Tumor-Penetrating iRGD Peptide Inhibits Metastasis

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Abstract

Tumor-specific tissue-penetrating peptides deliver drugs into extravascular tumor tissue by increasing tumor vascular permeability through interaction with neuropilin (NRP). Here, we report that a prototypic tumor-penetrating peptide iRGD (amino acid sequence: CRGDKGPDC) potently inhibits spontaneous metastasis in mice. The antimetastatic effect was mediated by the NRP-binding RXXK peptide motif (CendR motif), and not by the integrin-binding RGD motif. iRGD inhibited migration of tumor cells and caused chemorepulsion in vitro in a CendR- and NRP-1–dependent manner. The peptide induced dramatic collapse of cellular processes and partial cell detachment, resulting in the repellent activity. These effects were prominently displayed when the cells were seeded on fibronectin, suggesting a role of CendR in functional regulation of integrins. The antimetastatic activity of iRGD may provide a significant additional benefit when this peptide is used for drug delivery to tumors. Mol Cancer Ther; 14(1): 1–9. © 2014 American Association for Cancer Research.

Introduction

Tumor blood vessels are structurally defective (1). They often lack pericytes, and their basement membrane is abnormally loose. The irregular structure leads to leakiness and predisposes tumors to metastasis. The leakiness of tumor vasculature also results in high interstitial fluid pressure, which prevents drug penetration into tumor tissue (2). Poor drug penetration reduces the potential for antitumor efficacy and results in acquired drug resistance. Factors that increase vascular permeability, such as VEGF, bradykinin, and nitric oxide, improve drug distribution into tumor tissue, but can also promote metastasis by enhancing tumor cell access to and from blood vessels (2–4). miR105, a miRNA that disrupts vascular integrity and increases vascular permeability facilitates metastasis formation (5). Forced expression of miR105 in nonmetastatic tumor cells leads to enhanced vascular permeability and metastasis, whereas inhibition of miR105 in highly metastatic tumor cells suppresses metastasis.

Neuropilin-1 (NRP-1) and neuropilin-2 (NRP-2) are some of the key molecules that regulate vascular permeability (6). Tissue-penetrating peptides bind to NRPs through the consensus RXXR/K amino acid sequence, increasing vascular permeability and transport of molecules through tissue (7). The RXXR/K sequence motif is not active unless it occupies a C-terminal position in a peptide, therefore referred to as the C-end Rule (CendR motif) (8). Notably, many natural ligands for NRPs, such as VEGF and class 3 semaphorins, carry a CendR motif, and share various biologic activities with tissue-penetrating peptides (6, 7).

iRGD (cyclic CRGDK/RGPD/EC; the CRGDKGPDC form was used in this study) is a prototypic tumor-specific tissue-penetrating peptide, which delivers drugs deep into extravascular tumor tissue (9). Intravenously injected iRGD first targets αv integrins specifically expressed on tumor vasculature (10). iRGD is then proteolytically processed into CRGDK/R, exposing an active CendR motif at the C-terminus. The interaction of the CendR motif with NRPs initiates an active bulk transport system through the tumor tissue, allowing drugs conjugated to iRGD, and even free drugs coadministered with iRGD, to extravasate and spread within the tumor tissue (11). Thus, iRGD provides a simple way to enhance the therapeutic index of various anticancer drugs. However, the effect of iRGD on the permeability of tumor blood vessels has led to a hypothetical concern that iRGD may promote metastasis through antidromic tumor cell dissemination into the circulation (12). While our earlier studies have shown that iRGD does not promote seeding of nonmetastatic tumor cells (11), the effects of iRGD on metastatic tumors remain elusive. Here, we have examined the iRGD effects on spontaneous metastasis formation in mice bearing aggressive cancers.

