Inhibiting Interactions of Lysine Demethylase LSD1 with Snail/Slug Blocks Cancer Cell Invasion

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Abstract

The process of epithelial-mesenchymal transition (EMT) which is required for cancer cell invasion is regulated by a family of E-box–binding transcription repressors, which include Snail (SNAIL1) and Slug (SNAI2). Snail appears to repress the expression of the EMT marker E-cadherin by epigenetic mechanisms dependent on the interaction of its N-terminal SNAG domain with chromatin-modifying proteins including lysine-specific demethylase 1 (LSD1/KDM1A). We assessed whether blocking Snail/Slug-LSD1 interaction by treatment with Parnate, an enzymatic inhibitor of LSD1, or TAT-SNAG, a cell-permeable peptide corresponding to the SNAG domain of Slug, suppresses the motility and invasiveness of cancer cells of different origin and genetic background. We show here that either treatment blocked Slug-dependent repression of the E-cadherin promoter and inhibited the motility and invasion of tumor cell lines without any effect on their proliferation. These effects correlated with induction of epithelial and repression of mesenchymal markers and were phenocopied by LSD1 or Slug downregulation. Parnate treatment also inhibited bone marrow homing/engraftment of Slug-expressing K562 cells. Together, these studies support the concept that targeting Snail/Slug-dependent transcription repression complexes may lead to the development of novel drugs selectively inhibiting the invasive potential of cancer cells. Cancer Res; 73(1); 1–11. ©2012 AACR.

Introduction

Metastases represent the endpoint of a multistep process, the invasion–metastasis cascade, which leads to the dissemination of cancer cells to anatomically distant organs (1, 2). Whereas surgical resection and adjuvant therapy can cure well-confined primary tumors, metastatic disease is largely incurable because of its systemic nature and resistance to cytotoxic drugs, accounting for more than 90% of cancer mortality (1–3).

The ability of tumor cells to become “invasive” depends on the activation of an evolutionarily conserved developmental process known as epithelial-mesenchymal transition (EMT) through which tumor cells lose homotypic adhesion, change morphology, and acquire migratory capacity (4, 5). The EMT program involves the dissolution of adherent and tight junctions, loss of cell polarity, and dissociation of epithelial cell sheets into individual cells that exhibit multiple mesenchymal attributes, including heightened invasiveness (6), setting the stage for tumor cells to invade locally and spread to distant organs. EMT is thought to contribute to tumor progression, and aberrant expression of EMT regulator/inducers in cancer cells correlates with poor clinical outcomes and tumor aggressiveness (7–10). In addition to its clinical significance, altered expression of EMT regulators in cancer may identify new drug targets for development of novel anti-cancer therapies.

Features of EMT have been observed in breast (11), ovarian (12), colon (13), esophageal cancer (14), and in melanoma (15), and inducers of EMT in cancer cell lines include TGF-β, Wnt, Snail/Slug, Twist, Six1, and Zeb1/Zeb2 (16).

Downregulation 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 (17, 18). Several zinc-finger transcription factors such as Snail (19, 20) and Slug (21) can bind directly to these E-boxes to repress E-cadherin transcription.

The Snail family of transcription factors that includes Snail, Slug, and Smug is involved in physiologic and cancer-associated EMT. Slug contributes to invasion in melanomas (22) and in malignant mesotheliomas (23), its silencing inhibits neuroblastoma invasion in vitro and in vivo (24), its induction is required for the ability of Twist1 to promote invasion and metastasis, and the entire EMT process is...
blocked in the absence of Slug (25). Slug is overexpressed in numerous cancers, including leukemia, lung, esophageal, gastric, colorectal, pancreatic, breast, ovarian, prostate cancer, malignant mesothelioma, cholangiocarcinoma, hepatocellular carcinoma, and glioma (26). Elevated expression of Slug is associated with reduced E-cadherin expression, high histologic grade, lymph node metastasis, postoperative relapse, and shorter patients’ survival in a variety of cancers (26–28).

