A peptide inhibitor of synuclein-γ reduces neovascularization of human endometriotic lesions

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ABSTRACT: Endometriosis is a chronic painful gynecological condition characterized by adherence and growth of endometrium outside of the uterine cavity. Neovascularization is essential to the developing endometriosis lesion to support its growth. Synuclein-γ (SNCG), a protein implicated in cellular proliferation, is associated with a broad range of malignancies as well as endometriosis. We hypothesized that SNCG plays an important role in the neovascularization and growth of endometriosis and blocking of SNCG will interfere with survival of endometriotic lesions in a mouse model. We developed SP012, a novel 12 amino acid peptide inhibitor of SNCG. SP012 inhibited three-dimensional endothelial cell tube formation in a dose-dependent manner. Using intravital microscopy, SP012 was shown to be successfully delivered to human endometriotic lesions in a xenograft mouse model in vivo. A lymphoid (BALB/c-Rag2−/−Il2rg−/−lacking T, B and NK cells) mice were surgically induced with human endometriotic lesions and treated with SP012 or phosphate-buffered saline control. SP012 treated endometriotic lesions had decreased growth, development and vascularization at the time of necroscopy. Endometriotic lesions treated with SP012 also had fewer isolectin (+) microvessels. These results, using a mouse model, indicate that SNCG plays a role in the neovascularization and subsequent growth of human endometriotic lesions. Targeting SNCG function using peptide inhibitor might provide a potential therapeutic option for the treatment of endometriosis in the future.

Key words: angiogenesis / endothelial cells / endometriosis / mouse model / synuclein-gamma

Introduction

Endometriosis is a gynecological disease characterized by the estrogen-dependent growth of endometrium outside of the uterus. Affecting females mostly during their reproductive years, it causes pelvic pain, dyspareunia, dysmenorrhea, and is strongly associated with infertility. Retrograde menstruation (Sampson, 1927), the movement of menstruating endometrium from the uterus through the Fallopian tubes into the peritoneal cavity, is the widely accepted theory by which endometrium is found outside the uterus. Once in an ectopic location, endometrium adheres to the surrounding tissue and begins to grow. The growing endometriosis lesion must establish a vascular network, via angiogenesis and/or post-natal vasculogenesis (Laschke et al., 2011; Virani et al., 2013) to support its metabolic needs. Indeed, a defining characteristic of an endometriosis lesion is a robust blood supply (Nisolle et al., 1993). Angiogenesis, the growth of blood vessels from pre-existing blood vessels, is a complex physiological process (Risau, 1997). The process of angiogenesis within endometriotic lesions is not fully understood, but three general mechanisms have been proposed: sprouting, elongation and intussusception. The extension of new vessel branches from pre-existing capillaries requires proteolytic degradation of extracellular matrix, proliferation and migration of endothelial cells, and ultimately the formation of capillary tubules with open lumens to bring blood to the site of the angiogenic stimuli (Risau, 1997). A number of growth factors/cytokines exert chemotactic and proliferative effects on endothelial cells and their surrounding pericytes. New blood vessel formation is a relatively fragile process, subject to disruptive interference at multiple levels.

Synuclein-γ (SNCG), a protein implicated in cellular proliferation (Singh and Jia, 2008), has previously been shown to have elevated expression in endothelial cells of endometriosis lesions (Singh et al., 2008). SNCG, a member of the synuclein family of neuronal proteins (Lavedan, 1998; George, 2002), is involved in cellular proliferation by interacting with the mitotic checkpoint kinase BubR1, and inhibiting its regulatory role on cellular proliferation (Gupta et al., 2003). SNCG has also been shown to induce expression of key matrix metalloproteinases (MMP-2, and MMP-9) (Surgucheva et al., 2003), which are enzymes involved in both endometriosis disease progression (Han et al., 2012) and angiogenesis (Stetler-Stevenson, 1999). In vitro experiments have...
implicated SNCG in estrogen receptor signaling (jiang et al., 2004), an essential element in the estrogen-dependent growth of endometriosis lesions.

We postulate that elevated levels of SNCG in endometriosis lesions play a key role in the cellular proliferation essential to angiogenesis and subsequent disease progression. Here we demonstrate the development of SP012, a novel peptide inhibitor of SNCG, which inhibits angiogenesis in vitro and reduces neovascularization of human endometriotic lesions in a xenograft mouse model of endometriosis in vivo.