Materials and Methods

Peptides, cells, tumor models, and in vivo treatment studies

Peptides were synthesized as described previously (8, 9, 11). PC-3 human prostate cancer cells were purchased from and authenticated by ATCC. GFP-PC-3 cells were prepared by infecting...
the PC-3 cells with GFP lentiviruses (9, 13). LM-PmC cells were prepared by stable mCherry transduction in LM-P cells, which were derived from liver metastasis of pancreatic ductal adenocarcinoma (PDAC) in KrasG12D/+;LSL-Trp53R172H/+;Pdx-1-Cre (KPC) mice and authenticated as described earlier (14). Both cell lines were cultured in DMEM supplemented with 10% FBS and penicillin/streptomycin, and used for no longer than 6 months before being replaced. Tumour mouse models were created by orthotopic injections of 1 million GFP-PC-3 or LM-PmC cells into nude mice 2 weeks (GFP-PC-3) or 1 week (LM-PmC) before the initiation of the treatment study. The mice were intravenously treated every other day with 4 μg/kg of peptides or vehicle (PBS) alone. After 21 days (GFP-PC-3) or 14 days (LM-PmC) of treatment, the mice were dissected under deep anaesthesia, imaged under UV light with an Illumatool Bright Light System LT-9900 (Lightools Research), and perfused through the heart with PBS containing 1% bovine serum albumin (BSA) before harvesting tissues. All animal experimentation was performed according to procedures approved by the Animal Research Committee at Sanford-Burnham Medical Research Institute (La Jolla, CA).

Flow cytometry

The experiments were performed as described previously (9). The primary antibodies were rabbit anti-human NRP-1 b1b2 prepared in-house as by immunizing rabbits with a human NRP-1 b1b2 protein, goat anti-human NRP-2 (R&D Systems), mouse anti-human αvβ3 (LM609) (EMD Millipore), mouse anti-human αvβ5 (P1F6) (EMD Millipore), rat anti-mouse αv (RMV-7; eBioscience), rat anti-mouse α5 (MHR5; SouthernBiotech), mouse anti-human α5β1 (IBSB; Thermo Scientific), mouse anti-human β1 (TS2/16) (eBioscience), mouse anti-human active β1 (HLITS-4; EMD Millipore), rat anti-mouse β1 (H1M1b-1; eBioscience), and rat anti-mouse active β1 (9EG7; BD Biosciences). The primary antibodies were detected with corresponding secondary antibodies conjugated to Alexa488, 594, or 647 (Molecular Probes). The cells bodies were detected with corresponding secondary antibodies prepared in-house as by immunizing rabbits with a human NRP-2 (R&D Systems), rabbit anti-human NRP-1 b1b2 or control IgG for 4 hours. The cells were washed with warm PBS, 4,6-diamidino-2-phenylindole (DAPI; Molecular Probes), and Temperature Controlled Time Lapse System (Olympus IX81 Wide Field and Fluorescence Microscope equipped with CO2 and Temperature Controlled Time Lapse System (Olympus America).

For some cases, the cells were treated with 10 μg/mL of anti-NRP-1 b1b2 or control IgG (Abcam) for 30 minutes before seeding and throughout the assay. After incubation in a CO2 incubator at 37°C for 24 hours, the cells on the upper side of the membranes were gently wiped off, and the membranes were fixed in methanol and stained with hematoxylin and eosin. The membranes were mounted on glass slides, and imaged under a light microscope. The total number of cells that migrated to the lower side of the membranes was determined by counting cell numbers under low magnification (×100).

