell-bearing, or 4T1 cell-bearing mice), compared to those co-cultured with EMT6 cells (Fig. 3A). 4T1 cells co-cultured with splenic MDSCs provided activated signals either in the same chamber (lower) or a different chamber (upper) in a Transwell culture assay (Fig. 3B-i), implying that contact-independent factors were important for activation of splenic MDSCs. To confirm the critical role of soluble factors derived from metastasizing breast cancer cells, conditioned media (CM) from breast cancer cells (4T1-CM and EMT6-CM) were applied to splenic MDSC cultures. 4T1-CM, but not EMT6-CM, enhanced the production of IL-6 by splenic MDSCs (Fig. 3B-ii). 4T1-CM increased IL-6 transcription in splenic MDSCs from both 4T1cell- and EMT6 cell-bearing mice; EMT6-CM and recombinant IL-6 only slightly induced the transcription of IL-6 (Supplementary Fig. S2). Exposure of splenic MDSCs to 4T1-CM induced the activation of several signaling pathways, including Stat3, NF-κB, JNK, ERK, and p38 pathways (Supplementary Fig. S3). Using

inhibitors of each pathway, I found that the NF-κB, JNK, and p38 signaling pathways were important in the production of IL-6 by activated MDSCs (Fig. 3C). Importantly, confocal microscopic analysis of tissues from 4T1 cell-bearing mice revealed that MDSCs inside the primary tumor and lung strongly expressed IL-6 while those in spleen tissues from the same mice expressed little IL-6 (Fig. 3D). In summary, metastasizing, but not non-metastasizing, tumor-derived factors induced MDSCs to produce more IL-6, and full activation of recruited MDSCs occurred in the primary tumor site and metastatic organs in the vicinity of metastasizing cancer cells.

Activated MDSCs confer on breast cancer cells invasive potential and stimulate distant metastasis through IL-6 trans-signaling

Next, I evaluated whether activated MDSCs in the metastasizing tumor microenvironment affect breast cancer cell behavior. I cultured 4T1 and EMT6 cells in conditioned media from splenic MDSCs cultivated in the presence of 4T1-CM or EMT6-CM (4T1/MDSC-CM and EMT6/MDSC-CM, respectively) (Fig. 4A). 4T1 cells cultured with splenic MDSC-CM showed mild phosphorylation of Stat3. Moreover, 4T1 cells cultured with 4T1/MDSC-CM, but not EMT6/MDSC-CM, showed greatly increased Stat3 phosphorylation within 10 min (Fig. 4B). Stat3 phosphorylation levels were increased for 48 h in 4T1 cells cultured in the presence of 4T1/MDSC-CM (Fig. 4C and unpublished data). Similar results were obtained for 4T1 cells co-cultured with splenic MDSCs, but not for 4T1 cells cultured in the presence of recombinant IL-6 (Fig. 4D). These data suggest that IL-6 was important in inducing Stat3 phosphorylation in 4T1 cells, but that factors

other than IL-6 from tumor-infiltrating MDSCs were needed for persistent Stat3 phosphorylation.

The recent characterization of IL-6 trans-signaling (21) suggests that tumor microenvironments may provide soluble IL-6Rα as well as IL-6 to maximally induce cancer cell aggressiveness through highly augmented IL-6 signaling, which is implicated in tumor cell survival, cancer stem cell characteristics, and EMT phenotypes important for successful distant metastasis of cancer cells (5,6). To evaluate whether recruited and activated MDSCs in the tumor microenvironment provide enhanced soluble IL-6Rα, I measured levels of soluble IL-6Rα secreted from *ex vivo*-cultured splenic MDSCs from naïve, EMT6 cell-bearing, and 4T1 cell-bearing mice. Compared to those from naïve and EMT6 cell-bearing mice, splenic MDSCs from 4T1 cell-bearing mice produced more soluble IL-6Rα in *ex vivo* culture (Fig. 5A-i). In contrast, splenic MDSCs from naïve, EMT6 cell-bearing, and 4T1 cell-bearing mice expressed similar levels of surface IL-6Rαchain (Fig. 5A-ii).

Production of soluble IL-6Rα involves cell surface-associated proteases. Adam family proteases, especially Adam10 and Adam17, have been implicated in IL-6 trans-signaling (22,23). Non-stimulated splenic MDSCs from 4T1 cell-bearing mice expressed increased levels of both Adam10 and Adam17 compared to MDSCs from EMT6 cell-bearing mice and naïve mice (Fig. 5B). When I cultivated splenic MDSCs from 4T1 cell-bearing mice in the presence of protease inhibitors, levels of the membrane-bound form of IL-6Rα increased and those of soluble IL-6Rα levels decreased (Fig. 5C). To confirm the expression of Adam17 and IL-6Rα by MDSCs *in vivo*, I

analyzed spleen tissues, primary tumor masses, and metastatic lesions in the lungs from 4T1 cell-bearing mice. Confocal microscopy showed that MDSCs in the spleen, primary tumor sites, and lung expressed increased levels of Adam17 and IL-6Rα on their surfaces in 4T1 cell-bearing mice compared to those in EMT6 cell-bearing mice (Fig. 5D, Supplementary Fig. S4, and data not shown). Thus MDSCs that were expanded and recruited in the metastasizing tumor-bearing mice were already capable of soluble IL-6Rα production, even in the spleen, a site remote from the metastasizing cancer cells. Taken together with the increased IL-6 levels only in the vicinity of metastasizing tumor cells, these findings suggest that IL-6 trans-signaling occurs preferentially in primary tumor sites and the metastatic lung but not in the spleen.

To evaluate whether IL-6 trans-signaling is important for activation of 4T1 breast cancer cells, I cultivated 4T1 cells in the presence of IL-6 and/or soluble IL-6Rα and evaluated the individual and combined effects of a blocking anti-IL-6R antibody (which blocks both conventional IL-6 signaling and IL-6 trans-signaling) and a gp130-Fc fusion protein (which only blocks IL-6 trans-signaling). When applied individually, IL-6, but not soluble IL-6R α, increased Stat3 phosphorylation in 4T1 cells. Treatment with both IL-6 and soluble IL-6Rα further increased the phosphorylation of Stat3, implying that IL-6 trans-signaling functioned in 4T1 cell activation (Fig. 6A-i). Inhibition of IL-6 trans-signaling with gp130-Fc blocked Stat3 phosphorylation as efficiently as the IL-6R antibody (Fig. 6A-i). To further confirm the role of IL-6 trans-signaling in the interaction of breast cancer cells and MDSCs, 4T1 cells were cultured in the presence of

4T1/MDSC-CM. gp130-Fc fusion protein treatment inhibited Stat3 phosphorylation in 4T1 cells to an extent comparable to IL-6R antibody treatment (Fig. 6A-ii). The enhanced IL-6 signaling mediated by the cancer cell-MDSC interaction augmented 4T1 breast cancer cell aggressiveness. 4T1 cells cultivated with 4T1/MDSC-CM showed exaggerated invasiveness in a Matrigel invasion assay, a response that was blocked by gp130-Fc treatment (Fig. 6B). To investigate the role of IL-6 trans-signaling in *in vivo* metastasis, I administered gp130-Fc to the tumor-bearing mice. Continuous in fusion of gp130-Fc (5 or 10μg/ea/14days), starting from the day following cancer cell injection, reduced primary tumor growth in the mammary fat pads (Fig. 6C-i) and lung metastasis in dose-dependent manner (Fig. 6C-ii). These findings support the critical role of IL-6 trans-signaling in breast cancer cell invasiveness and metastasis *in vivo*. As increased IL-6 trans-signaling in 4T1 cell-bearing mice was suggested to occur in the primary tumor sites and metastatic lung (Fig. 3D), I evaluated whether increased IL-6 trans-signaling in the lung also affected the efferent phase of cancer cell metastasis. Treatment with gp130-Fc (2.5μg/bolus, i.v.) on day 4 after intravenous cancer cell injection decreased the lung metastasis of 4T1 cancer cells compared to vehicle-treated controls (Fig. 6D).