Materials and Methods

Ethics

Ethics approval for this study was obtained from the Health Sciences Research Ethics Board, Queen’s University, Kingston, ON, Canada (Approval No. ANAT-029-09). Written informed consent from all subjects was obtained prior to sample collection. All the procedures employing use of animals were approved by the Queen’s University Animal Care Committee (Animal utilization protocol number 2009-066-R3-A1).

Acquisition of human eutopic and ectopic samples

Paraffin-embedded sections of normal human endometrium and endometriosis samples in the proliferative phase were obtained from Kingston General Hospital. Endometrial biopsies were obtained from women with no known endometrial pathology or women with endometriosis during the gynecology clinics held at the Kingston General Hospital. Endometrial biopsies were acquired and stored at 4°C until time for engraftment into experimental mice.

Immunohistochemistry of SNCG in eutopic and ectopic endometrium

Tissue samples were fixed in 10% buffered formalin prior to embedding in paraffin wax. Embedded samples were sectioned at 5 μm, and adhered to positive charged glass slides overnight at 37°C. Sections were deparaffinised in xylene, and rehydrated in decreasing concentrations of ethanol and double deionized water (ddH2O). Sections were then incubated in sodium citrate antigen retrieval buffer (0.01 M, pH 6.0 in phosphate-buffered saline (PBS)) at 95°C for 15 min. Sections were rinsed in PBS and endogenous peroxidase activity was blocked using 1% hydrogen peroxide in PBS for 15 min. Following a PBS rinse, sections were blocked in 1% bovine serum albumin (BSA) for 60 min at room temperature. Sections were then incubated in primary antibody (Goat anti-Human SNCG, 0.002 mg/ml, Santa Cruz Biotechnology, Santa Cruz, CA, USA) overnight at 4°C in a humidified chamber. After a PBS rinse, slides were incubated with secondary antibody (Donkey anti-Goat-horse radish peroxidase, 0.000667 mg/ml, Santa Cruz Biotechnology) for 60 min at room temperature. Sections were washed in PBS and covered with 3,3′-diaminobenzidine (DAB) liquid chromagen substrate system (DAKO, Markham, ON, Canada) for 2 min. Finally, counterstaining was carried out using hematoxylin (Gill’s method, Fisher Scientific, Fair Lawn, NJ, USA) for 10 s and then rinsed in tap water. Sections were dehydrated in increasing concentrations of ethanol, cleared in xylene and coverslipped using Permount mounting media (Electron Microscopy Sciences, Hatfield, PA, USA).

Synthetic peptides

Cell permeable peptides (TAT-SP012: GYGRKKRRQRRRNSALHVASQHG and control TAT: GYGRKKRRQRRR) were synthesized as peptide amides by AmbioPharm, Inc. (North Carolina, USA). The identity was verified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. The purity of the peptides was ~99.5% as assessed by analytical reverse-phase high-performance liquid chromatography. The Gly and Tyr residues were incorporated upstream of TAT sequence (GRKKRRQRRR) for easier identification of fluorescein isothiocyanate (FITC) by anti-FITC antibodies during co-immunoprecipitation experiments. The peptides were dissolved in PBS solution, aliquoted and stored at ~80°C until required for use.

Co-immunoprecipitation: detection of SNCG association with internalized peptides

T47D cells were treated with 10 μM FITC-TAT-SP012 or FITC-TAT alone to observe the association of cell permeable SP012 with endogenously expressed SNCG in cells. After 4 h incubation, cell lysate was prepared as described previously (Singh et al., 2007) followed by co-immunoprecipitation using 10 μg of goat anti-SNCG antibody. Western blotting was performed with a monoclonal anti-FITC antibody (BioLegend, San Diego, CA, USA; 1:500 dilution) and then subsequently probed with 1:10000 dilution of a goat anti-mouse antibody (Santa Cruz Biotechnology, Inc.).

Cloning and expression of GFP-SP012

A DNA fragment of SP012 was cloned into an expression vector pEGFP-C2 to produce an enhanced green fluorescent protein (EGFP)-SP012 fusion protein. Cytoplasmic expression of EGFP-SP012 fusion protein was confirmed by green fluorescent signals in cells transfected with pEGFP-SP012. The correct molecular mass of EGFP-SP012 was verified by western blotting with anti-GFP antibody.