Chemorepulsion assay

Silver nanoparticles (AgNPs; diameter, 70 nm) were prepared as described previously (16). In brief, AgNPs synthesized by polyvinylpyrrolidone/ethylene glycol reduction method were precipitated with acetone, and redissoled in water. The AgNPs were then coated with NeutrAvidin (NA)–5-kDa polyethylene glycol (PEG)–Orthopyridyl disulfide (OPSS; NA, Thermo Scientific; N-Hydroxysuccinimide-PEG-OPSS, JenKem Technology USA), backfilled with lipoic-3-kDa PEG-amine (Nanocs), and labeled with amine-reactive CE555-succimidyl ester dye (Biotium Inc.). The NA-AgNPs were then coated with biotinylated peptides or with free biotin (Sigma-Aldrich). iRGD-AgNPs, CRGDC-AgNPs, and AgNPs without any peptides were blotted along the periphery of glass-bottom wells by applying 10 μL drops of nanoparticle solutions on the glass surface and drying the drops in a laminar airflow chamber for 5 minutes. The wells were briefly rinsed with PBS three times, and 2 × 105 LM-PmC cells in full culture DMEM were seeded using a cloning cylinder in the center of the wells in close proximity with the AgNPs, or in some experiments, on top of the AgNP-covered areas. The cells were allowed to attach to the glass surface in a CO2 incubator for 3 hours before the cylinder was removed, and further cultured in DMEM with 1% BSA for 24 hours. The wells were then subjected to live cell imaging by taking time-lapse images every 15 minutes for 48 hours with an Inverted IX81 Wide Field and Fluorescence Microscope equipped with CO2 and Temperature Controlled Time Lapse System (Olympus America).

Cell attachment assay

The assays were performed following a protocol described elsewhere (17). Cells resuspended in DMEM containing 1% BSA were treated with peptides for 30 minutes at 37°C under mild rotation. The cells were seeded in 96-well plates coated with human fibronectin (R&D Systems) or bovine collagen type I (BioPioneer) at 4 × 105 cells/mL (LM-PmC) or 2 × 105 cells/mL (GFP-PC-3), and allowed to attach to the wells for 30 minutes at 37°C in a CO2 incubator in the presence of the peptides. In some cases, the cells were treated with the peptides in the presence of 10 μg/mL of anti-NRP-1 b1b2 or control IgG, or treated solely with anti-human β1 (EMD Millipore) or anti-mouse β1 (eBioscience) integrin subunit antibodies or control
IgG (Abcam). After the incubation, the cells were treated with 0.25% crystalline trypsin for 2 minutes, and the reaction was stopped by adding 0.5 mg/mL soybean inhibitor. The wells were gently washed with warm DMEM to remove detached cells, and the cells that remained attached to the wells were cultured in full DMEM containing 0.5 mg/mL of MTT. Two hours later, the cells were lysed with a buffer containing dimethylsulfoxide and methanol at 1:1 volume ratio and absorbance at 595 nm was read with a microplate reader.

In vitro cell retraction assay

Cells were cultured on fibronectin-coated coverslips for 1 hour at 37°C in a CO2 incubator, and peptides were added at a final concentration of 10 μmol/L. The cells were cultured for another 30 minutes at 37°C in a CO2 incubator, fixed in 4% PFA, stained with a rabbit anti-phospho-paxillin pTyr118 antibody (Pierce Biotechnology) and DAPI, and imaged under a Fluoview 500 confocal microscope. The anti-phospho-paxillin pTyr118 was detected with an Alexa488 donkey anti-rabbit antibody (Molecular Probes).

Statistical analysis

Data were analyzed by the two-tailed Student t test, one-way ANOVA followed by a suitable post hoc test or a Pearson test. The results are summarized in Supplementary Table S1.

Results

iRGD inhibits prostate cancer metastasis in a CendR-motif-dependent manner

We tested the effect of the iRGD peptide (cyclic CRGDKGPDC) on metastasis by giving intravenous injections of iRGD to mice bearing orthotopic xenograft tumors of GFP-labeled PC-3 human prostate cancer cells (GFP-PC-3), which develop spontaneous metastases in various organs (13). GFP-PC-3 cells express high levels of cell surface αv integrins and NRPs (Fig. 1A). In vivo, the blood vessels and tumor cells in orthotopic and metastatic GFP-PC-3 tumors are effective targets for iRGD (9, 11). Intravenous iRGD injections repeated every other day over 3 weeks significantly inhibited spontaneous metastasis (Fig. 1B and C). The antimetastatic effect of iRGD was dependent on the NRP-binding RXXK CendR motif because RGDfV, a conventional cyclic RGD peptide that lacks a CendR motif (10, 11), and iRGDD (CRGDDGPKC), a scrambled iRGD with a disrupted CendR motif (11), did not inhibit metastasis. iRGD slightly inhibited primary tumor growth (Fig. 1D). However, no significant correlation was found between the weight of the primary tumor and metastatic burden (Supplementary Fig. S1A), indicating that delayed growth of the primary tumor was not a significant cause of the iRGD-mediated metastasis inhibition. The finding that iRGD treatment alone has minor effect on primary tumor growth is consistent with previous reports (9, 11).