Together, these studies imply that pharmacologic inhibition of Snail/Slug-regulated transcription repression would block migration and invasion of tumor cells. Because the effects of Slug on transcription may depend on the interaction of its N-terminal SNAG repressor domain with chromatin-modifying proteins such as lysine-specific demethylase 1 (LSD1; ref. 29), we assessed whether treatment with Parnate, an enzymatic inhibitor of LSD1, or TAT-SNAG, a cell-permeable peptide corresponding to the SNAG domain of Slug, blocks Slug-dependent repression of the E-cadherin promoter and inhibits the motility and invasion of tumor cell lines. We provide here proof-of-principle for the concept that pharmacologic inhibition of Slug-dependent transcription repression suppresses the expression of morphologic and molecular markers of EMT and blocks the migration and invasion of tumor cells of different histologic and genetic backgrounds.

Materials and Methods

Plasmids and antibodies

E-Cadherin promoter-LUC: the human E-cadherin promoter (−233 to −1 from the ATG start site; E-box at nt −204 to −198) was cloned by blunt-end ligation in the Smal site of pGL3-basic plasmid by PCR with forward (FW): 5'-ggtcggcagcagcc-3' and reverse (RV): 5'-ggtcggcagcagcc-3' primers.

pcDNA3-Slug: this plasmid was generated by PCR amplification of human Slug coding sequence with Slug-CDS-FW (5’-ctggctgtcagctggcggagc-3’) and Slug-CDS-RV (5’-ctagttgtccacctgagc-3’) primers.

The pTAT-SNAG plasmid was generated by inserting oligodeoxynucleotides encoding the highly conserved 9 amino acid sequence MPBSFLKVK of the SNAG domain, flanked by engineered Xhol and EcoRI sites, into the Xhol–EcoRI sites of pTAT-HA vector (kind gift of Dr. S.F. Dowdy). The pTAT-HA vector alone was used as negative control.

Fusion peptides were produced by Escherichia coli strain (DE3)pLyS8 (Novagen) after a 3-hour 1 mmol/L isopropyl β-D-1-thiogalactopyranoside (IPTG) induction at 37°C. The cell pellet was lysed in 50 mmol/L NaH₂PO₄, 300 mmol/L NaCl, 10 mmol/L imidazole, pH 8.0, and sonicated. Fusion peptides were purified from clear lysates using the batch/gradient-flow method with NINhA Agarose (Qiagen) and eluted from the resin with lysis buffer containing 200 mmol/L imidazole. Buffer exchange in PBS was conducted on purified peptides using PD-10 Desalting Columns (GE Healthcare); peptides were stored at −80°C.

Cell lines and treatments

Cell lines used in this study were tested for mycoplasma contamination (PCR Mycoplasma detection set, Takara Bio Inc.) every 3 months. 293T and HTLA230 (human neuroblastoma) cells were cultured in Dulbecco’s Modified Eagle’s Medium (Invitrogen); parental and p53-null HCT116 (human colon carcinoma) and parental or derivative (pBabe-puro or pBABE-Slug) Colo205 (human colon carcinoma) cell lines (8) were cultured in RPMI-1640 (Invitrogen) supplemented with 10% heat-inactivated FBS, 100 units/mL penicillin, 0.1 mg/mL streptomycin, 2 mmol/L L-glutamine, at 37°C, 5% CO₂. Cell lines 293T, HCT-116, and Colo205 were obtained from American Type Culture Collection and characterized by DNA fingerprinting and isozyme detection; cell line HTLA230 was obtained from Dr. G. Raschella’s laboratory and was previously described (24). The HCT116 p53-null cell line was kindly provided by Dr. B. Vogelstein (Johns Hopkins University, Baltimore, MD). HTLA230 cells lentivirally transduced with pLKO-shGFP or pLKO-shSlug were previously described (24). LSD1-silenced HCT116 cells were generated by lentiviral transduction with plasmid pGIPZ-shLSD1-H6 (Open Biosystems).

