

## Inhibition of the Interleukin-11-STAT3 Axis Attenuates Hypoxia-Induced Migration and Invasion in MDA-MB-231 Breast Cancer Cells

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Although interleukin-11 (IL-11) has been reported to be elevated in hypoxic tumors and has been associated with a poor prognosis in various cancers, little is known about its precise role in promoting metastasis in hypoxic tumors. In the present study, the molecular mechanism underlying the effects of IL-11 on MDA-MB-231 breast cancer cells migration and invasion in relation to metastasis under hypoxic conditions has been defined. Inhibition of IL-11 expression or function using small interfering RNA (siRNA) or a neutralizing antibody attenuated hypoxic MDA-MB-231 breast cancer cell migration and invasion through down-regulation of matrix metalloproteinases (MMPs) and activation of epithelial-to-mesenchymal transition (EMT) related gene expression. In addition, hypoxia-induced IL-11 increased STAT3 phosphorylation and STAT3 knockdown suppressed hypoxic MDA-MB-231 breast cancer cell invasion due to reduced MMP levels and reprogrammed EMT-related gene expression. These results suggest that one of the hypoxic metastasis pathways and the regulation of this pathway could be a potential target for novel cancer therapeutics.

**Key Words:** Hypoxia, Interleukin-11, Invasion, Migration, STAT3

### INTRODUCTION

The occurrence of metastasis, initiated by cancer cell migration and invasion, is the primary cause of an increased death rate and poor prognosis in patients with cancer [1]. It is becoming clear that intratumoral hypoxia, a common feature of the tumor microenvironment, is a critical mechanism that promotes metastasis and, thereby, increases tumor aggressiveness. Indeed, many genes related to cancer metastasis, such as epithelial-mesenchymal transition (EMT)-regulating factors (E-cadherin, N-cadherin, vimentin, and ZEB1), extracellular matrix remodeling enzymes (matrix metalloproteinase 1 (MMP-1), MMP-3, and lipoxygenases (LOX)), and membrane adhesion molecules (ANGPTL4, integrins, and caveolin-1) are increased in hypoxic tumors [2-5]. Although, hypoxia is an important factor in cancer metastasis, a better understanding of hypoxia underlying mechanisms is necessary to develop therapeutic strategies that target metastasis in hypoxic tumors.

Interleukin-11 (IL-11), a member of the IL-6 family of cytokines, plays an important role in tumor development and metastasis, which contributes to poor prognosis in patients with cancer [6]. Elevated IL-11 expression has frequently been observed in various solid tumors with an aggressive phenotype [6] and is known to be involved in metastasis in breast cancer [7,8], gastric carcinoma [9], and colorectal carcinoma [10]. Mechanistically, several signal transduction pathways such as oncogenic Ras activation regulate IL-11 expression levels [11]. IL-11 expression levels are also regulated by tumor microenvironment features, including hypoxia [12,13], suggesting that elevated IL-11, via tumor promoting signals, may play an important role during cancer progression. Indeed, the autocrine production of IL-11 is involved in tumorigenicity in hypoxic cancer cells through the phosphorylation of signal transducer and activator of transcription 1 (STAT1) [12]. However, whether the increased IL-11 observed in hypoxic cancer cells is a potential target for reduction of cancer metastasis has not been studied.

STAT3 is a transcription factor, which is phosphorylated and activated by numerous cytokines, growth factors, and oncogenic signaling pathways [14-16]. STAT3 signaling increases the expression of genes involved in cell survival, migration, invasion, and angiogenesis in cancer, which is

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**ABBREVIATIONS:** BSA, bovine serum albumin; DMEM, Dulbecco's Modified Eagle Medium; EMT, epithelial-to-mesenchymal transition; FBS, fetal bovine serum; HIF, hypoxia inducible factor; IL, Interleukin; LOX, lipoxygenase; MMP, matrix metalloproteinase; qRT-PCR, quantitative real time PCR; siRNA, small interfering RNA; STAT, signal transducer and activator of transcription.

associated with a poor prognosis in patients with cancer. In particular, transcriptionally activated STAT3 promotes cancer cell migration and invasion through the elevation of *MMP-2*, *MMP-9*, and EMT-related gene expression levels, which induces extracellular matrix remodeling and mesenchymal properties during cancer metastasis [14,17]. STAT3 activation is associated with metastasis in various types of tumors, including colorectal carcinoma [17], renal cell carcinoma [18,19], and malignant melanoma [20]. Although STAT3 has been reported to be phosphorylated and activated in hypoxic cancer cells [21], the stimuli involved in its activation are still poorly understood.

Based on this information, we hypothesized that IL-11 expression induced by hypoxia may contribute to tumor cell migration and invasion via the STAT3 signaling pathway. In addition to confirming our hypothesis, we demonstrated that the inhibition of IL-11 or STAT3 attenuates invasiveness of hypoxic cancer cells by down-regulating *MMP-2*, *MMP-9*, and EMT-related gene expression. These results suggest that the hypoxia-IL-11/STAT3 pathway may contribute to cancer metastasis, resulting in a poor prognosis for patients with cancer, and could be a potential target for cancer treatment.