Figure 1.

iRGD inhibits spontaneous metastasis in a prostate cancer mouse model. A, neuropilin and αv integrin expression in GFP-PC-3 human prostate cancer cells analyzed by flow cytometry. The profiles represent the values of cells incubated with isotype control (red) or appropriate neuropilin (NRP) or integrin antibodies (blue) as primary antibodies. B–D, mice bearing orthotopic GFP-PC-3 tumors implanted 2 weeks earlier received intravenous injections of 4 μmol/kg of iRGD, a scrambled iRGD with a disrupted CendR motif (iRGDD: CRGDDGPKC), or a conventional non-CendR RGD peptide (RGDfV), or PBS, every other day for 21 days. The mice were necropsied after the treatment and viewed under a fluorescence imager (B). PT, primary tumor; L, liver. Metastatic burden was analyzed by quantifying fluorescence intensity with ImageJ (C). Weight of primary tumors (D). n = 5 per group. One of three experiments that gave similar results is shown. Error bars, mean ± SEM. Statistical analyses were performed with ANOVA: n.s., not significant; *, P < 0.05; **, P < 0.01.
iRGD and another tumor-penetrating CendR peptide, iNGR, inhibit pancreatic cancer metastasis

We also tested iRGD in a transplantable PDAC mouse model. The model was created by orthotopic inoculation of mCherry-labeled LM-P (LM-PmC) cells. The LM-PmC cells were established from a liver metastasis of de novo PDAC in KPC mice, which carry k-Ras and p53 mutations that are commonly found in human PDACs (14, 18). The cells express α integrins and NRPs, and are effectively targeted by iRGD in vitro and in vivo (Supplementary Fig. S2). iRGD also inhibited metastasis in this model (Fig. 2A and B), whereas iRGDD, and another non-CendR cyclic RGD peptide, CRGDC (19), did not, indicating dependence of the activity on the CendR motif in iRGD. We also tested another tumor-specific CendR peptide, iNGR (CRNGRGPDC), which targets a variant CD13 in tumor vessels with its NGR motif, and accomplishes NRP-dependent tumor penetration with its CendR motif (20). iNGR effectively internalized into LM-PmC cells in vitro and targeted LM-PmC tumors in vivo (Supplementary Fig. S2B and C). Prolonged systemic treatment with this peptide also inhibited metastasis in the LM-PmC model, further supporting the role of the CendR motif in the activity. Neither iRGD nor iNGR affected primary tumor growth in this model (Fig. 2C).

Conventional RGD peptides block tumor cell seeding to tissues by interfering with α integrin functions, inhibiting experimental metastasis created from intravenously injected tumor cells (10). However, these monomeric peptides have little effect on spontaneous metastasis, which involves the entire metastatic cascade (21). Even RGD-coated nanoparticles, which bind to integrins with higher avidity and have a longer half-life than the free peptide, have limited effect on spontaneous metastasis unless the particles are loaded with antineoplastic drugs (22). Our results are in line with these earlier findings. CRGDC has a similar affinity to α integrins as iRGD (9), and inhibits tumor cell attachment to vitronectin as effectively as iRGD (Supplementary Fig. S3). RGDfV has a nearly 10-fold higher affinity to α integrins than CRGDC (23), and is an even more potent inhibitor of tumor cell attachment to vitronectin than iRGD and CRGDC. Neither CRGDC nor RGDfV inhibited spontaneous metastasis at the dose used for the CendR peptides (refer to Figs. 1 and 2), indicating that the iRGD effects are not mediated by the inhibition of αv-dependent cell attachment.