Finally, to confirm whether the strong and persistent Stat3 phosphorylation in MDSC-potentiated cancer cells is crucial to spontaneous tumor metastasis, I generated Stat3-knockdown 4T1 (4T1_shStat3) cells. Greatly increased invasiveness in a Matrigel invasion assay was observed in control 4T1 (4T1_Con) cells, but not in 4T1_shStat3 cells, after treatment with 4T1/MDSC-CM, although reduced Stat3 expression itself had no effect on

cancer cell invasiveness (Fig. 7A and 7B). Primary tumor growth in the mammary fat pads was reduced in 4T1_shStat3 cell-bearing mice compared to 4T1_Con cell-bearing mice, while the reduction in distant lung metastasis was more dramatic. 4T1_shStat3 cell-bearing mice exhibited few metastases (Fig. 7C and 7D).

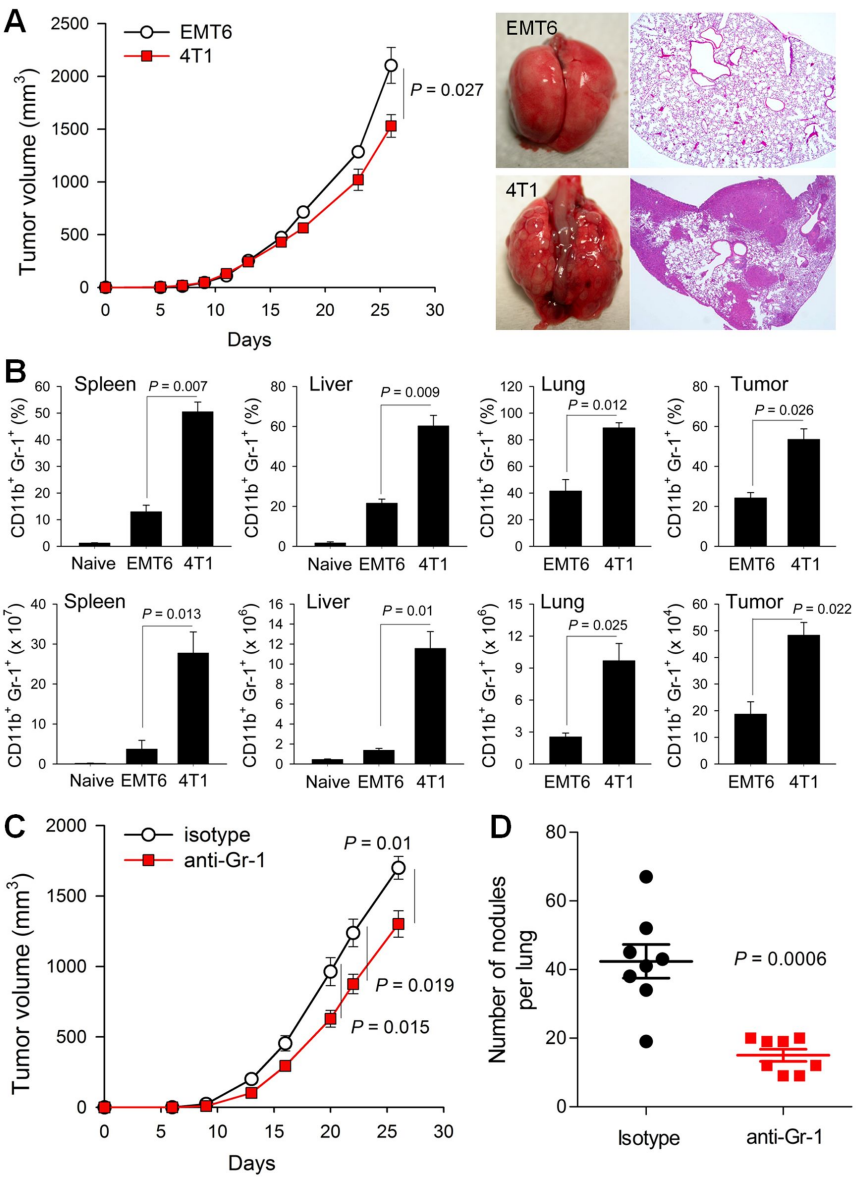


Figure 1. Metastatic cancer cells facilitate recruitment of MDSCs. A, EMT6 and 4T1 cells were injected into the mammary fat pads of BALB/c mice. (i) Primary tumor growth (n=8). (ii) Representative photographs of lungs 26 days after cell injection (H&E). B, the percentages and absolute numbers of MDSCs ($CD11b^+Gr\text{-}1^+$)at19days(n=4). C, 4T1 cell-bearing mice were treated intraperitoneally with anti-Gr-1 antibodies. Primary tumor growth (n=8). D, (i) numbers of tumor nodules in the lungs at 26 days and (ii) representative photographs of lungs from 4T1 cell-bearing mice at 26 days.