## METHODS

### Cell culture and transfection

MDA-MB-231 (human mammary carcinoma), HCT116 (human colorectal carcinoma), H1299 (human non-small lung carcinoma), A375 (malignant melanoma), and HepG2 (human hepatocellular carcinoma) cell lines were obtained from the ATCC (Manassas, VA, USA). Cancer cells were cultured in high glucose DMEM containing 10% fetal bovine serum (FBS) and antibiotics in the presence of 20% O<sub>2</sub>/5% CO<sub>2</sub> (normoxia) or 1% O<sub>2</sub>/5% CO<sub>2</sub> (hypoxia). Small interfering RNAs against human IL-11 or STAT3 was transiently transfected into cancer cells using Lipofectamine 2000 (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions, and transfected cells were then incubated for 48 h before being used in experiments.

### Small interfering RNAs (siRNAs) and reagents

Small interfering RNAs against IL-11 (sc-39636) or STAT3 (sc-29493) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Recombinant human IL-11 and IL-11 neutralizing antibody were purchased from R&D Systems (Minneapolis, MN, USA). Recombinant human IL-11 was reconstituted in PBS containing 0.5% bovine serum albumin (BSA) before being used in experiments.

### Immunoblotting and antibodies

Total proteins were extracted in a buffer containing 1% IGEPAL, 150 mM NaCl, 20 mM HEPES (pH 7.9), 10 mM NaF, 0.1 mM EDTA and protease inhibitor cocktail. Total proteins were electrophoresed on SDS-polyacrylamide gels and transferred to Immobilon-P membrane (Millipore, Billerica, MA, USA). The membranes were then incubated with primary antibodies overnight at 4°C, incubated with HRP-conjugated secondary antibodies for 1 h at room temperature, and visualized using an Enhanced Chemiluminescence Detection kit (Pierce, Rockford, IL, USA). Antibodies

recognizing STAT3 and pTyr-STAT3 (Tyr705) were purchased from Cell Signaling Technology (Danvers, MA, USA).

### Quantitative real-time PCR (qRT-PCR)

Total RNA was isolated with Trizol (Life Technologies), and used for cDNA synthesis using high capacity cDNA reverse transcription kit (Life Technologies). qRT-PCR was performed using SYBR Green PCR Master Mix (Life Technologies). Experimental Ct values were normalized to 36B4, and mRNA expression was calculated relative to 36B4 expression. The primer sequences were as follows: *IL-11*; 5'-CTCAGCACGACCAGGACC-3' (right) and 5'-GGACAGGGAAGGGTTAAAGG-3' (left), *CAIX*; 5'-CAGCAACTGCTCATAGGCAC-3' (right) and 5'-CTTTGCCAGAGTTGACGAGG-3' (left), *GLUT1*; 5'-GGCATTGATGACTC-CAGTGTT-3' (right) and 5'-ATGGAGCCCAGCAGCAA-3' (left), *VEGF*; 5'-AGCTGCGCTGATAGACATCC-3' (right) and 5'-CTACCTCCACCATGCCAAGT-3' (left), *LOX*; 5'-TG-CAGTCTATGTCTGCACC-3' (right) and 5'-CTATGGCTACCACAGGCGAT-3' (left), *MMP-2*; 5'-GGAAAGCCAGGATCCATTTT-3' (right) and 5'-ATGCCGCCTTTAACTGGAG-3' (left), *MMP-3*; 5'-CAATTTTCATGAGCAGCAACG-3' (right) and 5'-AGGGATTAATGGAGATGCC-3' (left), *MMP-9*; 5'-TTGGTCCACCTGGTTCAACT-3' (right) and 5'-ACGACGTCTTCCAGTACCGA-3' (left), E-cadherin; 5'-GACCGGTG-CAATCTTCAAA-3' (right) and 5'-TTGACGCCGAGAGCTCAC-3' (left), N-cadherin; 5'-CCACCTTAAAATCTGCAGGC-3' (right) and 5'-GTGCATGAAGGACAGCCTCT-3' (left), vimentin; 5'-ATTCCACTTTGCGTTCAAGG-3' (right) and 5'-CTTCAGAGAGAGGAAGCCGA-3' (left), fibronectin; 5'-ACCTCGGTGTTGTAAGGTGG-3' (right) and 5'-CCATAAAGGCAACCAAGAG-3' (left).

### Conditioned media preparation and secreted IL-11 measurement

To prepare conditioned media, MDA-MB-231 cells were seeded into 100 mm tissue culture dishes and allowed to reach 60% confluence. The medium was then replaced by serum-free Dulbecco's Modified Eagle Media (DMEM) and the cells were cultured under normoxic or hypoxic conditions for 72 h. Next, conditioned medium fractions were collected and centrifuged at 13,000 g at 4°C for 10 min, and concentrated 10 times using centrifugal filter unit (Millipore). The concentrated conditioned medium was used freshly for *in vitro* cell migration, invasion assay, or for secreted IL-11 protein measurement. IL-11 protein levels were measured using the human IL-11 Quantikine ELISA kit purchased from R&D Systems according to the manufacturer's instructions. IL-11 level was normalized to the total protein level in each sample.