iRGD inhibits tumor cell migration in vitro

We next studied the effect of iRGD and other CendR peptides on tumor cell motility in search for a possible mechanism of the antimitastatic activity. iRGD and iNGR, as well as RPARPAR, a non–tumor-specific NRP-binding CendR peptide (8), significantly inhibited random migration of LM-PmC and GFP-PC-3 cells in Transwell assays (Fig. 3A and B). An anti-NRP-1 b1b2 antibody, which blocks CendR peptide binding to the NRP-1 b1b2 domain (24), dose dependently inhibited the effects of the peptides. CRGDC and RGDfV had no effect on cell migration at the dose used for the CendR peptides. None of the CendR peptides affected tumor cell proliferation in vitro (Supplementary Fig. S4).

iRGD has chemorepulsive properties

Tumor cell migration in the Transwell system was also inhibited when iRGD was added only to the lower chamber (the chamber into which the cells migrate), suggesting that iRGD has chemorepulsive properties (Fig. 3C). Accordingly, we developed a chemorepulsion assay based on synthetic AgNPs to further study tumor cell response to iRGD. iRGD-coated AgNPs (iRGD-AgNPs), CRGDC-coated AgNPs (CRGDC-AgNPs), and plain AgNPs were immobilized on a glass surface, and tumor cells were seeded in the center in close proximity to the AgNPs using a cloning cylinder (Supplementary Fig. S5). Live cell imaging showed that LM-PmC cells were repelled by iRGD-AgNPs (Fig. 4A and Supplementary Movie SM1). Imaging at single-cell level showed dramatic collapse of cellular protrusions and inhibition of migration beyond the boundary line of iRGD-AgNPs (Supplementary Movies SM2 and SM3). Single cells that landed on surfaces coated with iRGD-AgNPs lost contact with the surface and died likely through anoikis (10), while cells that attached to noncoated surfaces spread and remained adherent. In contrast, the cells were attracted to CRGDC-AgNPs, in line with the capability of traditional RGD peptides to serve as a scaffold for cell attachment, spreading, and...
survival (ref. 10; Supplementary Movie SM4). The cells randomly invaded areas coated with plain AgNPs (Supplementary Movie SM5). We next seeded cells onto surfaces coated densely with the AgNPs to force migration on the AgNPs. The cells migrated approximately 2-fold slower on iRGD-AgNPs than on plain AgNPs or CRGDC-AgNPs (Fig. 4B). These results show that iRGD has chemorepulsive properties, and confirm its inhibitory effects on cell migration.

iRGD inhibits tumor cell attachment to fibronectin through interaction with NRP-1

Semaphorin 3A (Sem3A), also known as collapsin, is a chemorepulsive glycoprotein that collapses and paralyzes neuronal growth cones to repel axons (25). Interestingly, Sem3A and other class-3 semaphorins have an internal CendR sequence (RNR/PR), which interacts with NRPs upon proteolytic activation by furin (26–28). Sem3A and semaphorin 3F (Sem3F) also repel non-neuronal cells. The apparent mechanism is weakening of cell attachment to fibronectin mediated by β1 integrins, leading to collapse of cellular processes, and inhibition of cell migration and metastasis, all in a NRP-dependent manner similar to the iRGD activities (28–31). The semaphorins also possess other iRGD-like properties such as enhancing vascular permeability and internalization into cells (3, 28, 29). A NRP-binding 22 amino acid peptide made from the C-terminus of Sema3A serves as a tumor-penetrating drug delivery carrier similar to iRGD (32). Thus, it appears that iRGD utilizes a similar mechanism as Sema3A in collapsing cellular processes and repelling tumor cells.