Cells were treated with 100 μmol/L trans-2-phenylcyclopropylamine hydrochloride (Parnate; PCPA), a nonselective monoaminooxidase (MAO-A/B) inhibitor and an LSD1 enzymatic inhibitor (#P8511, Sigma-Aldrich), or with 50 nmol/L monoaminoxidase (MAO-A/B) inhibitor and an LSD1 enzymatic inhibitor (3).

Immunofluorescence

A total of 5 × 10⁶ cells for each time point were seeded on a glass cover in a 12-well microtitre plate. At the end of the treatment with Parnate, cells were fixed with 4% paraformaldehyde in PBS, permeabilized by incubation with PBS + 0.01% Triton X-100 (Sigma) for 5 minutes, and stained with anti-E-cadherin or anti-β-catenin antibody (#610181 and #610153, respectively; BD Transduction Laboratories) in PBS + 5% horse serum. Primary antibody was Alexa Fluor 488–conjugated anti mouse antibody (# A11001; Invitrogen) in PBS + 5% horse serum. Nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI). Photographs were taken using a Zeiss Axiosoph fluorescence microscope equipped with Axiosvision software version 4.6 (Carl Zeiss).

Immunoprecipitation

For immunoprecipitation, a 70% to 90% confluent 100-mm dish (10⁶–10⁷ cells) of 293T cells transfected with HA-LSD1-Flag and 3 pcDNA3-Slug expression plasmids (3 μg each) were lysed in 1 mL of lysis buffer (50 mmol/L Tris-HCl, pH 7.4, with 150 mmol/L NaCl, 1 mmol/L EDTA, 1% Triton X-100, and 0.1% SDS).
protease inhibitors). Cell lysate (3 mg) was incubated at 4°C with 60 μL of anti-Flag M2 Affinity Gel resin (#A2220, Sigma Aldrich) prepared according to the manufacturer’s protocol. PCPA (100 μmol/L) or pTAT-SNAG/pTAT peptide (10 μg/mL) was added to the immunoprecipitation buffer and the immunoprecipitation conducted after overnight (PCPA experiment) or 2-hour (pTAT peptides experiment) incubation.

Beads were then washed with lysis buffer, and the immunoprecipitated protein complexes were resolved by 8% SDS-PAGE in absence of 2-mercaptoethanol.

Luciferase assay
293T and HCT116 cells were transiently transfected using, respectively, ProFection Mammalian Transfection System-Calcium Phosphate (Promega) or SuperFect Transfection Reagent (Qiagen), with 3 μg of reporter plasmid E-cadherin promoter-LUC, 1 μg of pcDNA3 empty vector or pcDNA3-Slug expression plasmid, and 1:50 Renilla luciferase plasmid to account for variation in transfection efficiencies. Twenty-four hours after transfection, cells were treated with 100 μmol/L PCPA for 24 hours or with 50 nmol/L of pTAT peptides for 6 hours. Firefly and Renilla luciferase activity was recorded on a luminometer using the Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer’s protocol. Results are expressed as luciferase activity normalized for Renilla activity and represent the means ± SD of 3 (PCPA) or 2 (pTAT-SNAG or pTAT peptide) experiments.

Migration and invasion assay
For the wound-healing assay, cells were plated to confluence in a 6-well plate, and the cell surface was scratched using a pipette tip. Then, cells were treated with 100 μmol/L PCPA or with 50 nmol/L pTAT-SNAG or pTAT added at 2-hour intervals for 12 hours followed by a final dose 24 hours after scratching the cell surface, allowed to repopulate the scratched area for 3 days, and photographed using a digital camera mounted on an inverted microscope (magnification, ×5). Accurate wounds measurements were taken at 0 and 72 hours to calculate the migration rate according to the equation: percentage wound healing = [(wound length at 0 hour) – (wound length at 72 hours)]/(wound length at 0 hour) × 100. Two independent experiments were carried out.