### In vitro migration and invasion assay

Cancer cell migration and invasion assays were performed using Transwell chambers purchased from Sigma-Aldrich (St. Louis, MO, USA). For the migration assay, cells in 0.1 ml of FBS-free medium were seeded in the upper chamber and the lower chamber was filled with complete culture medium as a chemotactic agent, and cells were then incubated for 6 h. For invasion assay, cells (3×10<sup>3</sup>) in 0.1 ml of FBS-free medium were seeded in the upper chamber of an 8-μm Matrigel coated chamber (BD Biosciences, San

Jose, CA, USA) and incubated for 24 h. Cells that migrated and invaded were then stained with hematoxylin and eosin. The filter membranes, containing the migrating cells, were then placed on a glass slide and analyzed using an Olympus IX51 microscope (Olympus, Tokyo, Japan).

### Statistical analysis

The data are presented as means and standard deviations. All the experiments were analyzed using Prism 5.0 software (GraphPad Software, Inc., San Diego, CA, USA), and Student *t*-test was used to compare the differences between the groups, with  $p < 0.05$  considered to be statistically significant.

## RESULTS

### Hypoxia induces IL-11 mRNA and protein expression

To understand the functional role of IL-11 in hypoxic cancer cells, we confirmed whether IL-11 was elevated in vari-

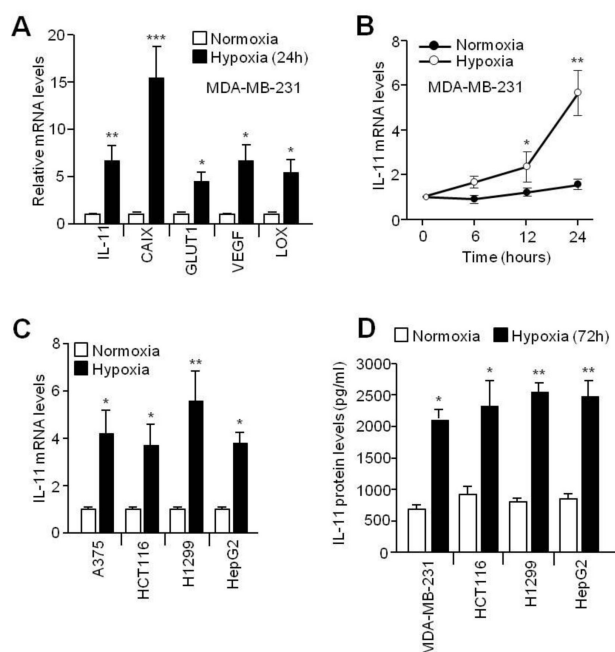
ous cancer cell lines. We found that IL-11 as well as hypoxia inducible factor (HIF)-1 $\alpha$  target genes were strongly increased in hypoxic MDA-MB-231 cells (Fig. 1A). To examine the time-dependent effects of hypoxia on IL-11 expression, MDA-MB-231 cells were incubated under hypoxic conditions for specific times. Fig. 1B shows that *IL-11* mRNA levels increased under hypoxic conditions at the 24-h time point. Because cancer is heterogeneous in nature, different types of cancer cell lines were tested. As expected, *IL-11* mRNA levels strongly increased in several cancer cell lines under hypoxic conditions (Fig. 1C). To examine the effect of hypoxia on the production of IL-11, we measured IL-11 protein levels in the culture medium. Consistent with the mRNA levels, levels of *IL-11* protein, which is produced and secreted from cancer cells, increased under hypoxic conditions (Fig. 1D). These results suggest that IL-11 production, both at the transcriptional and protein level, increases under hypoxic conditions in several cancer cell lines.