Human umbilical vein endothelial cell SNCG immunofluorescence

Human umbilical vein endothelial cells (HUVECS, 1.0 × 10^6) were added to six-well plates (Corning Incorporated, Corning, NY, USA) containing glass coverslips (Fisher Scientific). Cells were allowed to adhere to the coverslip for 24 h. Coverslips were fixed with 75% ethanol for 2 min, rinsed in PBS and incubated with primary antibody (Goat anti-Human SNCG, 0.002 mg/ml, Santa Cruz Biotechnology) for 1 h at room temperature. Coverslips were washed with PBS, incubated with secondary antibody (Donkey anti-Goat-PE, 0.001 mg/ml, Santa Cruz Biotechnology) for 30 min at room temperature and mounted onto glass slides using Prolong Gold antifade reagent with 4′,6-Diamidino-2-phenylindole (DAPI, Invitrogen, Eugene, OR, USA). Cells were then photographed using an epifluorescent microscope AxioVision SE64 Rel. 4.8 (Carl Zeiss Canada Ltd, Toronto, ON, Canada).

In vitro angiogenesis assay

The effect of SP012 on angiogenesis was assessed in vitro using an endothelial cell tube formation assay (Cell Biolabs, San Diego, CA, USA) as previously described (Nakamura et al., 2012). In brief, 1.0 × 10^4 HUVECs were seeded onto 50 μl of solidified extracellular matrix gel, an extract prepared from Engelbreth-Holm-Swarm sarcoma from mice, which contains laminin collagen type IV, heparin sulfate proteoglycans and entactin. Cells were incubated with different concentrations of SP012 (2.5, 5, 150, 300, 600 ng/ml) or PBS control. Cells were washed with PBS and a staining solution containing FITC was added. Cells were incubated at 37°C for 30 min, washed with PBS and visualized with fluorescent confocal microscopy (Leica TCS SP2 Multi Photon; Leica Microsystems, Concord, ON, Canada). A semi-quantitative assessment was performed using the ImageJ Pro Plus software version 6.0 (NIH, Bethesda, MD, USA). An independent observer traced the area of the endothelial cell...
tube structures, and calculated the integrated optical density expressed in arbitrary units.

**Intravital fluorescent microscopy**

Alymphoid mice engrafted with human endometriotic lesions (described later) were given i.p. injections of 25 mg/kg SP012-FITC. After 8 h, mice were anesthetized with ketamine and xylazine, and a catheter was inserted into the jugular vein. The peritoneal wall was exteriorized, and the human endometriotic lesion was placed on a fluorescent confocal microscope platform (Leica TCS SP2 Multi Photon; Leica Microsystems). Rhodamin-6 g (1 mg/ml, Sigma-Aldrich, Oakville, ON, Canada) was injected into the catheter to visualize blood vessels.

**Alymphoid xenograft mouse model of endometriosis**

Six- to eight-week old female alymphoid (BALB/c-Rag2−/−Il2rg−/−lacking T, B and natural killer cells) mice were induced with human endometriotic lesions as previously described (Nakamura et al., 2012). Breeding pairs of these mice were kindly provided by Dr. M. Ito (Central Institute for Experimental Animals, Kawasaki, Japan). All experiments were performed under protocols approved by the Queen’s University Institutional Animal Care Committee. In brief, on Day 0, 60-day slow-release estradiol 17-acetate pellets (15 mg/pellet; Innovative Research of America, Sarasota, FL, USA) were implanted s.c. to support the growth of estrogen-dependent endometrial tissue. On Day 5 endometriotic lesions were surgically induced by placing non-pathological eutopic endometrium collected by tissue biopsy at Kingston General Hospital. Endometrium was divided up into ~1 mm³ pieces and stored on ice for 30–60 min in PBS until surgery. A small incision was made in the abdomen of each mouse and two tissue pieces were dropped into the peritoneal cavity.

On Day 12, mice began to receive daily i.p. injections of 25 mg/kg SP012 (in 100 μl of PBS, n = 4) or PBS control (n = 4). This continued until Day 26. On Day 27, animals were sacrificed and endometriotic lesions were harvested and visualized using a dissecting microscope. Tissues were subsequently snap frozen in OCT cryomatrix (Thermo Scientific, Kalamazoo, MI, USA), and stored at −80°C for histological analysis. Two lesions per mouse were analyzed at three different depths throughout the endometriotic lesion for all histology experiments. In a separate experiment mice received daily injections of SP012 or PBS from Day 12 to Day 17, and then used for intravital microscopy confocal experiments as described.