LM-PmC and GFP-PC-3 cells attach to fibronectin predominately through a β1 integrin-mediated mechanism (Fig. 5A). The cells express high levels of surface β1 integrins, including α5β1, which primarily recognizes fibronectin (Fig. 5B; ref. 10). A large proportion of their β1 integrins are in an active conformation. iRGD inhibited the tumor cell attachment to fibronectin in a dose-dependent manner (Fig. 6A and Supplementary Fig. S6A). Most conventional RGD peptides bind both to αv and α5β1 integrins (19, 23), whereas the RGD motif of iRGD is selective for αv integrin binding (9), suggesting that iRGD-induced cell retraction is not due to direct competition with fibronectin. Instead, the iRGD effects were reversed by anti-NRP-1 β1β2 antibodies, indicating a NRP-1–dependent mechanism (Fig. 6A and Supplementary Fig. S6A). CRGDC, which competes with fibronectin in α5β1

Figure 3.

iRGD inhibits tumor cell migration in Transwell assays. LM-PmC (A and C) or GFP-PC-3 (B and C) cells were seeded on the upper side of a Transwell filter, and the number of cells that migrated to the other side of the filter was quantified. A and B, iRGD, non-CendR RGD peptides (CRGDC and RGDfV), or non-RGD CendR peptides (iNGR: CRNGRGPDC or RPARPAR) at a final concentration of 10 μmol/L or PBS was added to both upper and lower wells. C, iRGD or CRGDC at a final concentration of 10 μmol/L or PBS was added only to lower wells. Anti-NRP-1 β1β2 or control IgG was added to some of the wells. n = 3 per experiment. Nontreated columns were considered as 100%. Error bars, mean ± SEM; statistical analyses, ANOVA; *, P < 0.05; **, P < 0.01; ***, P < 0.001. Statistics against the nontreated columns are shown unless otherwise noted.
binding (19), inhibited tumor cell attachment to fibronectin in an RGD motif–dependent manner (Fig. 6B and Supplementary Fig. S6B). Anti-NRP-1 b1b2 had no effect on this activity. CRGDK, a post-cleavage mimic of iRGD, which binds to NRP-1 but has negligible RGD activity (9), inhibited cell attachment to fibronectin. iNGR and RPARPAR, CendR peptides without an RGD motif were also effective, but RPARPAR-NH2, an RPARPAR variant with a blocked C-terminus that lacks affinity to NRPs (8), was not. Immunocytochemistry revealed that iRGD dramatically collapsed cellular protrusions on fibronectin-coated surfaces (Fig. 6C and Supplementary Fig. S6C). The cell retraction was accompanied by partial disassembly of focal adhesions, integrin-containing structures that mediate cell spreading and motility on fibronectin, and other extracellular matrices (10, 33, 34). Two other CendR peptides, iNGR and RPARPAR, also caused collapse of cellular processes, whereas RPARPAR-NH2 was inactive. These results suggest the possibility that iRGD inhibits tumor cell attachment to fibronectin by functional regulation of β1 integrins through interaction with NRP-1. Importantly, iRGD did not affect cell attachment to collagen type-I (Supplementary Fig. S7), which is mainly recognized by α1β1 and α2β1 integrins (35), suggesting that iRGD does not universally affect β1 integrins but selectively regulate fibronectin-binding integrins. Earlier studies have reported NRP-1–mediated endocytic removal of cell surface α5β1, which supports our observations (36).

**Discussion**

Here, we show that iRGD, the prototypic tumor-penetrating CendR peptide, is a potent inhibitor of metastasis, and provide evidence that a likely mechanism underlying the activity is inhibition of tumor cell migration and chemorepulsion mediated through binding of the CendR peptide motif to NRP-1. This study reveals a potentially important and useful antimetastatic activity of iRGD, and removes the hypothetical concern (12) that iRGD might enhance metastasis.