For invasion assays, cells were plated (10⁵ cell/chamber) in BD BioCoat Matrigel invasion chambers (BD Biosciences). In
the upper chamber, medium was supplemented with 2% heat-
inactivated FBS. In the lower chamber, 20% FBS was used as
chemoattractant. A solution of 100 μmol/L PCPA or 50 nmol/L
pTAT-SNAG or pTAT (at 2-hour intervals for 12 hours) was
added in the upper chamber. After 24 hours, medium was
removed and chambers washed twice with PBS; noninvading
cells were removed from the upper surface of the membrane by
scrubbing with a cotton-tipped swab; invading cells were
fixed with 3.7% formaldehyde in PBS for 2 minutes, washed with PBS
twice, permeabilized with methanol for 20 minutes, washed
twice with PBS, stained with 0.05% crystal violet for 15 minutes,
and washed twice with PBS. Ten fields for each chamber were
photographed using a digital camera mounted on an inverted
microscope (magnification, ×10) and invading cells were
counted in each field. Experiments were carried out in
duplicate and repeated twice.

Real-time quantitative PCR analyses

For real-time quantitative PCR (qPCR), total RNA was
isolated from Parnate or TAT-SNAG-treated cells using the
RNeasy Mini kit (Qiagen). After digestion with RNase-free
DNase (Roche Applied Science), RNA (4 μg) was reverse-
transcribed using SuperScript III Reverse Transcriptase kit
(Invitrogen), and first-strand cDNA used as PCR template.
Reactions were done in triplicate, and RNA was extracted
from 2 separate experiments.

Figure 2. Effect of Parnate on migration, invasion, and proliferation of tumor cell lines. A and B, microphotographs show repopulation of wounded area of untreated and Parnate-treated HCT116 and HTLA230 cells; right, histograms represent accurate wound measurements taken at 0 and 72 hours for each treatment to calculate the migration rate; data are presented as means ± SD from 2 independent experiments; histograms show invasion inhibition (expressed as % of untreated cells taken as 100) by Parnate treatment (C); microphotographs show representative fields of Giemsa-stained lower membranes of the Boyden chambers; histograms show number of untreated and Parnate-treated cells counted by trypan blue exclusion (D); representative of 3 independent experiments carried out in triplicate. *, statistical significance (P < 0.05; Student t test).

A HCT116

T0 NT Day3 25 μmol/L Day3 50 μmol/L Day3 100 μmol/L Day3

B HTLA230

T0 NT Day3 25 μmol/L Day3 50 μmol/L Day3 100 μmol/L Day3

C HCT116

NT PCPA % Invasion NT PCPA % Invasion

D HCT116

T0 T24 T48 NT PCPA N° of cells x 10^6

NT PCPA N° of cells x 10^6

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Homing/engraftment assay of K562 cells in NOD/SCID mice

For the homing/engraftment assay, NOD/SCID mice (NOD.CB17-Prkdc scid/J, Charles River Laboratories International, Inc.; 5/group) were injected (2 x 10^6 cells/mouse) with untreated or PCPA-treated (16 hours, 100 μmol/L) Slug-K562 cells. Twenty-four hours later, mice were sacrificed, bone marrow harvested, and number of K562 cells determined by GFP positivity and by methylcellulose colony formation assays (50,000 cells/plate) in the absence of hemopoietic cytokines to allow growth of K562 cells only.

Statistical analyses

Data (means ± SD, 2 or 3 experiments) were analyzed for statistical significance by unpaired, 2-tailed Student t test. P < 0.05 was considered statistically significant.