**Isolectin IB4 immunofluorescence**

Isolectin-IB4, a pan endothelial cell marker extracted from Bandeiraea simplicifolia, was used to localize blood vessels in human endometriotic lesions. Frozen tissues in cryomatrix were sectioned at 5 μm onto positive charged glass slides. Sections were air dried for 5 min, fixed in 70% ethanol for 2 min, rinsed in ddH2O for 2 min and blocked in 1% BSA in PBS for 40 min. Sections were rinsed in PBS and then incubated with isolecitin-IB4 conjugated with Alexa Fluor 488 (0.0033 mg/ml in PBS, Invitrogen-Life.

**Figure 1** Immunohistochemistry for SNCG in human eutopic endometrium, myometrium and ectopic endometrium. SNCG had limited expression in eutopic endometrium (A), but was primarily expressed in perivascular areas of myometrium (B), and ectopic endometrium (mural cells highlighted in (C), endothelial cells highlighted in (D)). Semi-quantitative analysis (E) showed a significant increase in SNCG expression in ectopic compared with eutopic endometrium. Images are magnified ×200, and scale bars represent 200 μm. N = 5; *p < 0.05. Data are representative of three independent experiments. Data are represented as mean plus or minus SD.
Technologies, Burlington, ON, Canada) for 2 h. Sections were rinsed in PBS and coverslipped with Prolong Gold antifade reagent with DAPI (Invitrogen). Sections were photographed under an epifluorescence microscope using AxioVision SE64 Rel. 4.8 (Carl Zeiss Canada Ltd). Isolectin-Ib4 fluorescence was assessed semi-quantitatively using the ImageJ Pro Plus software version 6.0 (NIH). The area of the endometriotic lesion was traced out using a free hand drawing tool by an independent observer, and the integrated optical density expressed in arbitrary units was calculated.

**Statistical analysis**

Data were analyzed using GraphPad Prism 6 (GraphPad Software, La Jolla, CA, USA) by Student’s t-test or one-way analysis of variance (ANOVA) with a Holm-Sidak post hoc analysis. Error bars represent SD and a P-value <0.05 was considered significant.

**Results**

**Expression of SNCG is elevated in endometriosis**

Using immunohistochemistry we detected limited expression of SNCG in eutopic endometrium (Fig. 1A), but robust expression in perivascular areas of myometrium (Fig. 1B) or ectopic endometrium (Fig. 1C and D). Semi-quantitative analysis revealed that ectopic endometrium had a significantly higher expression of SNCG compared with eutopic endometrium (Fig. 1E). These results demonstrate that SNCG is elevated in endometriosis lesions and its localization in blood vessels suggests a role in their vascularization.

**SP012 is taken up by human endometriotic lesions in vivo**

We investigated if SP012 could be taken up by human endometriotic lesion cell types in vivo in a mouse model. First we showed by co-immunoprecipitation that SP012 binds to SNCG (Fig. 2A). In alymphoid mice with human endometriotic lesions, a strong FITC signal was seen within the endometriotic lesion 8 h after i.p. injection of SP012-FITC (Fig. 2C), but no signal was seen in the adjacent peritoneal wall (Fig. 2B). Using immunohistochemistry we demonstrated that SNCG is not expressed in the mouse peritoneal wall (Supplementary data, Fig. S1). These results indicate that SP012 is specifically taken up by cell types of human endometriotic lesions in vivo.

**SP012 inhibits three-dimensional endothelial tube formation**

We confirmed that SNCG is expressed in HUVECs using immunofluorescence (Fig. 3A and B). Using a three-dimensional in vitro angiogenesis assay, we found that increasing concentrations of SP012 completely inhibited three-dimensional tube formation in HUVECs (Fig. 3C–I). Using a water-soluble tetrazolium salt (WST)-1 cell proliferation assay, we also found that increasing concentrations of SP012 reduced HUVEC proliferation (data not shown).

**Figure 2** Western blot showing coimmunoprecipitation of the SNCG-SP012-FITC complex. Coimmunoprecipitation was conducted using anti-SNCG antibody as described in Materials and Methods. We observed evidence of SNCG in an immunoprecipitate complex in the presence of SP012-FITC (top panel, left lane) in contrast to control, TAT-FITC (top panel, right lane), when using a FITC antibody. The cell lysate shows the presence of SNCG and peptides (FITC, right lane). SP012-FITC injected into the peritoneal cavity of mice induced with human endometriotic lesions had FITC fluorescence in the endometriotic lesion (C), but not in adjacent peritoneal wall (B). Scale bars represent 200 μm.