Of the two active motifs in iRGD, the NRP-binding CendR motif is important for the antimetastatic activity, whereas the integrin-binding RGD motif appears to be only needed to bring the peptide to the tumors or tumor cells. The evidence includes the
lack of antimetastatic activity by RGD peptides with no CendR motif, and the effective metastasis inhibition by iNGR, which uses a non-integrin receptor to home to tumors and is then processed to bind to NRP (20). Both NRP-1 and NRP-2 are highly expressed on tumor cells, and have been shown to play a role in metastasis (37). iRGD and iNGR bind to both NRP-1 and NRP-2 (20), suggesting the possibility that the metastasis inhibition is a combined effect through both NRPs.

Cell migration is a critical step in the metastatic cascade (38). It involves dynamic regulation of cellular protrusions, which mediate cell adhesion, provide traction to move, and sense environmental cues that attract or repel the cells (34, 39). Some cell migration inhibitors function as chemorepellents by collapsing frontal cellular protrusions to eliminate forward traction, which helps the cells to change direction (40). Various chemorepellents, including the natural CendR molecule Sema3A, inhibit metastasis by suppressing tumor invasion (28–30, 41). Our live cell imaging study showed that iRGD provided cues to retract cellular protrusions, and consequently repelled migrating tumor cell populations. The effect was dependent on the NRP-binding CendR motif because CRGDC, a non-CendR control peptide, was ineffective, and chemorepulsion by iRGD in Transwell assays was significantly inhibited by a blocking anti-NRP-1 b1b2 antibody. These findings demonstrate the CendR-dependent chemorepulsive properties of iRGD, and provide a potential mechanism to the antimetastatic effects.

The retraction of cellular protrusions induced by iRGD appears to involve a NRP-1–mediated regulation of cell adhesion, which is prominently displayed on fibronectin-coated surfaces. The tumor cells we used in this study attach to fibronectin mainly through b1 integrins, suggesting that the deadhesion of the protrusions induced by iRGD was through negative regulation of fibronectin-binding b1 integrins. Importantly, the effects of iRGD on the deadhesion is clearly through CendR–NRP-1 interactions, as an anti-NRP-1 b1b2 antibody significantly blocked the iRGD effects, and other NRP-binding CendR peptides were effective while a CendR variant that lacks affinity to NRP was not. Thus, our results describe a string of CendR-mediated events, i.e., regulation of fibronectin-binding integrins, retraction of protrusions, and decreased avidity to fibronectin, which may contribute to the chemorepulsive properties of iRGD.

Our results question a general perception that NRPs are coreceptors that do not signal (37). NRPs lack a cytoplasmic signaling motif. Thus, it is thought that their central role is to modulate the functions of signaling receptors, such as VEGF receptor and plexin. However, the activities of the tumor-penetrating peptides we have
used in this study suggest independent signaling by NRPs. The effects of our peptides resemble those of Sem3A with an important difference: Semaphorins are large proteins that have activities other than NRP binding; whereas the small size of our peptides and the fact that their only common denominator is the presence of a CendR motif, strongly suggest that NRP binding alone is responsible for the antimitastatic and cell-repelling activities. Supporting this possibility, recent studies demonstrate that NRPs alone are capable of mediating various effects, such as endocytosis and phosphorylation of signaling adaptor molecules (6). Tumor-penetrating peptides may be ideal tools for dissecting intrinsic NRP functions.

The dose and administration schedules we used to establish the antimitastatic effects of iRGD (and inNGR) were identical to those we have previously used to promote drug delivery into tumors (11). Thus, our results suggest that tumor-penetrating CendR peptides will simultaneously achieve metastasis inhibition when used as an adjuvant therapy to promote tumor-specific drug delivery. The antimitastatic effects of iRGD could be useful in preventing initial metastasis, in suppressing additional metastasis when metastasis has already taken place, and in overcoming the prometastatic side effects of antiangiogenic therapies (41, 42).

Disclosure of Potential Conflicts of Interest
K.N. Sugahara, V.R. Kotamraju, T. Teesalu, and E. Ruoslahti have ownership interest (including patents) in CendR Therapeutics Inc. No potential conflicts of interest were disclosed by the other authors.

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References

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