### IL-11 contributes to cancer cell motility and invasion under hypoxic conditions

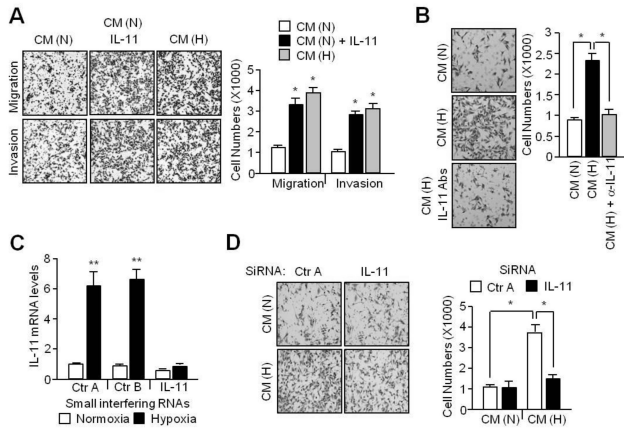
Because IL-11 is thought to be involved in tumor metastasis [8-10], we hypothesized that hypoxia-induced IL-11 may contribute to cancer cell migration and invasion. To prove this hypothesis, we tested IL-11 effect on cell migration and invasion in hypoxic cancer cells. Cell migration and invasion strongly increased when the cells were incubated in the presence of conditioned medium collected and 10 times concentrated from cancer cells cultured under hypoxic conditions (hypoxia-conditioned medium; Fig. 2A). In addition, we confirmed that human recombinant IL-11 treatment increased cancer cell migration and invasion under normoxia condition (Fig. 2A). To examine whether hypoxia-induced IL-11 expression contributed to the hypoxic cancer cell invasiveness, we tested the effect of an IL-11 neutralizing antibody on cancer cell invasiveness in the presence of hypoxia-conditioned medium. Fig. 2B shows that IL-11 sequestration by the neutralizing antibody significantly inhibited cancer cell invasion, originally increased by incubation in presence of hypoxia-conditioned medium. IL-11 silencing using a siRNA was performed to determine the role of IL-11 during cell invasion in hypoxic cancer cells. Fig. 2C shows that IL-11 siRNA completely suppressed IL-11 expression levels in hypoxic cancer cells. Consistent with the effect of the IL-11 neutralizing antibody (Fig. 2B), siRNA-mediated IL-11 inhibition also dramatically suppressed the cancer cell invasion induced by culture of the cells in the presence of hypoxia-conditioned medium (Fig. 2D). These results suggest that hypoxia-induced IL-11 expression is required for cancer cell migration and invasion under hypoxic conditions.

### STAT3 phosphorylation by hypoxia-induced IL-11 transcriptionally activates MMPs and alters EMT-related gene expression

To determine the effective concentration of IL-11 required to induce STAT3 phosphorylation, MDA-MB-231 breast cancer cells were stimulated with increasing concentration of IL-11. Fig. 3A indicates that 2 ng/ml of recombinant IL-11 was sufficient to increase STAT3 phosphorylation. Because IL-11 has been shown to be strongly correlated with increased STAT3 activation in human cancers

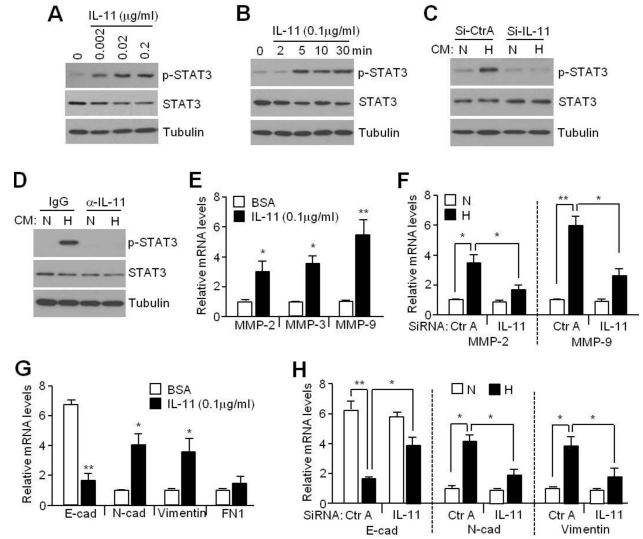


**Fig. 1.** IL-11 increases in response to hypoxia. (A) MDA-MB-231 cells were incubated under hypoxic (1% O<sub>2</sub>) or normoxic (20% O<sub>2</sub>) conditions for 24 h and indicated mRNA levels were analyzed by qRT-PCR. Values represent mean $\pm$ SD of three independent experiments performed in triplicate; \* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $p < 0.001$ . (B) MDA-MB-231 cells were incubated under hypoxic or normoxic conditions for the indicated time. *IL-11* mRNA levels were analyzed by qRT-PCR. Values represent mean $\pm$ SD of three independent experiments performed in triplicate; \* $p < 0.05$  and \*\* $p < 0.01$ . (C) Indicated cells were incubated under hypoxic or normoxic conditions for 24 h. Values represent mean $\pm$ SD of three independent experiments performed in triplicate; \* $p < 0.05$  and \*\* $p < 0.01$ . (D) Cells were incubated under hypoxic or normoxic conditions for 72 h. The cultured medium was collected to analyze the concentrations of secreted IL-11 protein as described in materials and methods. Values represent mean $\pm$ SD of three independent experiments performed in triplicate; \* $p < 0.05$  and \*\* $p < 0.01$ .



