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Role of the IL-8/CXCR2 axis in promoting vasculogenic mimicry in triple-negative breast cancer through epithelial-mesenchymal transition

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Abstract

Vasculogenic mimicry (VM) — a phenomenon where tumor cells form *de novo* vascular networks independent of endothelial cells — supports the growth of highly aggressive tumors, including triple-negative breast cancer (TNBC). Evidence indicates that the interleukin 8 (IL-8)/CXCR2 axis and epithelial-mesenchymal transition (EMT) independently promote VM in TNBC. This study tested the hypothesis that the IL-8/CXCR2 axis promotes VM via EMT. Tissue samples from 71 female breast cancer cases (age range: 50 – 55 years), comprising 37 TNBC and 34 non-TNBC cases, were retrieved from the Department of Pathology at the University of Ghana Medical School. Out of the 37 TNBC tissues, 10 expressed either IL-8, CXCR2, or vimentin exclusively; three expressed all three markers; and 14 did not express any of these markers. For the non-TNBC tissues, 31 expressed CXCR2 (alone or co-expression with IL-8 and/or vimentin), 27 co-expressed IL-8 with CXCR2 and/or vimentin, and two expressed all three markers. Notably, 24 of the 37 non-TNBC tissues co-expressed IL-8 and CXCR2. A phi coefficient analysis displayed an inverse relationship between CXCR2 or IL-8 expression and TNBC and a positive correlation between vimentin expression and TNBC. Inhibiting the IL-8/CXCR2 axis with the CXCR2 antagonist SB225002 suppressed VM in MDA-MB-231 breast cancer cells. In addition, inhibiting the IL-8/CXCR2 axis using SB225002 and siRNA-mediated CXCR2 knockdown suppressed EMT by upregulating E-cadherin expression and downregulating N-cadherin and vimentin expression. Our findings suggest that the IL-8/CXCR2 signaling axis promotes VM in TNBC by facilitating EMT. Therefore, therapies targeting IL-8/CXCR2 may be used to suppress EMT and VM in TNBC simultaneously.

Keywords: Epithelial-mesenchymal transition; Interleukin-8; CXC chemokine receptor 2; Triple-negative breast cancer; Vasculogenic mimicry

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1. Introduction

Breast cancer accounts for over 1 million diagnosed cancer cases worldwide and is the leading cause of cancer-related deaths among women globally.[1] The disease is classified

based on stage, histological presentation, and molecular subtypes. The molecular subtypes, defined by the St. Gallen International Breast Cancer Conference in 2013, include luminal A (estrogen receptor [ER]/progesterone receptor [PR]+, human epidermal growth factor receptor-2 [HER2]-, Ki67+ < 20%), luminal B (ER/PR+ < 20%, HER2-, Ki67+ ≥ 20%); HER2+ B2 (ER/PR+, HER2 overexpression), HER2 overexpression (ER-, PR-, HER2 overexpression), and basal-like triple-negative breast cancer (TNBC) (ER-, PR-, HER2-).[2] TNBC is the most threatening of all the molecular subtypes, as it is highly aggressive and has poor survival outcomes. In addition, TNBC is difficult to treat because it lacks three cell surface receptors (i.e., ER, PR, and HER2), which are often targeted during treatment.[3]

When the diameter of a solid tumor reaches >2 mm, new blood vessels are formed to maintain sufficient blood supply to the tumor; otherwise, the tumor becomes necrotic.[4] Vasculogenesis and angiogenesis, which involve endothelial cells, are the primary means by which blood supply to the tumor is kept constant. In 1999, Maniotis *et al.*[5] described a phenomenon known as vasculogenic mimicry (VM) in malignant melanoma when they found that tumor cells can form *de novo* vessel-like channels independent of endothelial cells. These vessel-like channels stain positive for periodic acid-Schiff (PAS) but negative for CD31 and CD34 antigens. Two distinct VM types have been identified: tubular and patterned matrix types. Tubular VM is characterized by tumor cells that form perfused tube-like channels covered with secretory glycoprotein. Patterned matrix VM is characterized by tumor cells that are wrapped by PAS-positive matrix proteins of blood vessels.[4,6] VM is associated with high tumor grade, metastasis, and aggressive subtypes like TNBC and is a promising target for anti-tumor therapy.[7]