Results

Effect of Parnate on the Slug-regulated E-cadherin promoter

Previous studies have shown that LSD1 interacts with the SNAG domain of Snail (29–31) through its C-terminal amine oxidase domain (29); thus, we first assessed whether the monoamine oxidase/LSD1 inhibitor Parnate blocks the interaction between Slug and LSD1 in 293T cells co-transfected with expression vectors encoding human HA-Flag-LSD1 and Slug. As shown in Fig. 1A and B, LSD1 and Slug readily interacted in the absence of Parnate, but their association was suppressed when the cell lysate was treated with this compound; the effect of Parnate was specific because it did not block the interaction of LSD1 with endogenous p53 (ref. 32; Fig. 1A and B). Because the Snail–LSD1 interaction may be necessary for Snail-dependent repression of the E-cadherin promoter (29–31), we conducted luciferase assays in 293T and HCT116 colon cancer cells to test whether ectopically expressed Slug represses the activity of the E-cadherin promoter and if treatment with Parnate blocks the effect of Slug. Indeed, E-cadherin promoter–driven luciferase activity was repressed by Slug in both cell lines and treatment with Parnate blocked the effect of Slug (Fig. 1C and D); the activity of the E-cadherin promoter was also enhanced in cells treated with Parnate only (Fig. 1C and D), probably due to inhibition of endogenous Snail/Slug proteins.

Effect of Parnate on migration and invasion of tumor cell lines

We then asked whether treatment with Parnate impairs the migration and invasion of tumor cell lines. In a wound-healing assay, untreated colon cancer HCT116 and neuroblastoma HTLA230 cells filled almost completely the wounded area 3 days after scratching the cell monolayer, whereas treatment with different doses of Parnate markedly suppressed repair of the wound (Fig. 2A and B). The inhibitory effect of Parnate on migration and invasion of tumor cell lines.
The repopulation of the wounded area was not due to decreased proliferation because growth of untreated and Parnate-treated tumor cells was undistinguishable (Fig. 2D).

The effect of Parnate on cell invasion was tested using Matrigel-coated Boyden chambers; Parnate-treated HCT116, HTLA230, and Colo205 colon cancer cells (parental and Slug-expressing) were markedly less invasive of the untreated counterpart (20%–38% residual invasion; Fig. 2C), but their proliferation rates were identical (Fig. 2D, right). Because the cell lines used in the invasion assays have a wild-type \( p53 \) gene and the \( p53 \)-null genotype promotes the invasiveness of cancer cells (33, 34), we assessed whether treatment with Parnate also suppressed the migration and invasion of \( p53^{-/-} \) HCT116 cells (35). Indeed, migration and invasiveness of these cells was also markedly inhibited by Parnate treatment (Supplementary Fig. S1).

The concept that the effects of Parnate depend on disruption of LSD1 and Snail/Slug activity was validated in LSD1- and Slug-silenced tumor cell lines; as shown in Supplementary Fig. S2, LSD1-silenced HCT116 cells exhibited lower migration and invasion.

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**Figure 4.** Expression of EMT markers detected by real-time qPCR in Parnate-treated (12 hours) tumor cells. Data represent the mean ± SD of 2 experiments. Changes in the expression induced by Parnate treatment are all statistically significant except for those of occludin and desmoplakin in Colo205-Slug cells.
invasion than control HCT116-shGFP cells; likewise, Slug-silenced HTLA230 cells were markedly less invasive than HTLA230-shGFP cells; moreover, they expressed higher levels of the epithelial markers E-cadherin, occludin, and desmplakin and lower levels of the mesenchymal markers vimentin and N-cadherin (Supplementary Fig. S3).