**Fig. 2.** IL-11 is essential for the hypoxia-induced cancer cell migration and invasion. (A) MDA-MB-231 cells were seeded in the upper side of Transwell chambers and the lower part of the chamber was filled with conditioned medium collected from MDA-MB-231 cells exposed to normoxia or hypoxia for 24 h. Photographs of migrated or invaded cells were captured at 20 $\times$  magnification under a microscope (left). A quantitative analysis of the cell numbers is shown (right). Values represent mean $\pm$ SD of three independent experiments performed in triplicate; \* $p$ <0.05. (B) Conditioned medium from MDA-MB-231 cells was pre-incubated with normal serum or IL-11 neutralizing antibody (0.5  $\mu$ g/ml) for 1 h prior to the experiment. MDA-MB-231 cells were seeded in Matrigel-coated Transwell chamber and incubated with conditioned medium in the presence or absence of the IL-11 neutralizing antibody for 16 h. Values represent mean $\pm$ SD of three independent experiments performed in triplicate; \* $p$ <0.05. (C) Transfected MDA-MB-231 cells were incubated under normoxic or hypoxic conditions for 24 h. *IL-11* mRNA levels were analyzed by qRT-PCR. Values represent mean $\pm$ SD of three independent experiments performed in triplicate; \*\* $p$ <0.01. (D) Conditioned medium was collected from MDA-MB-231 cells transfected with a siRNA against control (40 nM) or IL-11 (40 nM) under normoxic or hypoxic conditions. MDA-MB-231 cells were seeded in Matrigel-coated Transwell chamber and incubated in the presence of conditioned medium for 16 h. Values represent mean $\pm$ SD of three independent experiments performed in triplicate; \* $p$ <0.05.

[7,12,13], we tested the effect of IL-11 produced by hypoxic cancer cells on STAT3 phosphorylation and activation. STAT3 phosphorylation was strongly increased by human recombinant IL-11 treatment (Fig. 3B) and by incubation of the cells with conditioned medium derived from hypoxic cancer cells (Fig. 3C). Moreover, hypoxia-conditioned medium, collected from IL-11 suppressed cancer cells, did not increase STAT3 phosphorylation (Fig. 3C). Consistent with this finding, STAT3 phosphorylation increased by hypoxia-conditioned medium treatment was completely abolished by the IL-11 neutralizing antibody (Fig. 3D), suggesting that IL-11 plays an essential role in hypoxia-induced STAT3 phosphorylation. STAT3 activation has been shown to promote tumor progression by transcriptionally regulating the expression of genes related to the extracellular matrix remodeling, such as *MMP-2* and *MMP-9* [14]. Here, we examined the effect of IL-11 on MMP mRNA expression. Fig. 3E shows that human recombinant IL-11 strongly increased *MMP-2*, *MMP-3*, and *MMP-9* mRNA levels in highly metastatic breast cancer cells. To examine whether IL-11 is required for MMP expression in hypoxic cancer cells, we measured *MMP-2* and *MMP-9* mRNA levels in IL-11 sup-