One factor that contributes to VM in breast cancer is epithelial-mesenchymal transition (EMT), which involves an alteration in the cytoskeletal dynamics of epithelial cells as they differentiate into mesenchymal cells.[6] During this transition, phenotypic features of epithelial cells, like apical-basal polarity and cell-to-cell contact, are replaced with phenotypic characteristics of mesenchymal cells, like extensively flattened and elongated features.[8] In addition, epithelial cell markers, like E-cadherin, zonula occludins-1, and α-catenin, are downregulated, while mesenchymal cell markers, like N-cadherin, fibronectin, and vimentin, are upregulated.[9] EMT results in enhanced cell motility, invasiveness, increased metastatic propensity, and drug resistance.[10] EMT can be induced by various stimuli, including tumor-stromal cell interactions, growth factors, and cytokines.[11,12] Evidence suggests that coordinated expression of the cytokines interleukin-1β (IL-1β) and tumor necrosis factor-α drives EMT in breast cancer through the PI3K/AKT signaling pathway, leading to disease relapse.[12,13] Another cytokine implicated in breast cancer EMT is IL-6. Studies have demonstrated that IL-6 can promote EMT in breast cancer by aberrantly activating the JAK/STAT3 pathway. This results in inflammation, increased aggressiveness, and progression of the disease.[14-16] The human tumor growth factor-β (TGF-β) has also been demonstrated to induce EMT, leading to the progression and dissemination of breast cancer.[17,18]

Apart from EMT, the pro-inflammatory cytokine interleukin-8 (IL-8/CXCL8) is another factor that promotes VM. IL-8 binds to CXC chemokine receptor (CXCR) 1 and 2 and induces angiogenesis and neutrophil recruitment.[19] IL-8 is overexpressed in TNBC and correlates with drug resistance, tumor recurrence, and immune evasion.[20,21] The IL-8/CXCR2 signaling axis has also been reported to promote VM in glioblastoma multiforme cells[22,23] and breast cancer cells.[24]

Studies have revealed a relationship between EMT and IL-8 secretion. The induction of EMT through TGF-β[25] or SNAIL overexpression[26] promotes IL-8 expression. On the other hand, IL-8 signaling enhances EMT by upregulating the expression of SNAIL, SLUG, and vimentin and downregulating the expression of E-cadherin.[27-29] Given the role of the IL-8/CXCR2 axis in EMT and VM, the IL-8/CXCR2 axis may act in consonance with EMT to promote VM. Herein, we demonstrate that the IL-8/CXCR2 axis promotes VM in TNBC via EMT.

2. Methods

2.1. Tissue microarray construction

Formalin-fixed, paraffin-embedded breast cancer tissues from 80 mastectomy cases were randomly selected from the archives of the Department of Pathology at the University of Ghana Medical School for this study. The tissues were used to construct tissue microarray blocks using the T-Sue™ Microarray Paraffin Block (Simport Scientific Inc., Canada), following published protocols.[30] The microarray blocks were sectioned, and the sections were fixed onto poly-L-lysine-coated slides (Leica, USA) for staining.

2.2. Hematoxylin and eosin staining

Hematoxylin and eosin (H&E) staining (CDH, India) was performed to confirm the presence of tumors in the breast cancer tissues. The sections were deparaffinized in xylene, then rehydrated in decreasing alcohol concentrations and rinsed with water. The sections were stained with hematoxylin for 2 min, rinsed with water for 5 min, and then stained with eosin for 3 min. Finally, the stained sections were dehydrated in increasing alcohol

concentrations, dipped in xylene, and mounted with distyrene-plasticizer-xylene (DPX) (Molychem, India). The slides were viewed independently by two pathologists. In the case of discordant results, a third pathologist was consulted to resolve the disagreement.

2.3. Immunohistochemical staining

Antigen retrieval was performed using tris-EDTA (pH 9.0) (Sigma Aldrich, USA) at 95°C for 20 min, and endogenous peroxidase blocking was performed using 3% H_2O_2 (BDH Prolabo, Belgium) for 10 min. The tissue sections were incubated overnight at 4°C with rabbit monoclonal antibodies for ER (cat. no.: 790-4325), PR (cat. no.: 790-2223), and HER2/neu (cat. no.: 790-2991) (all purchased from Roche Diagnostics, Netherlands), as well as mouse monoclonal antibodies for IL-8 (cat. no.: ab34100), CXCR2 (cat. no.: ab89254), CD34 (cat. no.: ab54208), and vimentin (cat. no.: ab8979) (all purchased from Abcam, United Kingdom [UK]). The sections were then stained with OptiView HQ Universal Linker (Roche Diagnostics, Netherlands) for ER, PR, and HER2/neu or HRP-tagged goat anti-mouse IgG H&L (cat. no.: ab205719; Abcam, UK) for IL-8, CXCR2, CD34, and vimentin. Sections were counterstained with hematoxylin and mounted using DPX. The intensity of ER and PR staining was recorded using the Allred score, while HER2 was recorded using the ASCO/CAP guidelines.[31]

2.4. PAS/CD34 double staining

PAS staining was performed using the PAS stain kit (Abcam, UK), following the manufacturer's protocol, and counterstaining was performed using Modified Mayer's hematoxylin (Ventana Roche, USA) for 3 min. The slides were rinsed with running tap water for 2 min, followed by the addition of a bluing reagent for 30 s. After washing with distilled water, the tissue sections were dehydrated using increasing concentrations of ethanol, dipped in xylene, and mounted with DPX.