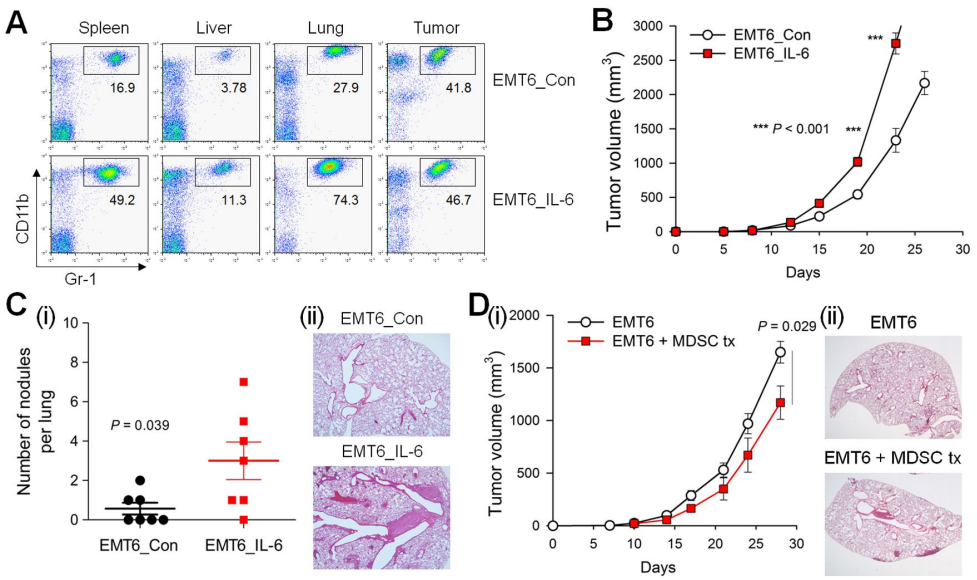


Figure 2. Induction of recruitment and reconstitution of MDSCs in non-metastasizing EMT6 cell-bearing mice enhanced cancer cell metastasis. A-C, EMT6_Con and EMT6_IL-6 cells were injected into the mammary fat pads of BALB/c mice. A, MDSCs were analyzed at 21 days. B, primary tumor growth (n=7). C, (i) numbers of metastatic nodules in the lungs at 26 days (n=7) and (ii) lung sections at 26 days (H&E). D. EMT6 cells were injected into the mammary fat pads. Three days later, mice were intravenously injected with splenic MDSCs (5×10^6/mouse) from 4T1 cell-bearing mice, a total of nine times. (i) primary tumor growth (n=5) and (ii) representative photographs of lungs at 26 days.

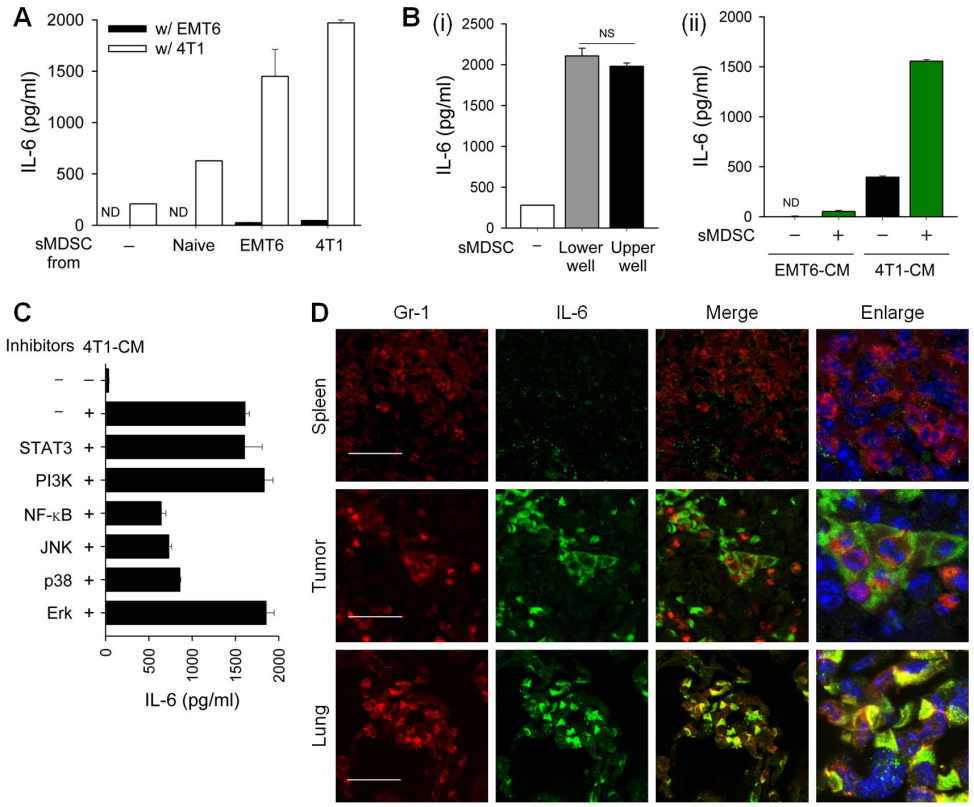


Figure 3. Metastasizing cancer cell-derived factors induced excessive IL-6 production by MDSCs. A, splenic MDSCs (4×10^5) were co-cultured with 4T1 cells (1×10^4) or EMT6 cells (1×10^4) for 48h. IL-6 levels in the culture supernatants were measured by ELISA. B, splenic MDSCs from 4T1 cell-bearing mice were co-cultured with 4T1 cells for 48h in Transwell systems (i) or exposed to conditioned media (CM) for 24 h (ii). C, splenic MDSCs were cultured with 4T1-CM in the presence of signaling inhibitors for 24 h. D, immunofluorescence staining of Gr-1 (red) and IL-6 (green) in the spleen, tumors and lungs of 4T1 cell-bearing mice. Scale bar=30 μm

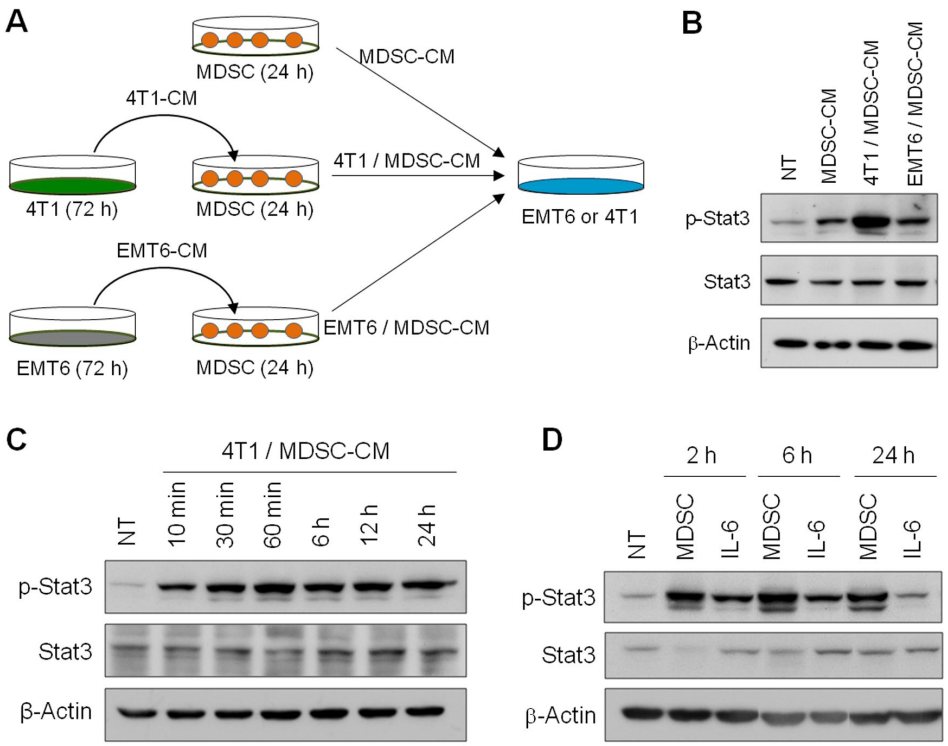


Figure 4. Stimulation of MDSCs with metastasizing 4T1 cell-derived factors induced persistent Stat3 phosphorylation in cancer cells. A, splenic MDSCs from 4T1 cell-bearing mice were cultivated in the presence of 4T1-CM or EMT6-CM. The conditioned media (MDSC-CM, 4T1/MDSC-CM, EMT6/MDSC-CM) were harvested and applied to 4T1 and EMT6 cancer cells. B-C, phospho-Stat3 and Stat3 levels in 4T1 cells exposed to each CM for 10 min (B) and for the indicated periods of time (C). D, 4T1 cells (1×10^4) were co-cultured with splenic MDSCs (4×10^5) from 4T1 cell-bearing mice or recombinant mouse IL-6 (1ng/ml). Phospho-Stat3 and Stat3 levels in 4T1 cells were determined by Western blotting after the removal of MDSCs.