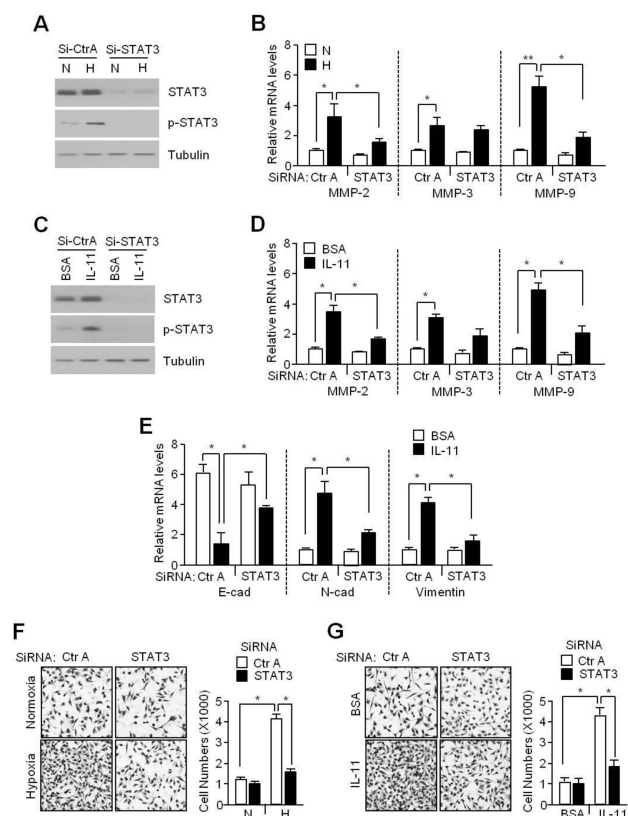


**Fig. 3.** Hypoxia-induced IL-11 leads to STAT3 phosphorylation. (A) MDA-MB-231 cells were incubated with human recombinant IL-11 for the indicated concentration, and (B) time. Indicated protein levels were analyzed by western blot analysis. (C) Conditioned medium was collected from MDA-MB-231 cells, which had been transfected with control or IL-11 siRNA and incubated under normoxic or hypoxic conditions. Non-transfected MDA-MB-231 cells were incubated with conditioned medium for 30 min as indicated. (D) MDA-MB-231 cells were incubated with conditioned medium, which had been pre-incubated with normal serum or the IL-11 neutralizing antibody (0.5  $\mu$ g/ml) for 30 min. (E) MDA-MB-231 cells were incubated with recombinant IL-11 for 24 h and mRNA expression levels were then measured by qRT-PCR. Values represent mean $\pm$ SD of three independent experiments performed in triplicate; \* $p$ <0.05 and \*\* $p$ <0.01. (F) MDA-MB-231 cells were transfected with the indicated siRNAs and incubated under normoxic or hypoxic conditions for 24 h. Values represent mean $\pm$ SD of three independent experiments performed in triplicate; \* $p$ <0.05 and \*\* $p$ <0.01. (G) Indicated mRNA levels were measured by qRT-PCR. Values represent mean $\pm$ SD of three independent experiments performed in triplicate; \* $p$ <0.05 and \*\* $p$ <0.01. (H) MDA-MB-231 cells were transfected with siRNA against IL-11 and incubated under normoxic or hypoxic conditions for 24 h. Values represent mean $\pm$ SD of three independent experiments performed in triplicate; \* $p$ <0.05 and \*\* $p$ <0.01.

pressed cells. We here found that IL-11 suppression decreases hypoxia-induced *MMP-2* and *MMP-9* expression (Fig. 3F). Because hypoxia leads to mesenchymal properties through EMT, causing metastasis in several cancer types, we examined whether IL-11 is involved in hypoxia-induced EMT. Fig. 3G shows that recombinant IL-11 strongly reduced epithelial marker, E-cadherin, and increased mesenchymal markers, N-cadherin, and vimentin in MDA-MB-231 breast cancer cells. We additionally found that IL-11 suppression altered EMT-related gene expression under hypoxia (Fig. 3H), indicating that IL-11 is required for EMT in hypoxic cancer cells.

#### **STAT3 suppression results in a reduction of cancer cell invasion under hypoxia and IL-11 treatment**

STAT3 silencing using a siRNA significantly decreased *MMP-2* and *MMP-9*, but not *MMP-3* mRNA expression in cancer cells cultured under hypoxic conditions (Fig. 4A and



**Fig. 4.** STAT3 silencing attenuates hypoxia and IL-11-induced cancer cell invasion. (A) MDA-MB-231 cells were transfected with a siRNA against STAT3 and incubated under normoxic or hypoxic conditions for 24 h. Protein levels were analyzed by western blot. (B) Gene expression levels were analyzed by qRT-PCR. Values represent mean $\pm$ SD of three independent experiments performed in triplicate; \* $p$ <0.05 and \*\* $p$ <0.01. (C) Cells were incubated with BSA or recombinant IL-11 (0.1  $\mu$ g/ml) for 24 h. (D, E) Gene expression levels were analyzed by qRT-PCR. Values represent mean $\pm$ SD of three independent experiments performed in triplicate; \* $p$ <0.05. (F) Transfected MDA-MB-231 cells with a control or a STAT3 siRNA were seeded into Matrigel-coated Transwell chamber and incubated under normoxic or hypoxic conditions for 24 h. Values represent mean $\pm$ SD of three independent experiments performed in triplicate; \* $p$ <0.05. (G) Transfected MDA-MB-231 cells with a control or a STAT3 siRNA were seeded in Matrigel-coated Transwell chamber and incubated with BSA or IL-11 for 24 h. Values represent mean $\pm$ SD of three independent experiments performed in triplicate; \* $p$ <0.05.

B) and in IL-11 treated cancer cells (Fig. 4C and D). Additionally, we tested whether the IL-11-STAT3 pathway caused EMT under hypoxia. Fig. 4E shows that STAT3 silencing significantly increased E-cadherin, but decreased N-cadherin and vimentin, indicating that IL-11 stimulates EMT through the STAT3 pathway in MDA-MB-231 breast cancer cells. Consistently, cell invasion was completely abolished by STAT3 inhibition in cancer cells cultured under hypoxic conditions (Fig. 4F) and in IL-11-treated MDA-MB-231 breast cancer cells (Fig. 4G).

## DISCUSSION

In order for cancer cells to survive under hypoxic conditions, they initiate numerous adaptive processes such as angiogenesis, migration, invasion, and metabolic reprogramming [3]. These adaptation mechanisms result in resistance to radiation therapy and chemotherapy and tumor metastasis in hypoxic tumors, which contributes to the poor prognosis of patients with cancer. In fact, metastasis-promoting factors, such as the EMT-regulating factors, Twist 1, E-cadherin, N-cadherin, and vimentin, the extracellular matrix remodeling enzymes, MMP-1, MMP-3, and LOX, and the membrane adhesion molecules, ANGPTL4, integrins, and caveolin-1, are highly expressed in hypoxic tumors [2,3]. Although it is becoming clear that hypoxia causes tumor metastasis via the up-regulation of various genes, further studies are necessary to explain metastasis-promoting mechanisms.