2.5. Cell lines and culture

MDA-MB-231 cells, obtained from the American Type Culture Collection (ATCC), were maintained in Dulbecco's Modified Eagle Medium (DMEM), supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (all purchased from Gibco-Life Technologies, United States of America [USA]), at 37°C in a humidified atmosphere containing 5% CO_2.

2.6. Cell viability assay

An MTT assay was conducted to determine the effect of the CXCR2 antagonist SB225002 on cell viability. Briefly, the cells were seeded in 96-well plates at a density of 1×10^4 cells/well and incubated at 37°C for 24 h. The cells were treated with SB225002 (Thermo Fisher Scientific, USA) for 24, 48, and 72 h. Thereafter, 10 µL of 5 mg/mL MTT (Sigma-Aldrich, USA) was added to each well and incubated at 37°C for 4 h. Next, 100 µL of acidified isopropanol was added and incubated at 37°C for 30 min. Absorbance was measured at 570 nm with a Varioskan™ LUX multimode microplate reader (Thermo Fisher Scientific, USA). From the absorbance values, percent cell viabilities were calculated.

2.7. Tube formation assay

We coated 96-well plates with 50 µL Matrigel (Sigma-Aldrich, USA) per well and allowed it to polymerize at 37°C for 30 min. MDA-MB-231 cells (1.5×10^4 cells/well) were pretreated with SB225002 and added to the Matrigel-coated plates, followed by incubation at 37°C for 8 h. Images of the tubes formed in each well were captured with an OPTIKA® microscope (OPTIKA, Italy).

2.8. siRNA transfection

Cells were seeded in six-well plates at a density of 1×10^4 cells/well a day before transfection. Cells were then transfected with scrambled (control) siRNA or siRNA targeting CXCR2 (cat. no.: 4392420; Thermo Fisher Scientific, USA) using the Lipofectamine™ 2000 Transfection Reagent (Thermo Fisher Scientific, USA), according to the manufacturer's protocol. At 48-h post-transfection, the cells were harvested for further analyses.

2.9. Reverse-transcription quantitative polymerase chain reaction

MDA-MB-231 cells were seeded into six-well plates at a density of 1×10^6 cells/well, allowed to attach for 24 h, and then left untreated or treated with SB225002 or CXCR2-siRNA for 48 h. Total RNA was extracted using an RNeasy™ Mini Kit (Qiagen, USA), per the manufacturer's protocol. Using a Luna Universal One-Step RT-qPCR kit (New England Biolabs, USA), reverse-transcription quantitative polymerase chain reaction (RT-qPCR) was performed to determine the mRNA expression of IL-8, CXCR2, vimentin, E-cadherin, and N-cadherin. A QuantStudio™ RT-PCR System (Thermo Fisher Scientific, USA) was used for reverse transcription and amplification. Thermocycling conditions were reverse transcription (55°C for 10 min), initial denaturation (95°C for 1 min), 40 cycles of denaturation (95°C for 30 s), annealing (56°C for 30 s), and extension (60°C for 30 min). The housekeeping gene *β-actin* was used as an internal control. Primer sequences are listed in Table A1. QuantStudio™ Design & Analysis Software (Life Technologies, USA) was used to obtain cycle threshold (CT) values, and the $2^{-\Delta\Delta CT}$ method[32] was used to determine the relative expression of each gene.

2.10. Statistical analysis

Data were analyzed using GraphPad Prism 8.1 (GraphPad Software, USA). One-way analysis of variance (ANOVA) followed by Dunnett's *post hoc* test was used to compare differences between multiple groups. The phi coefficient analysis was used to determine the correlation between the expression of IL-8, CXCR2, or vimentin and TNBC. Data are presented as the mean ± *SD* of at least three independent experiments performed in triplicates. Group differences were considered statistically significant when $P \leq 0.05$.