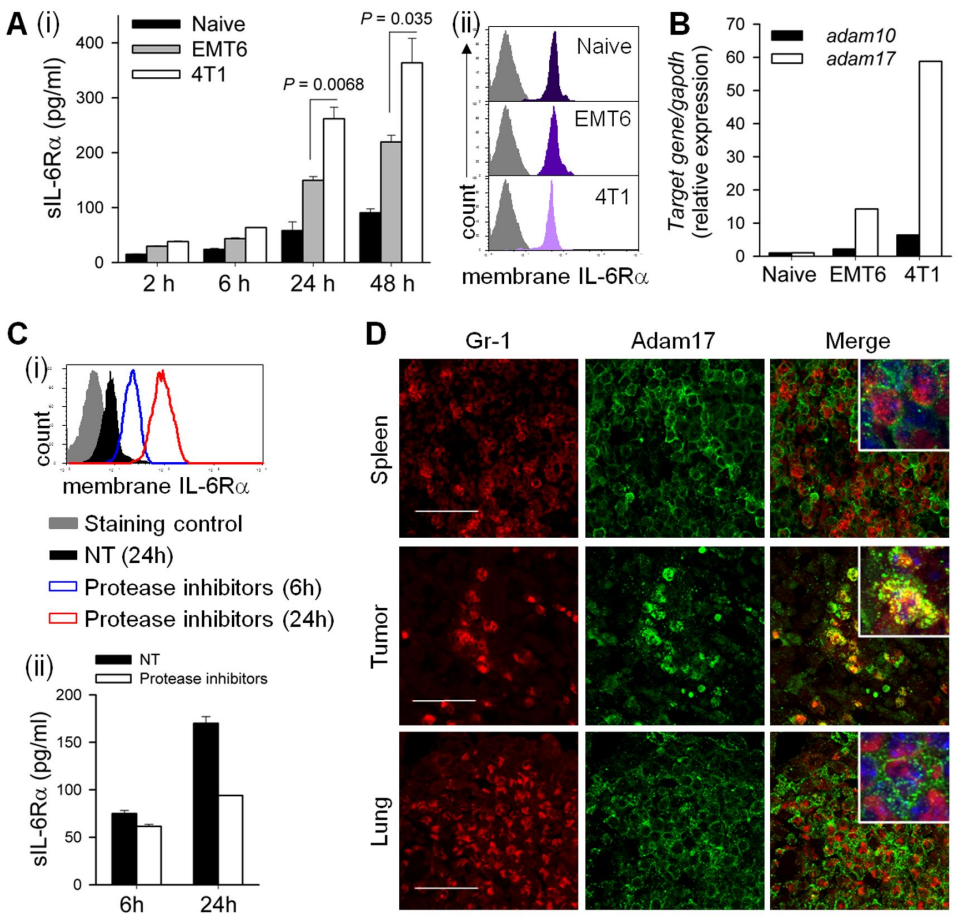


Figure 5. Increased shedding of sIL-6Rα by the MDSCs of tumor-bearing mice. A, soluble IL-6Rα levels in culture supernatants of splenic MDSCs were measured by ELISA (i) and surface IL-6Rα levels on splenic MDSCs were measured by FACS (ii). B, the mRNA expression of Adam10 and Adam17 in splenic MDSCs of naïve and tumor-bearing mice were determined by qRT-PCR. C, protease inhibitor cocktails were applied to cultures of splenic MDSCs from 4T1 cell-bearing mice for 6 or 24 h. (i) Membrane-bound IL-6Rα was detected by FACS. (ii) Soluble IL-6Rα levels were measured by ELISA. D, tissue sections were stained for Adam17 (green) and Gr-1 (red) to compare their localizations. Scale bar=30 μm.