Inflammatory cytokines produced in hypoxic tumors have been reported to cause tumorigenicity, angiogenesis, and metastasis [22]. As such, IL-11 is thought to be a potential target for cancer treatment. Indeed, Onnis et al. recently showed that IL-11 autocrine production mediates tumor development in hypoxic cancer cells using *in vitro* and *in vivo* models [12]. In addition, several reports have shown that IL-11 promotes cancer metastasis via the STAT3 pathway in breast, chondrosarcoma, colorectal cancer, and endometrial cancer [8,10,23,24]. However, the functional role of hypoxia-induced IL-11 for cancer metastasis in hypoxic cancer cells remains to be elucidated. In the present study, we showed that IL-11 autocrine production plays an important role in cancer cell motility and invasiveness under hypoxic conditions. Our results show that IL-11 mRNA and protein levels strongly increased in response to hypoxia in several cancer cell lines such as human breast adenocarcinoma (MDA-MB-231), malignant melanoma (A375), human colorectal carcinoma (HCT116), human non-small cell lung carcinoma (H1299), and human hepatocellular liver carcinoma (HepG2). Additionally, we found that conditioned medium derived from hypoxic cancer cells also stimulates cancer cell motility and invasiveness, and that this effect is blocked by an IL-11-neutralizing antibody or siRNA, suggesting that IL-11 autocrine production promotes cancer cell motility and invasiveness in response to hypoxia. Together, our results indicate that IL-11 autocrine production in hypoxic tumors could be used as a therapeutic strategy to prevent cancer metastasis. One exciting finding in the present study is that the autocrine hypoxia-induced IL-11 production significantly alters EMT-related gene expression such as E-cadherin, N-cadherin, and vimentin, suggesting that IL-11-STAT3 pathway may be involved in hypoxic tumor EMT.

Although STAT3 phosphorylation, which is associated with cancer progression, has been shown to be increased in MDA-MB-231 breast cancer cells under hypoxic conditions [21], the molecular mechanism by which STAT3 is phosphorylated and activated is not clearly understood. We demonstrated that IL-11 is an important mediator of STAT3 phosphorylation in hypoxic cancer cells. Indeed, our results show that conditioned medium derived from hypoxic cancer cells strongly increased STAT3 phosphorylation, and this STAT3 phosphorylation is blocked by an IL-11-neutralizing antibody or siRNA. Consistent with this finding, treatment of the cells with the IL-11 neutralizing antibody or siRNA also significantly altered *MMP-2*, *MMP-9*, and

EMT-related gene expression. These genes are potential targets induced by activated STAT3 in hypoxic cancer cells. In addition, we found that STAT3 silencing using a siRNA significantly decreased the invasiveness of hypoxic cancer cells, suggesting that the IL-11/STAT3 pathway is required for cancer cell invasiveness under hypoxic conditions.

Although, it is becoming clear that tumor inflammation, which involves communication between cancer and immune cells, causes cancer metastasis, the mechanisms underlying the effects of tumor inflammation on tumor progression are poorly understood. In addition, the gain of mesenchymal properties, referred as the "EMT," has become prominently implicated as a mean by which transformed epithelial cells can acquire the ability to invade, resist apoptosis, and disseminate [1]. Here, our results suggest that the IL-11-STAT3 pathway could be activated by tumor inflammation, and this signaling pathway may be involved in cancer metastasis by regulating remodeling of the extracellular matrix and EMT.

In summary, our results highlight the critical role of IL-11 in cancer cell migration and invasion under hypoxic conditions. These findings may help us understanding the molecular mechanism of cancer metastasis in hypoxic tumors and may lead us to develop effective therapeutic strategies for improving cancer treatment in the future.