3. Results

3.1. Classification of tumor cases according to molecular subtypes

Out of the 80 randomly selected cases, 71 were confirmed to contain tumors following H&E staining. The expression of ER and PR was identified by nuclear-positive staining (Figure 1A and B), whereas the expression of HER2 was identified by membrane-positive staining (Figure 1C). Tissues that did not stain for any of the three markers were classified as TNBC (Figure 1D). As displayed in Table 1, the confirmed tumor cases were classified as luminal A (ER+, PR+, HER2-), luminal B (ER+, HER2+, and/or PR+), HER2/neu (ER-, PR-, HER2+), or TNBC (ER-, PR-, HER2-). Out of the 71 cases, most were TNBC cases, followed by luminal A, HER2/neu, and luminal B subtypes. The mean age at diagnosis for the various subtypes was relatively identical (50.00 – 55.43 years). In addition, most patients had grade II breast tumors (Table 1).

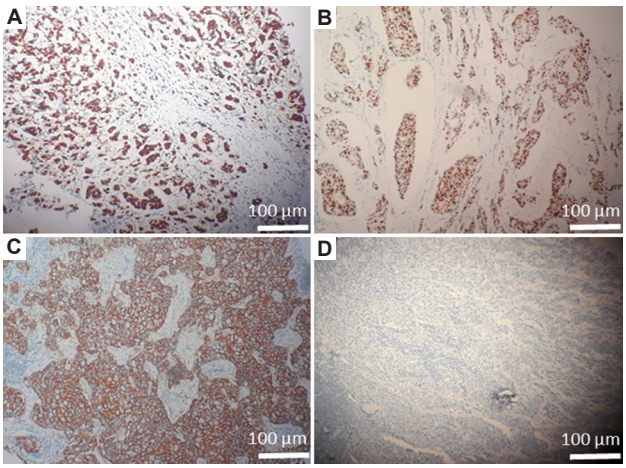

Figure 1. Classification of tumor cases according to molecular subtypes. Photomicrographs displaying nuclear-positive staining for (A) ER and (B) PR, and membrane-positive staining for (C) HER2/neu. Tumors negative for the three markers are classified as (D) TNBC. Magnification: ×40. Scale bars: 100 μm.
Abbreviations: ER: Estrogen receptor; PR: Progesterone receptor; HER2: Human epidermal growth factor receptor-2; neu: Neuroblastoma.

3.2. Staining of IL-8, CXCR2, vimentin, and PAS/CD34 in tumor samples

Tissue staining for IL-8, CXCR2, vimentin, and PAS/CD34 is displayed in Figure 2. The distribution of the expression of these markers between TNBC and non-TNBC cases is presented in Figure 3. Out of the 37 TNBC tissues, 10 expressed either IL-8, CXCR2, or vimentin; three tissues expressed all three markers; 14 did not express any of these markers. In the non-TNBC samples, 31 expressed CXCR2 (alone or co-expression with IL-8 and/or vimentin), 27 co-expressed IL-8 with CXCR2 and/or vimentin, and four expressed vimentin. Two tissues expressed all three markers, while two did not express any of these markers. Notably, almost all (24 of 37) non-TNBC tissues that expressed IL-8 also expressed CXCR2 (Figure 3).

3.3. Correlation between expression of markers and molecular subtypes

The correlation of IL-8, CXCR2, vimentin, and PAS+/CD34- expression with TNBC was analyzed using the phi coefficient. We identified an inverse relationship between CXCR2 ($r = -0.5767$; $P < 0.05$) or IL-8 ($r = -0.4460$; $P < 0.05$) expression with TNBC. Conversely, a positive correlation was noted between vimentin expression ($r = 0.2736$; $P < 0.05$) and TNBC. PAS+/CD34- double staining ($r = -0.1042$; $P > 0.05$) did correlate with TNBC (Table 2).

3.4. CXCR2 antagonist SB225002 reduces cell viability

To determine the effect of CXCR2 inhibition on cell viability, we performed an MTT assay after treating MDA-MB-231 cells with the CXCR2 antagonist SB225002. The results indicated that CXCR2 inhibition reduced cell viability with increasing concentrations of SB225002, especially after 48 and 72 h of treatment (Figure 4). The half-maximal inhibitory concentration (IC_{50}) of 69.29 ± 6.67 nM and 55.16 ± 4.513 μM was obtained for 48- and 72-h treatments, respectively.

3.5. Inhibition of the IL-8/CXCR2 axis suppresses VM

Next, we determined the importance of the IL-8/CXCR2 axis on VM. By performing a tube formation assay, we observed that increasing concentrations of the CXCR2 antagonist SB225002 progressively reduced the number of vessel-like channels formed (Figure 5). This suggests that inhibition of the IL-8/CXCR2 axis suppresses VM in MDA-MB-231 cells.