**Effect of Parnate on homing/engraftment of K562 cells**

We showed recently that expression of Slug is required for the homing/engraftment of Philadelphia K562 cells to the bone marrow (36); thus, we assessed whether treatment with Parnate suppresses the interaction between Slug and LSD1 in Slug-overexpressing K562 cells and inhibits the homing/engraftment of these cells to the bone marrow of NOD/SCID mice. As shown in Fig. 3A, anti-Slug Western blotting of LSD1 immunoprecipitations from untreated and Parnate-treated Slug-K562 cells indicate that the amount of Slug in complex with LSD1 was markedly reduced after treatment with Parnate; we assessed bone marrow homing/engraftment of untreated and Parnate-treated (16h) Slug-expressing K562 cells 24 hours after injection in NOD/SCID mice (2 x 10^6 cells/mouse; 5 mice/group) by measuring GFP positivity and colony formation in methylcellulose plates not supplemented with cytokines. As shown in Fig. 3B and C, treatment with Parnate caused a decrease (~50%) in the number of GFP-positive Slug-K562 cells migrating to the bone marrow (B) and of cytokine-independent clonogenic K562 cells (C). Because the hyaluronan receptor CD44 is required for the bone marrow homing/engraftment of BCR-ABL-expressing cells (37), we assessed CD44 levels in untreated and Parnate-treated K562 cells; as shown in Fig. 3D, Slug-K562 cells express higher levels of CD44 than parental cells; however, treatment with Parnate had no effect on CD44 expression, suggesting that the effect of Parnate on K562 homing/engraftment might be CD44-independent.

**Effect of Parnate on expression of epithelial and mesenchymal markers**

Parnate treatment of cancer cell lines should phenocopy the changes in the expression of epithelial and mesenchymal markers induced by Slug downregulation (Supplementary Fig. S3); indeed, Parnate treatment of HCT116, Colo205, and HTLA230 cells induced an increase in the expression of the epithelial markers E-cadherin, occludin, and desmplakin detected by real-time qPCR (Fig. 4). In HTLA230 cells, Parnate treatment induced also a decrease in the expression of the mesenchymal markers vimentin and N-cadherin (Fig. 4), whereas expression of these 2 markers in HCT116 and Colo205 cells was too low to be affected by the treatment (not shown). Expression of members of the miR-200 family, which regulate EMT via ZEB1 and is suppressed by ZEB1 (at least in the case of miR-200c; ref. 6) did not show significant variations after Parnate treatment of HCT116 cells (not shown). In Parnate-treated HCT116 cells, the increase in E-cadherin mRNA transcripts was accompanied by a corresponding increase (at least 2-fold) in protein levels (Fig. 5A). Immunofluorescence microscopy confirmed such increase...
and revealed a distinctive "zipper-like" pattern of E-cadherin expression, which is indicative of adherens junctions' formation between neighboring cells (Fig. 5B; compare untreated and Parnate-treated cells).

Treatment with Parnate also induced an increase in the expression of β-catenin as revealed by Western blotting and immunofluorescence (Fig. 5C and D, respectively).

**Effect of a cell-permeable SNAG domain Slug peptide**

The role of the SNAG domain of Slug for migration and invasion of cancer cells was also investigated upon treatment with a cell-permeable SNAG domain Slug peptide (TAT-SNAG) expected to function as a competitive inhibitor of the interaction between Slug and LSD1. Upon treatment with a single dose of 10 μmol/L, the TAT-SNAG peptide was readily taken up by HCT116 cells exhibiting a half-life of approximately 120 minutes (not shown).

First, we assessed whether the TAT-SNAG peptide added to the cell lysate of 293T cells co-expressing Slug and LSD1 blocked the interaction of these 2 proteins; as shown in Fig. 6A, the TAT-SNAG peptide markedly reduced the amount of Slug in complex with LSD1, whereas the TAT peptide alone had only a modest effect. Then, we conducted luciferase assays in HCT116 cells to test whether treatment with the TAT-SNAG peptide suppressed the inhibitory effect of Slug on the activity of the E-cadherin promoter; as expected, ectopically expressed Slug repressed E-cadherin promoter–driven luciferase activity, and treatment with the TAT-SNAG peptide blocked the effect of Slug (Fig. 6B).