## ACKNOWLEDGEMENTS

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## REFERENCES

- Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011;144:646-674.
- Tsai YP, Wu KJ. Hypoxia-regulated target genes implicated in tumor metastasis. *J Biomed Sci*. 2012;19:102.
- Semenza GL. Hypoxia-inducible factors: mediators of cancer progression and targets for cancer therapy. *Trends Pharmacol Sci*. 2012;33:207-214.
- Chan DA, Giaccia AJ. Hypoxia, gene expression, and metastasis. *Cancer Metastasis Rev*. 2007;26:333-339.
- Bertout JA, Patel SA, Simon MC. The impact of O<sub>2</sub> availability on human cancer. *Nat Rev Cancer*. 2008;8:967-975.
- Taniguchi K, Karin M. IL-6 and related cytokines as the critical lynchpins between inflammation and cancer. *Semin Immunol*. 2014;26:54-74.
- Ren L, Wang X, Dong Z, Liu J, Zhang S. Bone metastasis from breast cancer involves elevated IL-11 expression and the gp130/STAT3 pathway. *Med Oncol*. 2013;30:634.
- Kang Y, He W, Tulley S, Gupta GP, Serganova I, Chen CR, Manova-Todorova K, Blasberg R, Gerald WL, Massagué J. Breast cancer bone metastasis mediated by the Smad tumor suppressor pathway. *Proc Natl Acad Sci U S A*. 2005;102:13909-13914.
- Tsai CY, Wang CS, Tsai MM, Chi HC, Cheng WL, Tseng YH, Chen CY, Lin CD, Wu JI, Wang LH, Lin KH. Interleukin-32 increases human gastric cancer cell invasion associated with tumor progression and metastasis. *Clin Cancer Res*. 2014; 20:2276-2288.
- Calon A, Espinet E, Palomo-Ponce S, Tauriello DV, Iglesias M, Céspedes MV, Sevillano M, Nadal C, Jung P, Zhang XH, Byrom D, Riera A, Rossell D, Mangués R, Massagué J, Sancho E, Batlle E. Dependency of colorectal cancer on a TGF- $\beta$ -driven program in stromal cells for metastasis initiation. *Cancer Cell*. 2012;22:571-584.
- Shin SY, Choi C, Lee HG, Lim Y, Lee YH. Transcriptional regulation of the interleukin-11 gene by oncogenic Ras. *Carcinogenesis*. 2012;33:2467-2476.
- Onnis B, Fer N, Rapisarda A, Perez VS, Melillo G. Autocrine production of IL-11 mediates tumorigenicity in hypoxic cancer cells. *J Clin Invest*. 2013;123:1615-1629.
- Gao YB, Xiang ZL, Zhou LY, Wu ZF, Fan J, Zeng HY, Zeng ZC. Enhanced production of CTGF and IL-11 from highly metastatic hepatoma cells under hypoxic conditions: an implication of hepatocellular carcinoma metastasis to bone. *J Cancer Res Clin Oncol*. 2013;139:669-679.
- Huang S. Regulation of metastases by signal transducer and activator of transcription 3 signaling pathway: clinical implications. *Clin Cancer Res*. 2007;13:1362-1366.
- Bromberg J. Stat proteins and oncogenesis. *J Clin Invest*. 2002;109:1139-1142.
- Seo IA, Lee HK, Shin YK, Lee SH, Seo SY, Park JW, Park HT. Janus Kinase 2 Inhibitor AG490 inhibits the STAT3 signaling pathway by suppressing protein translation of gp130. *Korean J Physiol Pharmacol*. 2009;13:131-138.
- Rokavec M, Öner MG, Li H, Jackstadt R, Jiang L, Lodygin D, Kaller M, Horst D, Ziegler PK, Schwitalla S, Slotta-Huspenina J, Bader FG, Greten FR, Hermeking H. IL-6R/STAT3/miR-34a feedback loop promotes EMT-mediated colorectal cancer invasion and metastasis. *J Clin Invest*. 2014;124:1853-1867.
- Ito N, Eto M, Nakamura E, Takahashi A, Tsukamoto T, Toma H, Nakazawa H, Hirao Y, Uemura H, Kagawa S, Kanayama H, Nose Y, Kinukawa N, Nakamura T, Jinnai N, Seki T, Takamatsu M, Masui Y, Naito S, Ogawa O. STAT3 polymorphism predicts interferon- $\alpha$  response in patients with metastatic renal cell carcinoma. *J Clin Oncol*. 2007;25:2785-2791.
- Fang Z, Tang Y, Fang J, Zhou Z, Xing Z, Guo Z, Guo X, Wang W, Jiao W, Xu Z, Liu Z. Simvastatin inhibits renal cancer cell growth and metastasis via AKT/mTOR, ERK and JAK2/STAT3 pathway. *PLoS One*. 2013;8:e62823.
- Steder M, Alla V, Meier C, Spitschak A, Pahnke J, Fürst K, Kowtharapu BS, Engelmann D, Petigk J, Egberts F, Schäd-Trcka SG, Gross G, Nettelbeck DM, Niemetz A, Pützer BM. DNP73 exerts function in metastasis initiation by disconnecting the inhibitory role of EPLIN on IGF1R-AKT/STAT3 signaling. *Cancer Cell*. 2013;24:512-527.
- Pawlus MR, Wang L, Hu CJ. STAT3 and HIF1 $\alpha$  cooperatively activate HIF1 target genes in MDA-MB-231 and RCC4 cells. *Oncogene*. 2014;33:1670-1679.
- Mamlouk S, Wielockx B. Hypoxia-inducible factors as key regulators of tumor inflammation. *Int J Cancer*. 2013;132:2721-2729.
- Li TM, Wu CM, Huang HC, Chou PC, Fong YC, Tang CH. Interleukin-11 increases cell motility and up-regulates intercellular adhesion molecule-1 expression in human chondrosarcoma cells. *J Cell Biochem*. 2012;113:3353-3362.
- Lay V, Yap J, Sonderegger S, Dimitriadis E. Interleukin 11 regulates endometrial cancer cell adhesion and migration via STAT3. *Int J Oncol*. 2012;41:759-764.