3.6. Inhibition of the IL-8/CXCR2 axis suppresses EMT

Given the influence of the IL-8/CXCR2 axis on VM, we investigated whether this effect involved EMT. Our

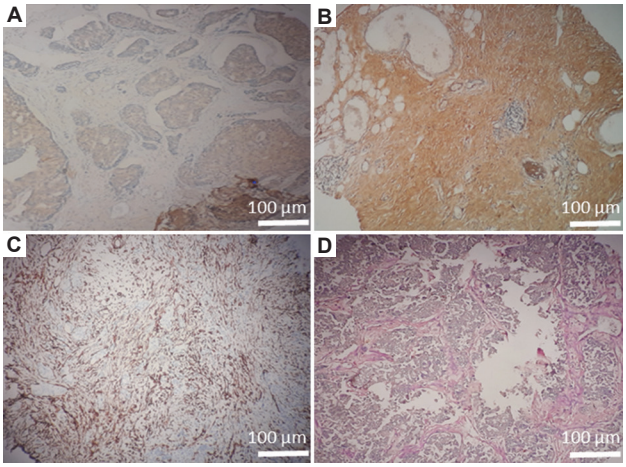

Figure 2. Staining of interleukin (IL)-8, CXCR2, vimentin, and periodic acid-Schiff (PAS)/CD34 in tumor samples. Photomicrographs displaying cytoplasmic staining for (A) IL-8, (B) CXCR2, (C) vimentin, and (D) PAS/CD34 in breast cancer tissues. PAS stain (purple) indicates tubular structures with no surrounding CD34+ cells. Magnification: ×40. Scale bars: 100 μm.

hypothesis posits that EMT, characterized by cytoskeletal remodeling and enhanced motility, may facilitate VM. We inhibited the IL-8/CXCR2 axis by suppressing CXCR2 activity using SB225002 and knocking down the *CXCR2* gene using siRNA. Inhibition of CXCR2 activity led to an increase in the expression of the epithelial marker E-cadherin and a decrease in the expression of the mesenchymal markers N-cadherin and vimentin. A similar finding was observed after *CXCR2* knockdown, indicating that the IL-8/CXCR2 axis is critical for EMT in breast cancer (Figure 6).

4. Discussion

VM supplies blood to tumor cells and is associated with aggressive breast cancer phenotypes and poor survival rates.[4] One of the promoters of VM is EMT, a process that involves cytoskeletal changes and increased motility of tumor cells.[4] Evidence indicates that IL-8 signaling enhances EMT by upregulating the expression of mesenchymal

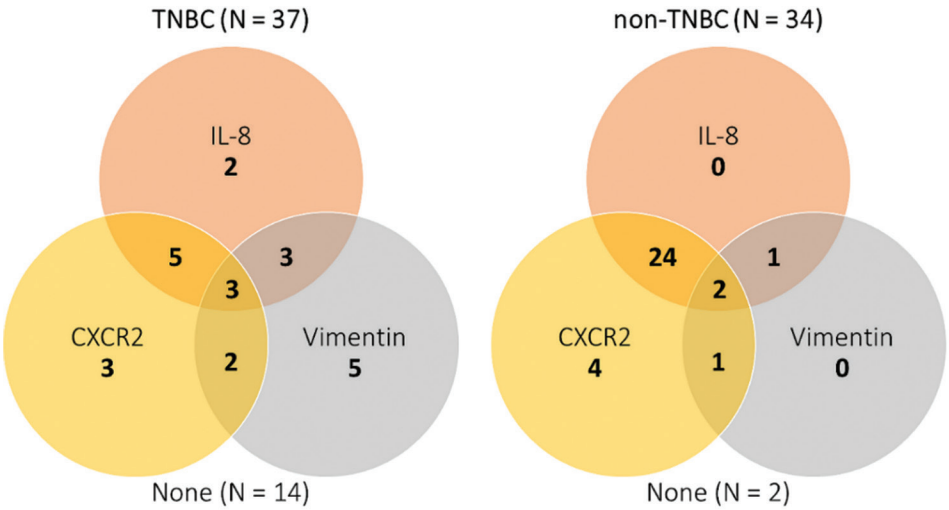

Figure 3. Venn diagram displaying the distribution of IL-8, CXCR2, and vimentin expression in triple-negative breast cancer (TNBC) and non-TNBC cases