We also investigated the effect of the cell-permeable TAT-SNAG peptide on the migration and invasiveness of HCT116 cells. In a wound-healing assay conducted as described above, treatment of HCT116 cells with the TAT-SNAG peptide markedly reduced repopulation of the wounded area compared with treatment with the TAT peptide alone (Fig. 6C); likewise, HCT116 cells incubated with the TAT-SNAG peptide in the upper part of Matrigel-coated chambers were markedly less invasive of those treated with the TAT peptide alone (Fig. 6D). Neither the TAT-SNAG nor the TAT peptide alone had any effect on the proliferation of HCT116 cells (Fig. 6E). Like Parnate-treated cells, TAT-SNAG–treated HCT116 cells exhibited a 2- to 3-fold increase in the expression of the epithelial markers E-cadherin, desmoplakin, and occludin (Fig. 6F and G). Treatment with the TAT-SNAG peptide also inhibited migration and invasiveness, but not proliferation, of p53-null HCT116 cells (Supplementary Fig. S4).

**Discussion**

The Snail and Slug proteins play an essential role in developmental (38) and cancer-associated EMT (19, 20, 39); mechanistically, the effects appear to be mediated by downregulation of the expression of a number of target genes (e.g., E-cadherin) whose promoters are bound via the C-terminal "zinc fingers" and repressed by chromatin-modifying proteins recruited by the N-terminal "SNAG domain." The histone demethylase LSD1 protein is one of those recruited to the E-cadherin promoter via the "SNAG domain" of Snail (29–31) and this interaction may be important for the EMT-inducing effects of Snail/Slug proteins because migration and invasion of cancer cells is similarly suppressed by either Snail or LSD1 RNA interference (29). We assessed directly the biologic consequences of disrupting protein interactions involving the SNAG domain of Snail/Slug in cancer cells treated with Parnate, an inhibitor of the amine oxidase domain of LSD1 required for its binding to Snail, or exposed to a cell-permeable peptide corresponding to a highly conserved segment of the "SNAG domain" which includes amino acids required for transcription repression. Parnate and the TAT-SNAG peptide blocked the interaction of LSD1 with Slug, rescued the Slug-dependent repression of the E-cadherin promoter activity, and suppressed the Slug/Slug-regulated motility and invasion of cancer cells of different origin and genetic background. Consistent with the concept that the biologic effects of Parnate and the TAT-SNAG peptide depend on disruption of the Slug–LSD1 interaction, silencing of LSD1 or Slug led to decreased migration and invasion of tumor cell lines and modulation of EMT marker levels (Supplementary Figs. S2 and S3). Together, these studies support the importance of the SNAG domain–LSD1 interaction for the EMT-inducing effects of Slug/Slug, although it cannot be excluded that Parnate or the TAT-SNAG peptide used in our assays may have functioned through other pathways or blocked other protein–protein interactions required for the biologic effects of these E-box–binding proteins.

Of interest, neither Parnate nor the TAT-SNAG peptide had any noticeable effect on proliferation of the cancer cell lines used in our studies; this indicates that their effects on the motility and invasion of cancer cells are specific and suggests...
that these compounds (or, more likely, their derivatives) may be used in combination with more conventional cytotoxic drugs or, individually, after surgery or chemotherapy to target residual metastasis-proficient cells. However, potential toxic effects on normal cells need to be examined because both compounds are likely to have pleiotropic effects on gene expression.

Parnate is a monoamine oxidase inhibitor approved by the U.S. Food and Drug Administration since 1985 for treatment of severe depression, but its use is limited by serious side effects caused by high concentration of serotonin and dopamine and paradoxical increase of epinephrine and norepinephrine levels. Moreover, the IC₅₀ of Parnate for inhibition of LSD1 appears to be higher than that for monoamine oxidase A and B (40, 41), possibly preventing prolonged treatments in patients with cancer.

The use described here of the TAT-SNAG peptide to target transcription repression complexes is conceptually similar to that of peptidomimetic inhibitors of the transcription repressor BCL6 which consist of the BCL6-binding domain of the N-CoR and SMRT corepressors (42). One of these, RI-BPI, has shown potent anti-lymphoma and anti-leukemia effects in vitro and in mice (43, 44).

In aggregate, our findings raise the possibility that improved approaches to target Snail/Slug-dependent transcription repression complexes may lead to the development of novel drugs selectively inhibiting the invasive potential of cancer cells.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

References