**SP012 reduces endometriotic lesion vascularity in a mouse model**

Having confirmed that SP012 affects angiogenesis in vitro, we then assessed the effect of SP012 on human endometriotic lesion neovascularization and growth in our xenograft alymphoid mouse model of endometriosis. When control mice (treated with PBS) were injected with Rhodamin-6G dye, there was an intricate network of blood vessels supplying the human endometriotic lesion (Fig. 4A–C). However, in SP012-treated mice, only one or two blood vessels were seen per field of view in human endometriotic lesions (Fig. 4D–F). To confirm these results, we performed a separate alymphoid xenograft mouse experiment, where we evaluated human endometriotic lesion microvessel density by examining fluorescence for the pan endothelial cell marker isoelectin-Ib4. Endometriotic lesions harvested at the time of necropsy from PBS-treated control mice (Fig. 5D and E) had robust vasculature, and were larger compared with lesions from SP012-treated mice (Fig. 5J). Isolectin-Ib4 stained specifically in cell types that had an endothelial like structure (Fig. 5K–M). Human endometriotic lesions from PBS-treated control mice had numerous isoelectin-Ib4 (+) blood vessels (Fig. 5A–C). However, human endometriotic lesions from SP012-treated mice had limited isoelectin-Ib4 fluorescence (Fig. 5F–H). Semi-quantitative analysis revealed a significant reduction in isoelectin-Ib4 fluorescence in SP012-treated mice (Fig. 5N, P < 0.05). Using proliferating cell nuclear antigen as a marker of cellular proliferation we showed that endometriotic lesions from SP012-treated mice (Supplementary
data, Fig. S2D–F) had less cellular proliferation compared with controls (Supplementary data, Fig. S2A–C). These results indicate that inhibition of SNCG by the peptide inhibitor SP012 prevents the neovascularization and growth of endometriotic lesions.

Discussion

In this study we have established a role for SNCG in endometriosis and demonstrated the effects of a novel SNCG inhibitor on angiogenesis of endometriosis in a xenograft mouse model. The importance of angiogenesis in endometriosis lesion establishment and disease progression is based on several observations. One of the hallmark features of endometritic lesions is a dense web of blood vessels (Nisolle et al., 1993). Eutopic endometrium is a rich source of angiogenic growth factors, as angiogenesis is essential in normal uterine function (Nap et al., 2004). However, ectopic endometrium has elevated expression of angiogenic growth factors, particularly vascular endothelial growth factor (VEGF), and the peritoneal fluid from females with endometriosis have increased levels of VEGF compared with normal females (McLaren et al., 1996). Studies using animal models have demonstrated that inhibiting angiogenesis by blocking VEGF or inducing endothelial cell apoptosis stops growth of endometriotic lesions in vivo (Edwards et al., 2013). Although the growth factors that stimulate angiogenesis have been extensively investigated in endometriosis, proteins involved in the machinery of cellular proliferation, an important step in angiogenesis, have not been investigated in endothelial cells of endometriosis lesions.

SNCG is implicated in cellular proliferation by binding to the mitotic spindle checkpoint kinase Bubr1. Under normal circumstances Bubr1 is present at the kinetochore during cellular proliferation, and maintains tension of the mitotic spindles and microtubules (Singh and Jia, 2008). Incorrect attachment leads to a ‘Stop’ signal, stopping the cell cycle and leading potentially to apoptosis. In cells where SNCG is expressed, SNCG binds to Bubr1 preventing the correct formation of the spindle checkpoint assembly complex, leading to continued cell proliferation (Singh et al., 2007).