Table 1. Molecular subtype classification of tumor cases

Variable	Molecular subtype (*n* [%])				Total
	Luminal A	Luminal B	HER2/neu	TNBC	
Number of cases	25 (35.2)	2 (2.8)	7 (9.9)	37 (52.1)	71 (100.0)
Tumor grade I	1 (1.4)	0 (0.0)	0 (0.0)	8 (11.3)	9 (12.7)
Tumor grade II	21 (29.6)	2 (2.8)	3 (4.2)	23 (32.4)	49 (69.0)
Tumor grade III	3 (4.2)	0 (0.0)	4 (5.6)	6 (8.5)	13 (18.3)
Mean age (years)	51.64	50.00	55.43	51.38	52.11±2.33*

Note: *Data presented as mean±*SD*.
Abbreviations: TNBC: Triple-negative breast cancer; HER2: Human epidermal growth factor receptor-2; neu: Neuroblastoma.

Table 2. Phi coefficient analysis of the correlation between markers and TNBC

Group	Phi coefficient analysis		
	r	95% confidence interval	*P*
CXCR2 versus TNBC	−0.5767	−0.7139 to −0.3968	<0.05
IL-8 versus TNBC	−0.4460	−0.6153 to −0.2374	<0.05
Vimentin versus TNBC	0.2736	0.0430 to 0.4765	<0.05
PAS/CD34 versus TNBC	−0.1042	−0.3295 to 0.1323	>0.05

Abbreviations: TNBC: Triple-negative breast cancer; IL-8: Interleukin 8; PAS: Periodic acid-Schiff.

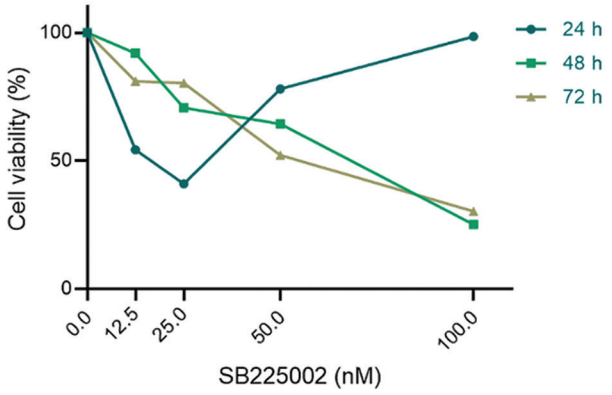

Figure 4. CXCR2 antagonist SB225002 reduces cell viability. MDA-MB-231 cells were treated with SB225002 for 24, 48, and 72 h. Cell proliferation was evaluated using an MTT assay.
Notes: Data are presented as mean ± *SD* of three independent experiments performed in triplicates.

markers and downregulating the expression of epithelial markers.[27,29] Moreover, the inhibition of IL-8/CXCR2 signaling suppresses VM in breast cancer cells.[24] Herein, we investigated the influence of the IL-8/CXCR2 signaling axis on VM in breast cancer through the regulation of EMT.

Our finding that the mean ages at diagnosis are similar between TNBC (51.38 years) and non-TNBC (52.36 years) patients is corroborated by a study conducted in Accra, Ghana, which reported mean ages of 51.7 years for TNBC patients and 52.9 years for non-TNBC patients.[33] This could be due to the age at which breast cancer develops in Ghanaian women or the age at which affected women report their ailment. Nonetheless, screening women who aged 50 and above could be beneficial for the early detection of breast cancers.

Notably, while only 13.5% of TNBC tissues coexpressed IL-8 and CXCR2, 70.6% of non-TNBC tissues coexpressed these markers. Moreover, an inverse relationship between CXCR2 or IL-8 and TNBC was observed. Indeed, IL-8 is highly expressed in ER- and HER2+ subtypes of breast cancer.[34,35] Our study thus confirms the crucial role of the IL8/CXCR2 axis in non-TNBC subtypes. In addition, a positive correlation was noted between vimentin and TNBC, which is supported by findings that vimentin is associated with TNBC.[36] Conversely, there was no correlation between PAS+/CD34- staining and TNBC. Although this suggests a low incidence of VM in our study