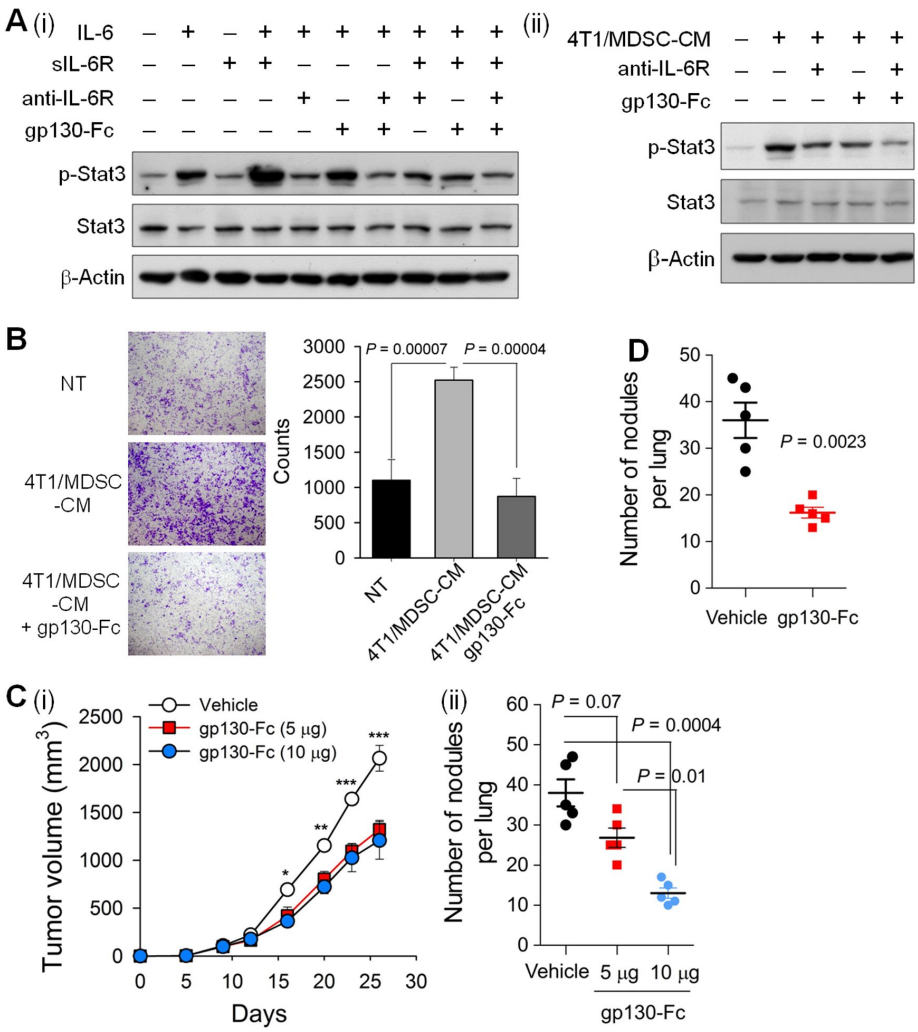


Figure 6. Activated MDSCs contributed to tumor invasiveness through IL-6 trans-signaling. A, 4T1 cells were treated with recombinant IL-6 plus soluble IL-6Rα (i) or 4T1/MDSC-CM (ii) for 30 min in the presence of anti-IL-6R blocking antibody or gp130-Fc. B, 4T1 cells were allowed to invade through Matrigel for 18 h in the presence or absence of 4T1/MDSC-CM and/or gp130-Fc (crystal violet). C, 4T1 cells were injected into the mammary fat pads. Some mice underwent continuous administration using osmotic mini-pumps (5 or 10 μg for 14 days). Primary tumor growth (i) and numbe rs of metastatic masses in the lungs at 26 days (ii). D, 4T1 cells were injected intravenously into BALB/c mice (n=5 mice per group). Some mice received gp130-Fc (2.5 μg) 4 days after cancer cell injection. Numbers of metastatic masses in the lungs at day 12 were determined. Values are the means ± SEM of each group. **P*<0.05,***P*<0.01,****P*<0.001

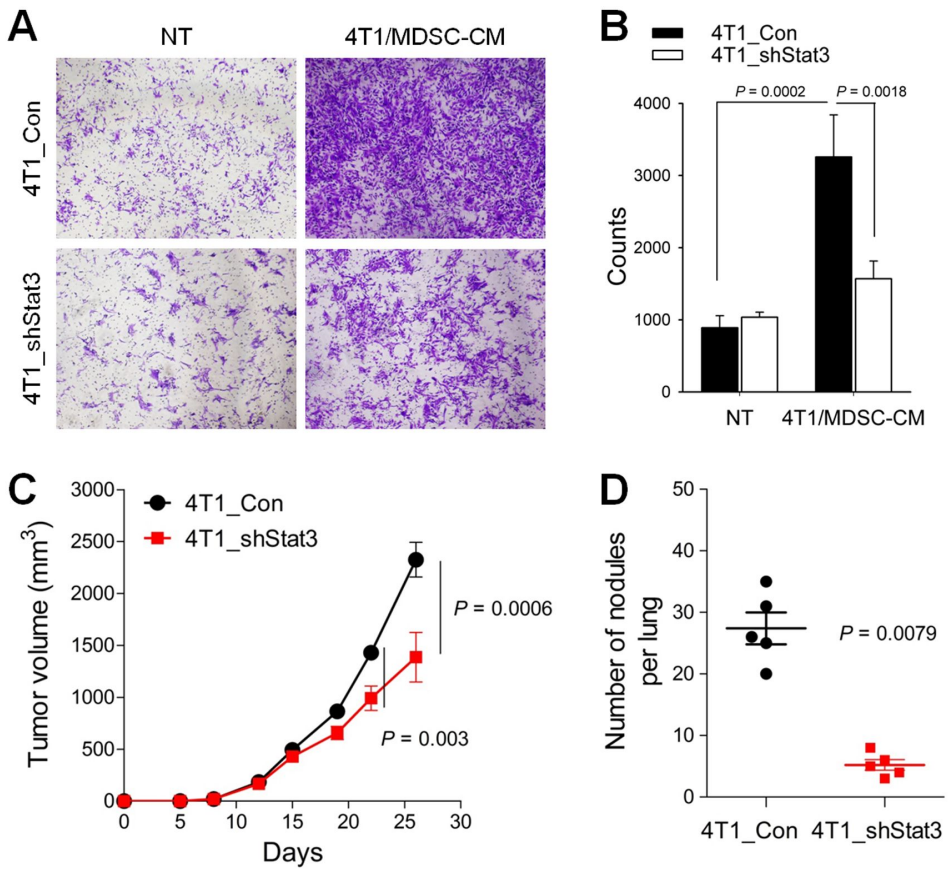
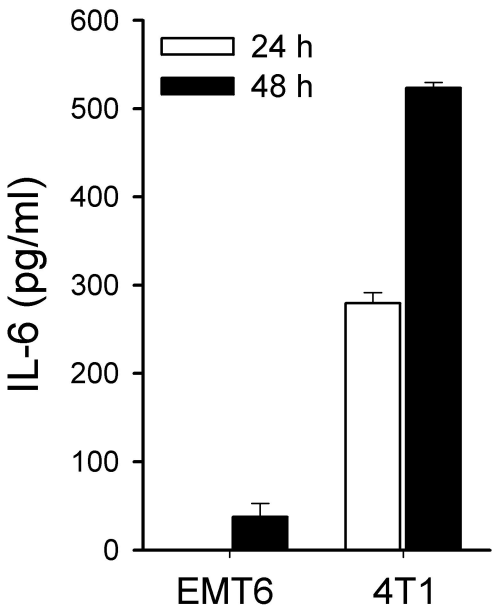
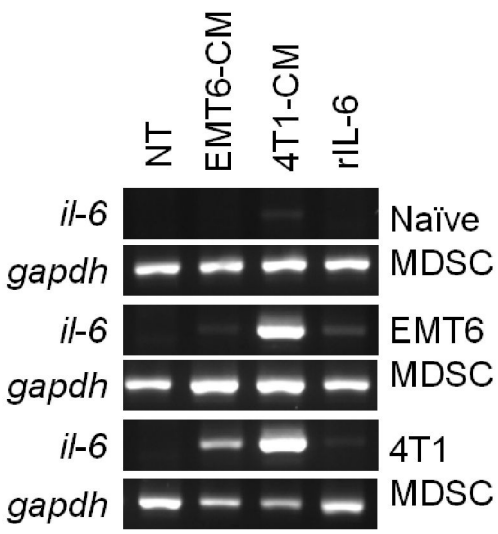


Figure 7. Stat3 phosphorylation in MDSC-activated cancer cells contributed to tumor invasiveness and distant lung metastasis. A-B, 4T1_Con and 4T1_shStat3 cells were allowed to invade through Matrigel for 18 h in the presence or absence of 4T1/MDSC-CM. A, invaded 4T1 cells (crystal violet). B, invaded cells were counted using ImageJ software. C-D, 4T1_Con and 4T1_shStat3 cells were injected into the mammary fat pads. C, primary tumor growth. D, numbers of metastatic masses in the lungs at 26 days. Values are the means ± SEM of each group (n=5).