Expression of SNCG was reported to be elevated in endothelial cells of endometriosis lesions compared with eutopic endometrium (Singh et al., 2008). In this study we have confirmed that SNCG is localized in peri-vascular areas of endometriosis lesions, and semi-quantitative analysis showed elevated expression in endometriosis compared with eutopic endometrium matched for menstrual stage. We proposed that blocking SNCG with a peptide inhibitor would limit cellular proliferation of endothelial cells, and ultimately hamper angiogenesis and growth of endometriotic lesions in our xenograft mouse model. In separate experiments, using both intravital confocal microscopy and traditional fluorescent histology, we found that alymphoid mice induced with human endometriotic lesions and treated with the novel peptide inhibitor of SNCG, SP012, had reduced neovascularization. Although we performed in vitro angiogenesis experiments to confirm that SP012 has a direct effect on endothelial cells, SP012 could also inhibit endometriotic lesion neovascularization indirectly. Inhibiting SNCG could affect MMP expression (Surgucheva et al., 2003), and estradiol signaling (Jiang et al., 2004), processes essential to endometriosis disease progression. Altered MMP or estradiol function could limit the growth of SNCG is expressed by HUVECs and SP012 ameliorates endothelial cell tube formation in vitro. Fluorescence of anti-human SNCG was specifically localized to human umbilical vein endothelial cells (HUVECs) (A), image (B) represents isotype control. Increasing concentrations of SP012 ((C) PBS control, (D) 2.5 ng/ml, (E) 5 ng/ml, (F) 150 ng/ml, (G) 300 ng/ml, (H) 600 ng/ml) completely inhibited HUVEC tube formation when cultured on a synthetic extracellular matrix. Scale bars represent 50 μm. (I) Semi-quantitative analysis of endothelial cell tube formation. Error bars represent standard deviation (SD). Data are representative of three independent experiments.
Figure 4  Intravital microscopy of human endometriotic lesions treated with SP012. Human endometriotic lesions in mice treated with PBS control had a dense web of blood vessels supplying the lesion (A–C). SP012 treated endometriotic lesions had only one or two blood vessels per field of view (D–F). Images with the FITC filter were added to provide contrast and allow better visualization of blood vessels. Images are magnified × 150 and scale bars represent 200 μm. Images are representative of two independent experiments.

Figure 5  Blood vessel growth and human endometriotic lesion development in alymphoid mice treated with SP012. Human endometriotic lesions obtained from mice dosed with PBS control, had numerous isolectin (+) blood vessels (A–C), compared with lesions obtained from mice treated with SP012 (F–H). Gross observation of the human endometriotic lesions from mice dosed with PBS control (D and E) showed robust vasculature and growth, compared with those treated with SP012 (I and J). (K–M) Representative high power fluorescent images of the pan endothelial cell marker isolectin-IB4. (N) Semi-quantitative analysis of isolectin fluorescence in human endometriotic lesions. Images K–M are magnified × 400, A–C and F–H are magnified × 50, and E and J are magnified × 17. White scale bars represent 100 μm, and black scale bars represent 1 mm. Error bars represent standard error of the mean. N = 4, *P < 0.05. Images and data are representative of two independent experiments.
endometriotic lesions, reducing the stimulus for blood vessel growth and decreasing the blood vessel density. Further studies are needed to confirm whether these alternative pathways play a role in SP012 mediated suppression of endometriotic lesion neovascularization.

The current therapeutic options for endometriosis have significant limitations. Surgical interventions are invasive, complicated and prone to a high recurrence rate. Medical interventions, most commonly affecting estradiol production by altering GnRH, are not compatible with pregnancy and have numerous side effects (Giudice, 2010). Anti-angiogenic therapy provides a novel therapeutic option to manage endometriosis. However, this approach also has the potential for negative consequences. Most of the current anti-angiogenic compounds tested for endometriosis either directly induce endothelial cell apoptosis, or bind and sequester VEGF, thereby affecting its biological activity. Although effective at preventing neovascularization of endometriotic lesions, these compounds also affect physiological processes that require angiogenesis, such as the menstrual cycle and wound healing. The expression of SNCG is elevated in blood vessels of endometriotic lesions compared with eutopic endometrium; therefore inhibiting its function might not have as significant an impact on physiological angiogenesis as other anti-angiogenic compounds. Mice treated with SP012 demonstrated no visible side effects (data not shown); however, further experiments are required to assess the effects of SP012 on physiological processes that require angiogenesis.

We have described the development of SP012, a novel peptide inhibitor of SNCG. SP012 is able to block angiogenesis in vitro, is taken up by human endometriotic lesions and prevents lesion neovascularization in vivo. Although further experiments are needed to define the mechanism by which SP012 affects neovascularization of endometriotic lesions, inhibition of SNCG with SP012 could be a novel therapeutic strategy to manage endometriosis.

**Supplementary data**

Supplementary data are available at http://molehr.oxfordjournals.org/.

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**Authors’ roles**


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**Conflict of interest**

V.S. holds stock in SYNG Pharmaceuticals, Inc., Canada and is also a director of SYNG Pharmaceuticals, Inc., Canada. This research was not sponsored by the company.

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