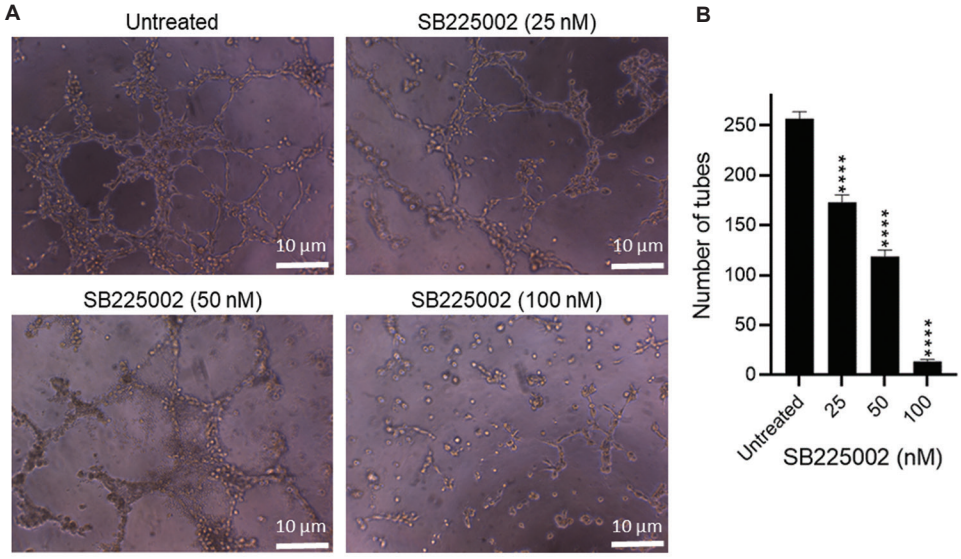

Figure 5. Inhibition of the IL-8/CXCR2 axis suppresses vasculogenic mimicry (VM). Cells were either left untreated or treated with SB225002 for 8 h. (A) Microscope images of the untreated and treated cells. (B) Number of vessel-like channels formed from the untreated and treated cells. Magnification: ×100. Scale bars: 10 µm.
Notes: The bar graph represents the mean ± *SD* of three independent experiments performed in triplicates. ****$P < 0.0001$ versus untreated cells.

