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Suppression of Invasion and Metastasis of Triple-Negative Breast Cancer Lines by Pharmacological or Genetic Inhibition of Slug Activity[1,2,3]

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Abstract

Most triple-negative breast cancers (TNBCs) exhibit gene expression patterns associated with epithelial-to-mesenchymal transition (EMT), a feature that correlates with a propensity for metastatic spread. Overexpression of the EMT regulator Slug is detected in basal and mesenchymal-type TNBCs and is associated with reduced E-cadherin expression and aggressive disease. The effects of Slug depend, in part, on the interaction of its N-terminal SNAG repressor domain with the chromatin-modifying protein lysine demethylase 1 (LSD1); thus, we investigated whether tranylcypromine [also known as trans-2-phenylcyclopropylamine hydrochloride (PCPA) or Parnate], an inhibitor of LSD1 that blocks its interaction with Slug, suppresses the migration, invasion, and metastatic spread of TNBC cell lines. We show here that PCPA treatment induces the expression of E-cadherin and other epithelial markers and markedly suppresses migration and invasion of TNBC cell lines MDA-MB-231 and BT-549. These effects were phenocopied by Slug or LSD1 silencing. In two models of orthotopic breast cancer, PCPA treatment reduced local tumor growth and the number of lung metastases. In mice injected directly in the blood circulation with MDA-MB-231 cells, PCPA treatment or Slug silencing markedly inhibited bone metastases but had no effect on lung infiltration. Thus, blocking Slug activity may suppress the metastatic spread of TNBC and, perhaps, specifically inhibit homing/colonization to the bone.

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Introduction

Treatment of breast cancer has substantially improved over the past 30 years due, in large part, to the development of more effective combination chemotherapy protocols, endocrine therapies, and human epidermal growth factor receptor 2–targeted therapies [1–4]. However, progress of patients with triple-negative breast cancer (TNBC), which are estrogen receptor–, progesterone receptor–, and human epidermal growth factor receptor 2–negative and represent 10% to 15% of the total, has been more limited because they cannot be treated with endocrine or targeted therapies. Although TNBC is histopathologically heterogeneous, the vast majority are high-grade invasive ductal carcinomas characterized by marked degrees of nuclear pleomorphism, lack of tubule formation, high number of mitotic cells, and high frequency of p53 mutations [5,6]. Microarray-based analysis of TNBCs has identified six reproducible gene expression subtypes, two of which, the mesenchymal-like and the mesenchymal stem–like, show enrichment for gene expression patterns associated with epithelial-to-mesenchymal transition (EMT) [7]. Such enrichment correlates with a propensity of TNBC cells to disseminate as indicated by increased expression of EMT markers in breast cancer circulating tumor cells (CTCs) [8]. The EMT is a process through which tumor cells lose homotypic adhesion, change morphology, and acquire migratory and invasive capacity [9,10]. EMT is thought to contribute to tumor progression, and aberrant expression of EMT regulator/inducers in cancer cells correlates with tumor aggressiveness and poor clinical outcomes [11]. Transcriptional repression of E-cadherin expression is a key event during EMT. The human E-cadherin promoter contains E-box elements that are required for regulation of its transcription [12]. The zinc-finger transcription factors (TFs) Snail [13], Slug [14], Zeb1 [15], and Zeb2 [16] can bind directly to these E-boxes and repress E-cadherin transcription. Slug contributes to invasion in many tumor types [17–20] and can cooperate with Twist or Sox9 in promoting invasion and metastasis [21,22]. Overexpression of Slug is detected in many tumors [23] including the basal and mesenchymal-type TNBCs [7,24,25] and is associated with reduced E-cadherin expression, high histologic grade, lymph node metastasis, post-operative relapse, and shorter patients' survival [26–28]. Moreover, Slug represses the expression of E-cadherin and of the cell-cell junction protein plakoglobin in TNBC cells [15,29] and its silencing suppresses the invasion of breast cancer cells [30].