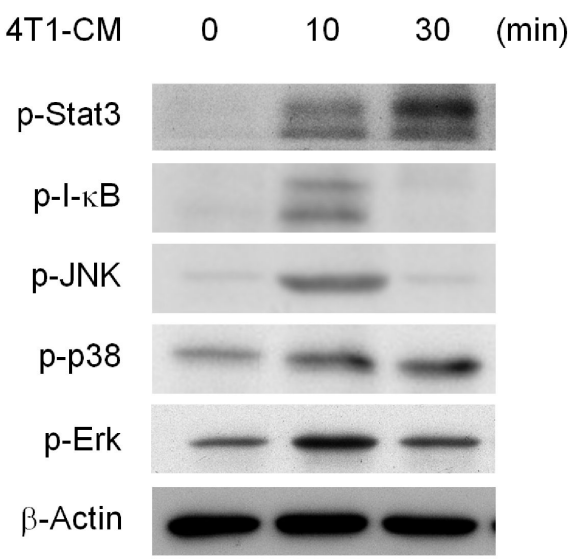
Supplementary Information



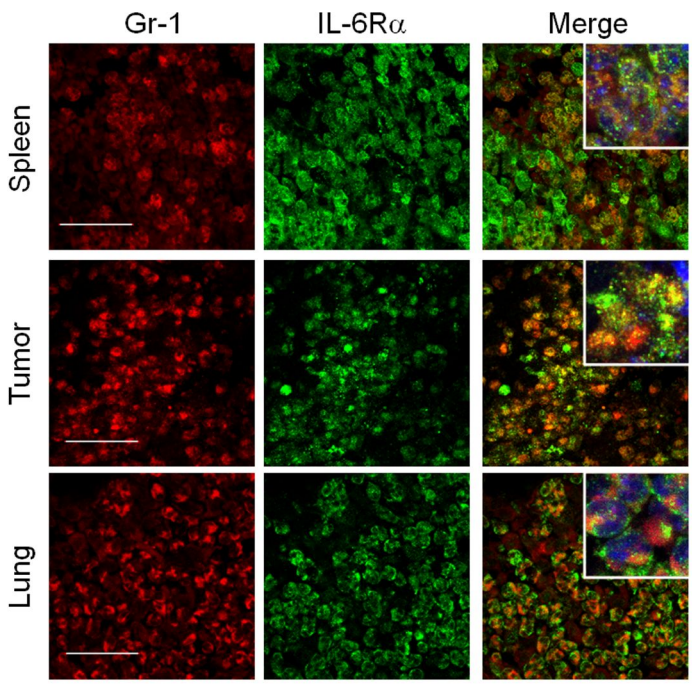
Supplementary Fig. S1. IL-6 levels in culture supernatants of cancer cells, as determined by ELISA.



Supplementary Fig. S2. Splenic MDSCs from naïve and tumor-bearing mice were treated with EMT6-CM, 4T1-CM, or recombinant IL-6 (1 ng/ml) for 6 h. IL-6 mRNA expression in 4T1-CM-treated splenic MDSCs was detected by RT-PCR.



Supplementary Fig. S3. Splenic MDSCs from 4T1 cell-bearing mice treated with 4T1-CM for the indicated periods of time. Signaling molecules were detected by Western blotting



Supplementary Fig. S4. Immunofluorescence staining of Gr-1 (red) and IL-6Ra (green) in spleen and tumor tissues from 4T1 cell-bearing mice. Scale bar=30 μm.

DISCUSSION

MDSCs have been suggested to constitute tumor-favoring microenvironments largely through their suppressive effects on innate and adaptive immunity and promotion of angiogenesis (8,9,13,14). In murine breast cancer cell model, 4T1 breast cancer cells recruited more MDSCs and metastasized more strongly compared to EMT6 cells, not only in syngeneic immunocompetent BALB/c mice, but also in immunodeficient NOG mice, in which T, B, and NK cells are defective (unpublished data) (25). This implies that MDSCs in 4T1 cell-bearing mice induced spontaneous distant metastasis of cancer cells independently of their suppressive effects on adaptive and natural-killer cell anti-tumorimmunity. Thus in this study, I provide evidence that MDSCs potentiated by metastasizing breast cancer cells directly enhance the aggressiveness of cancer cells though trans-signaling by upregulating both IL-6 and sIL-6Rα secretion in primary tumor sites and the metastatic lung.

Induced expression of IL-6 in EMT6 cancer cells caused recruitment of MDSCs in lymphoid organs, metastatic target organs, and primary tumor sites comparable to that caused by metastasizing 4T1 cancer cells, which implies that IL-6 or downstream signaling may be involved in this process. However, recruitment of MDSCs *perse* was not enough to guarantee that non-metastasizing breast cancer cells fully adopted metastatic capability. Transfer of splenic MDSCs from metastasizing 4T1 cell-bearing mice increased distant metastasis of non-metastasizing EMT6 cells but did not imbue them with the full metastatic capability of 4T1 cells. Based on the

above findings, I assume that additional factors from metastasizing breast cancer cells affected the homing of MDSCs into the tumor sites and increased the potency of recruited MDSCs (25,18,19,26). *In vitro* co-culture experiments showed that recruited MDSCs in the spleens of tumor-bearing mice required additional activation in the vicinity of metastasizing cancer cells, predominantly through contact-independent mechanisms. The outcome of activation of MDSC by metastasizing cancer cells *in vitro* can be summarized as exaggerated augmentation of IL-6 production by MDSCs (18,19). Immunofluorescence microscopy of different tissues from 4T1 cell-bearing mice indeed showed that MDSCs in the primary tumor mass and metastatic lung, but not in the spleen, expressed high level of IL-6. These findings suggest that recruited MDSCs may have different roles or function through different mechanisms depending on the recruited sites (lymphoid organs *versus* tumor sites, primary or metastatic) (27,28).

In contrast to the requirement for contact with metastasizing cancer cells for increased IL-6 production by MDSCs, the components necessary for increased soluble IL-6Rα production were increased in MDSCs in the remote sites of metastasizing tumor-bearing mice, but not those of non-metastasizing tumor-bearing mice. Expression levels of both IL-6Rα and the enzymes responsible for digesting the membrane form into soluble forms (Adam10 and Adam17) (22,23,29,30) were increased in the splenic MDSCs of 4T1 cell-bearing mice. Moreover, simple cultivation of splenic MDSCs from 4T1 cell-bearing mice increased the expression of soluble IL-6Rα compared to EMT6 cell-bearing mice. Thus at least four remote signals were secreted by metastasizing 4T1 cancer cells; these induced (i) recruitment of MDSCs to

various sites of tumor-bearing hosts, (ii) increased expression of IL-6Rα, (iii) increased expression of Adam family proteases, and (iv) highly increased expression of IL-6 by MDSCs (31,32,18,19,26). Further studies are needed to clarify the critical roles of the various mediators that may be involved in MDSC modulation.