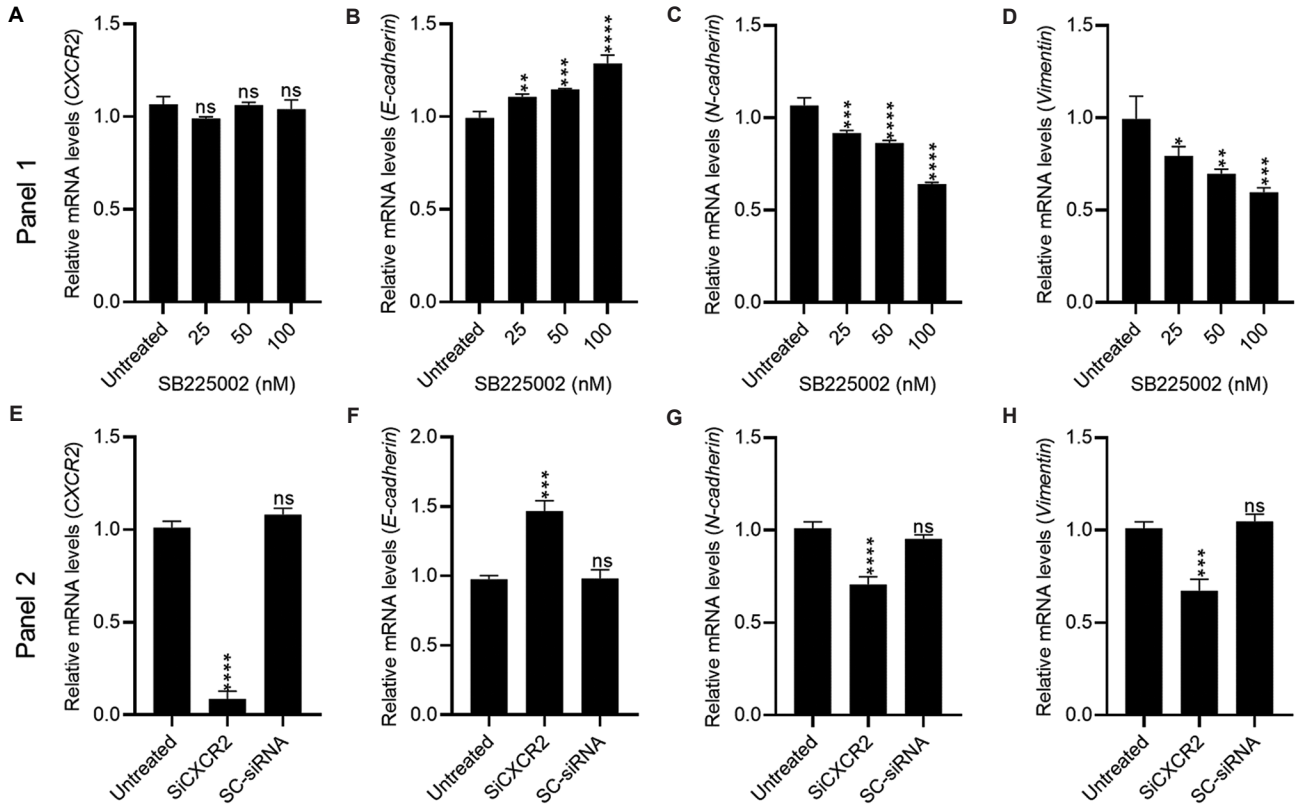

Figure 6. Inhibition of the IL-8/CXCR2 axis suppresses epithelial-mesenchymal transition (EMT). Cells were either left untreated or treated with (Panel 1) SB225002 or (Panel 2) siCRCX2 for 48 h. The mRNA levels of (A) CXCR2, (B) E-cadherin, (C) N-cadherin, and (D) vimentin were analyzed using reverse-transcription quantitative polymerase chain reaction (RT-qPCR).

Notes: Data are presented as mean ± *SD* of three independent experiments performed in triplicates. **P* < 0.05 versus untreated cells; ***P* < 0.01 versus untreated cells; ****P* < 0.001 versus untreated cells; *****P* < 0.0001 versus untreated cells.

Abbreviation: ns: Not significant.

population, these results should be interpreted cautiously. Recent research has reported that PAS+/CD34-staining may not conclusively prove the presence of VM due to the role of CD34 in other normal physiological processes.[37]

Similar to previous findings,[24] inhibiting CXCR2 activity with SB225002 reduced cell viability. SB225002 (N-(2-hydroxy-4-nitrophenyl)-N0-(2-bromophenyl) urea) is a non-peptide antagonist of CXCR2 and IL-8 receptor B (IL-8RB), and it disrupts the binding of IL-8 to CXCR2.[38,39] MDA-MB-231 cells express CXCR1/CXCR2,[40] and our results confirm the dependence of MDA-MB-231 cells on the IL-8/CXCR2 signaling axis. Inhibiting the IL-8/CXCR2 axis also suppressed VM, as evidenced by the reduced number of patterned vessel-like channels following CXCR2 inhibition. This observation confirms previous findings[24] and highlights the supportive role of the IL-8/CXCR2 signaling axis in breast cancer VM. The IL-8/CXCR2 axis triggers the activity of other signaling pathways, such as MAPK/ERK, PI3K/Akt/mTOR, and PLC/PKC, and some of these are associated with VM (e.g., PI3K/Akt).[41] Therefore, the inhibition of IL8-CXCR2 binding may have suppressed VM through these other pathways.

Our gene expression analyses revealed that the IL8/CXCR2 axis supports EMT in TNBC cell models. This is demonstrated by the upregulation of the epithelial marker E-cadherin and the downregulation of mesenchymal markers N-cadherin and vimentin following CXCR2 inhibition and knockdown. This indicates that one of the mechanisms through which the IL-8/CXCR2 axis promotes VM is through EMT. Given that EMT involves alterations in the cytoskeletal dynamics and tumor cell motility, its involvement in IL-8/CXCR2-mediated VM is expected.

5. Conclusion

This study established an inverse relationship between CXCR2 or IL-8 and TNBC and a positive correlation between vimentin and TNBC. Furthermore, the findings demonstrate that inhibiting the IL-8/CXCR2 axis suppresses VM in MDA-MB-231 cells by repressing EMT. This suggests

that targeted therapies against IL-8/CXCR2, like ABXIL-8 (a humanized antibody against IL-8) and the small-molecule inhibitor repertaxin, may be used to simultaneously suppress EMT and VM in TNBC. Nonetheless, further studies are warranted to validate these findings.

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Conflict of interest

The authors declare that they have no competing interests.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

All data from the study have been presented in the article and appendix.

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Appendix

Table A1. Primer sequences

Gene	Primer sequences (5'→3')	
	Forward	Reverse
β-actin	ACTCTTCCAGCCTTCCTTC	ATCTCCTTCTGCATCCTGTC
CXCR2	CACCGATGTCTACCTGCTGA	CACAGGGTTGAGCCAAAAGT
E-cadherin	ATTCTGATTCTGCTGCTCTTG	AGTAGTCATAGTCCTGGTCTT
IL-8	CACCGGAAGGAACCATCTCACT	TCAGCCCTCTTCAAAAACTTCTCC
N-cadherin	GGTGGAGGAGAAGAAGACCAG	GGCATCAGGCTCCACAGT
Vimentin	GTGCAGTTTTGCCAAGGAGT	CGAAGGTGACGAGCCATTTCC

Abbreviations: IL-8: Interleukin 8; CXCR2: CXC chemokine receptor.