Because Slug-regulated transcription repression depends, in part, on the interaction of its N-terminal SNAG repressor domain with chromatin-modifying proteins such as lysine demethylase 1 (LSD1) [31,32], inhibitors of this interaction may suppress the motility and invasion of TNBC cells. A previous study from our laboratories has shown that treatment with tranylcypromine [also known as trans-2-phenylcyclopropylamine hydrochloride (PCPA) or Parnate], an Food and Drug Administration-approved monoamine oxidase (MAO)/LSD1 enzymatic inhibitor [33], or TAT-SNAG, a cell permeable peptide that includes the highly conserved SNAG domain of Slug, blocks Slug-dependent repression of the E-cadherin promoter, suppresses the expression of morphologic and molecular markers of EMT, and inhibits the motility and invasion of tumor cells of different histologic and genetic backgrounds [34].

In this study, we extended these findings to investigate the effects of PCPA and the requirement of Slug or LSD1 expression for the migration, invasion, and EMT marker expression of TNBC cell lines *in vitro* and for metastatic spread in mouse models of TNBC.

We show here that TNBC cell lines MDA-MB-231 and BT-549 are highly dependent on Slug/LSD1 activity for their migration and invasion and that treatment with PCPA has anti-metastatic effects in orthotopic models of breast cancer and in immunodeficient mice injected intracardiacally with TNBC MDA-MB-231 cells.

Materials and Methods

Plasmids and Antibodies

TNBC cell lines were lentivirally transduced with the following plasmids: pLKO-SCR-sh and pLKO-Slug-sh [34] and pLKO-Twist2-sh and pGIPZ-LSD1-sh-H6 (purchased from Open Biosystems, Lafayette, CO).

Slug, LSD1, and anti–β-actin expression in transduced cell lines was detected by anti-Slug (Abgent, San Diego, CA; #AP2053a), anti-LSD1 (Abcam, Cambridge, UK; #ab17721), and anti–β-actin (Santa Cruz Biotechnology, Santa Cruz, CA; #sc-47778) antibodies.

Cell Lines and Treatments

Triple-negative mesenchymal-like human breast cancer MDA-MB-231 and BT-549 [7] and 4T1 (murine animal model for stage IV human breast cancer) cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Carlsbad, CA) supplemented with 10% heat-inactivated FBS, 100 units/ml penicillin, 0.1 mg/ml streptomycin, and 2 mM L-glutamine at 37 °C and 5% CO_2. Cell lines were obtained from American Type Culture Collection (ATCC, Manassas, VA) and characterized by DNA fingerprinting and isozyme detection. For *in vivo* experiments, cells were engineered to stably express enhanced green fluorescent protein (copGFP) using a lentiviral vector (pCDH-CMV-MCS-EF1-copGFP, SBI). Cells were treated with 100 μM PCPA, a non-selective MAO-A/B/LSD1 inhibitor. All lines were tested for mycoplasma contamination (PCR Mycoplasma Detection Set; Takara Bio Inc, Otsu, Shiga, Japan) every 3 months.

Proliferation Assay

Cell proliferation assay was performed using CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega, Madison, Wisconsin). Briefly, 5000 cells were seeded in 96-well flat bottom culture plates in 100 μl of culture medium. After 24, 48, 72, and 96 hours, 20 μl of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium solution was added to each well and plates were incubated for 3 hours in a humidified 5% CO_2 atmosphere. Absorbance at 492 nm was recorded using a 96-well plate reader. Proliferation of MDA-MB-231 and BT-549 cells was also measured by trypan blue exclusion. Each treatment was performed in triplicate, and the experiment was repeated at least twice.

Migration and Invasion Assay

For wound-healing assays, cells were plated to confluence onto a six-well plate and the cell surface was scratched using a pipette tip. Then, cells were treated with PCPA (100-500 μM), allowed to repopulate the scratched area for 1 to 3 days, and photographed using a digital camera mounted on an inverted microscope (magnification, ×5). Accurate wound measurements were taken at 0 and 72 hours to calculate the migration rate according to the equation: %wound healing = [(wound length at 0 hour) − (wound length at 72 hours)]/(wound length at 0 hour) × 100. Experiments were performed twice independently.

For invasion assays, cells were plated (10^5 cells per chamber) onto BD BioCoat Matrigel invasion chambers (BD Biosciences, San Josè,