The importance of IL-6 signaling in promoting tumorigenesis is well-documented, particularly for tumors associated with chronic inflammation such as colitis-associated colon cancer, pancreatic cancer, and hepatocellular carcinoma (33-36). In addition to these tumors, increased IL-6 signaling is preferentially found in basal-like breast cancers and high-grade tumors and is associated with a poor response to chemotherapy, increased distant metastasis in xenograft animal models, and decreased metastasis-free survival in patients (5,6). Thus, IL-6 signaling has been linked to tumor aggressiveness, including cancer stem cell phenotypes (5,6,37,38) and EMT phenotypes (39), drug resistance (40), and anoikis resistance; *i.e.*, contact-independent survival, which is required for travel through the vascular system (41). In addition to tumor-derived IL-6 autocrine signaling (5,6), paracrine IL-6 signaling with in tumor microenvironments has been highlighted recently. Mesenchymal stem cells constitute the cancer stem cell niche by providing IL-6 and CXCL7 (42,43). Paracrine IL-6signaling from tumor-infiltrating inflammatory cells is more important because these cells have a greater inflammatory cytokine secretion capacity, including IL-6 (44-46). The caveat for this paracrine signaling is that cancer cells should express sufficient receptor machineries to recognize the increased IL-6 supply from the microenvironments. In this way, aggressive cancer cells

exploited trans-signaling by inducing the expression of molecules responsible for production of soluble IL-6 receptors, similar to their non-cancerous counter parts, in which trans-signaling through additional soluble IL-6R signaling operates when additional signaling on top of baseline membrane IL-6R signaling is needed (47).

The site of MDSC function in the metastatic tumor-bearing mice requires further comment. In terms of sites of MDSC immunosuppressive activity, the available data are contradictory. Some authors suggest that lymphoid organs, including the liver, are the primary sites of MDSC accumulation and immunosuppression (27,48), while others emphasized effector sites such as inflammatory sites and tumors, but not lymphoid organs such as the spleen (28,49,26). In terms of MDSC function during the efferent phase of tumor metastasis and related angiogenesis, accumulation of MDSCs in the lung, a metastatic target organ, supported the effective engraftment of metastatic tumor cells at this site (15,19,50). Because the metastasis-promoting effects of MDSCs in this study occurred in the absence of adaptive immunity and natural-killer cell activity and there was no increase in IL-6 signaling in the spleen, MDSCs themselves must have directly increased tumor cell metastatic capability in the tumor sites, either primary tumors or metastases, but not in the lymphoid organs, affecting both the afferent and efferent phases of metastasis through exaggerated IL-6 trans-signaling.

REFERENCES

1. American Cancer Society. Breast Cancer Facts & Figures 2011−2012. <http://www.cancer.org/Research/CancerFactsFigures/BreastCancerFactsFigures/breast-cancer-facts-and-figures-2011−2012>

2. Hornberger J, Alvarado MD, Rebecca C, Gutierrez HR, Yu TM, Gradishar WJ. Clinical validity/utility, change in practice patterns, and economic implications of risk stratifiers to predict outcomes for early-stage breast cancer: a systematic review. *JNatlCancerInst*.2012;104(14):1068−1079.

3. El Saghir NS, Tfayli A, Hatoum HA, Nachef Z, Dinh P, Awada A. Treatment of metastatic breast cancer: state-of-the-art, subtypes and perspectives. *CritRevOncolHematol*.2011;80(3):433−449.

4. Fidler IJ. The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited. *NatRevCancer*.2003;3(6):453−458.

5. Oh K, et al. Transglutaminase 2 facilitates the distant hematogenous metastasis of breast cancer by modulating interleukin-6 in cancer cells. *BreastCancerRes*.2011;13(5):R96.

6. Sansone P, et al. IL-6 triggers malignant features in mammospheres from human ductal breast carcinoma and normal mammary gland. *JClinInvest*.2007;117(12):3988−4002.

7. Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. *NatRevImmunol*.2009;9(3):162−174.

8. Gabrilovich DI, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumours. *NatRevImmunol*.2012;12(4):253−268.

9. Marigo I, Dolcetti L, Serafini P, Zanovello P, Bronte V. Tumor-induced

tolerance and immune suppression by myeloid derived suppressor cells. *ImmunolRev*.2008;222(1):162−179.

10. Nagaraj S, Schrum AG, Cho HI, Celis E, Gabrilovich DI. Mechanism of T cell tolerance induced by myeloid-derived suppressor cells. *JImmunol*.2010;184(6):3106−3116.

11. Rodriguez PC, Ochoa AC. Arginine regulation by myeloid derived suppressor cells and tolerance in cancer: mechanisms and therapeutic perspectives. *ImmunolRev*.2008;222(1):180−191.

12. Tu S, et al. Overexpression of interleukin-1beta induces gastric inflammation and cancer and mobilizes myeloid-derived suppressor cells in mice. *CancerCell*.2008;14(5):408−419.

13. Montero AJ, Diaz-Montero CM, Kyriakopoulos CE, Bronte V, Mandruzzato S. Myeloid-derived suppressor cells in cancer patients: a clinical perspective. *JImmunother*.2012;35(2):107−115.

14. Sinha P, Clements VK, Ostrand-Rosenberg S. Interleukin-13-regulated M2 macrophages in combination with myeloid suppressor cells block immune surveillance against metastasis. *CancerRes*.2005;65(24):11743−11751.

15. Hiratsuka S, Watanabe A, Aburatani H, Maru Y. Tumour-mediated upregulation of chemoattractants and recruitment of myeloid cells predetermines lung metastasis. *NatCellBiol*.2006;8(12):1369−1375.

16. Kim S, et al. Carcinoma-produced factors activate myeloid cells through TLR2 to stimulate metastasis. *Nature*.2009;457(7225):102−106.

17. Yan HH, et al. Gr-1+CD11b+ myeloid cells tip the balance of immune protection to tumor promotion in the premetastatic lung. *CancerRes*.2010;70(15):6139−6149.

18. Chalmin F, et al. Membrane-associated Hsp72 from tumor-derived exosomes mediates STAT3-dependent immunosuppressive function of mouse and human myeloid-derived suppressor cells. *JClinInvest*.2010;120(2):457−471.

19. Deng J, et al. S1PR1-STAT3 signaling is crucial for myeloid cell colonization at future metastatic sites. *CancerCell*.2012;21(5):642−654.

20. Yang J, et al. Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. *Cell*.2004;117(7):927−939.

21. Chalaris A, Garbers C, Rabe B, Rose-John S, Scheller J. The soluble Interleukin 6 receptor: generation and role in inflammation and cancer. *EurJCellBiol*.2011;90(6−7):484−494.

22. Chalaris A, et al. Apoptosis is a natural stimulus of IL6R shedding and contributes to the proinflammatory trans-signaling function of neutrophils. *Blood*.2007;110(6):1748−1755.

23. Arndt PG, Strahan B, Wang Y, Long C, Horiuchi K, Walcheck B. Leukocyte ADAM17 regulates acute pulmonary inflammation. *PLoSOne*.2011;6(5):e19938.

24. Ito M, et al. NOD/SCID/gamma(c)(null) mouse: an excellent recipient mouse model for engraftment of human cells. *Blood*.2002;100(9):3175−3182.

25. van Deventer HW, et al. The inflammasome component NLRP3 impairs antitumor vaccine by enhancing the accumulation of tumor-associated myeloid-derived suppressor cells. *CancerRes*.2010;70(24):10161−10169.

26. Corzo CA, et al. HIF-1α regulates function and differentiation of myeloid-derived suppressor cells in the tumor microenvironment. *JExpMed*.2010;207(11):2439−2453.

27. Ilkovitch D, Lopez DM. The liver is a site for tumor-induced

myeloid-derived suppressor cell accumulation and immunosuppression. *CancerRes*.2009;69(13):5514−5521.

28. Haverkamp JM, Crist SA, Elzey BD, Cimen C, Ratliff TL. In vivo suppressive function of myeloid-derived suppressor cells is limited to the inflammatory site. *EurJImmunol*.2011;41(3):749-759.

29. Chalaris A, et al. ADAM17-mediated shedding of the IL6R induces cleavage of the membrane stub by gamma-secretase. *BiochimBiophysActa*.2010;1803(2):234−245.

30. Briso EM, Dienz O, Rincon M. Cutting edge: soluble IL-6R is produced by IL-6R ectodomain shedding in activated CD4 T cells. *JImmunol*.2008;180(11):7102−7106.

31. Gallina G, et al. Tumors induce a subset of inflammatory monocytes with immunosuppressive activity on CD8+ T cells. *JClinInvest*.2006;116(10):2777−2790.

32. Barsoum IB, et al. Hypoxia induces escape from innate immunity in cancer cells via increased expression of ADAM10: role of nitric oxide. *CancerRes*.2011;71(24):7433−7441.

33. Bollrath J, et al. gp130-mediated Stat3 activation in enterocytes regulates cell survival and cell-cycle progression during colitis-associated tumorigenesis. *CancerCell*.2009;15(2):91−102.

34. Grivennikov S, et al. IL-6 and Stat3 are required for survival of intestinal epithelial cells and development of colitis-associated cancer. *CancerCell*.2009;15(2):103−113.

35. Lang SA, et al. Targeting heat shock protein 90 in pancreatic cancer impairs insulin-like growth factor-I receptor signaling, disrupts an

interleukin-6/signal-transducer and activator of transcription 3/hypoxia-inducible factor-1alpha autocrine loop, and reduces orthotopic tumor growth. *ClinCancerRes*.2007;13(21):6459–6468.

36. Park EJ, et al. Dietary and genetic obesity promote liver inflammation and tumorigenesis by enhancing IL-6 and TNF expression. *Cell*.2010;140(2):197–208.

37. Korkaya H, Liu S, Wicha MS. Regulation of cancer stem cells by cytokine networks: attacking cancer's inflammatory roots. *ClinCancerRes*.2011;17(19):6125–6129.

38. Korkaya H, Liu S, Wicha MS. Breast cancer stem cells, cytokine networks, and the tumor microenvironment. *JClinInvest*.2011;121(10):3804–3809.

39. Yadav A, Kumar B, Datta J, Teknos TN, Kumar P. IL-6 promotes head and neck tumor metastasis by inducing epithelial-esenchymal transition via the JAK-STAT3-SNAIL signaling pathway. *MolCancerRes*.2011;9(12):1658–1667.

40. Wang Y, et al. Autocrine production of interleukin-6 confers cisplatin and paclitaxel resistance in ovarian cancer cells. *CancerLett*.2010;295(1):110–123.

41. Wang Y, et al. Interleukin-6 signaling regulates anchorage-independent growth, proliferation, adhesion and invasion in human ovarian cancer cells. *Cytokine*.2012;59(2):228–236.

42. Sasser AK, Sullivan NJ, Studebaker AW, Hendey LF, Axel AE, Hall BM. Interleukin-6 is a potent growth factor for ER-a-positive human breast cancer. *FASEBJ*.2007;21(13):3763–3770.

43. Liu S, et al. Breast cancer stem cells are regulated by mesenchymal stem cells through cytokine networks. *CancerRes*.2011;71(2):614−624.

44. Lesina M, et al. Stat3/Socs3 activation by IL-6 transsignaling promotes progression of pancreatic intraepithelial neoplasia and development of pancreatic cancer. *CancerCell*.2011;19(4):456−469.

45. Lo CW, et al. IL-6 trans-signaling in formation and progression of malignant ascites in ovarian cancer. *CancerRes*.2011;71(2):424−434.

46. Matsumoto S, et al. Essential roles of IL-6 trans-signaling in colonic epithelial cells, induced by the IL-6/soluble-IL-6 receptor derived from lamina propria macrophages, on the development of colitis-associated premalignant cancer in a murine model. *JImmunol*.2010;184(3):1543−1551.

47. Chen Q, et al. Fever-range thermal stress promotes lymphocyte trafficking across high endothelial venules via an interleukin 6 trans-signaling mechanism. *NatImmunol*.2006;7(12):1299−1308.

48. Watanabe S, et al. Tumor-induced CD11b+Gr-1+ myeloid cells suppress T cell sensitization in tumor-draining lymph nodes. *JImmunol*.2008;181(5):3291−3300.

49. Lesokhin AM, et al. Monocytic CCR2(+) myeloid-derived suppressor cells promote immune escape by limiting activated CD8 T-cell infiltration into the tumor microenvironment. *CancerRes*.2012;72(4):876−886.

50. Acharyya S, et al. A CXCL1 paracrine network links cancer chemoresistance and metastasis. *Cell*.2012;150(1):165−178.

국문 초록

암 미세 환경에서 특히 골수 유래의 골수기원억제세포 (MDSCs)와 암세포의 상호작용은 여러 가지 측면으로 종양 전이에 매우 중요합니다. 지난 연구들에서 골수기원억제세포는 암 세포에 유리한 환경을 조성한다고 이야기 되어 왔고, 이번 연구를 통해 골수기원억제세포가 암 세포의 aggressiveness를 증가시키고 자발성 전이를 이끈다는 것을 증명하였습니다. 이는 마우스의 모델을 이용하여 Interleukin-6를 높게 발현하는 유방암 세포가 in vivo상에서 더 많은 골수기원억제세포를 recruit하고, 또한 암 세포의 metastasizing capacity도 같이 증가한다는 사실을 증명했습니다. 전이성 종양유래인자는 골수기원억제세포의 IL-6 생산을 증가시키고 recruit된 골수기원억제세포는 tumor site와 전이된 기관에서 완전히 활성화되며, 또한 골수기원억제세포의 표면에 존재하는 Adam-family proteases의 발현을 도와 IL-6 수용체를 shedding시켜 IL-6의 트랜스 신호전달을 통해 유방암세포의 invasiveness와 distant metastasis을 촉진시킨다는 매커니즘을 확인하였습니다. 요약하자면 이번 연구를 통해 유방암세포와 골수기원억제세포는 synergistic mutual loop을 형성하여 feedback에 의해 골수기원억제세포가 직접적으로 유방암세포의 자발적인 전이를 이끄는 aggressiveness에 영향을 준다는 것을 밝혔습니다.

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주요어 : 골수기원억제세포 (MDSCs), 유방암 세포, IL-6 트랜스 신호전달, 암 전이

학번 : 2011-21912