Apoptosis in Human Fibrosarcoma Cells Is Induced by a Multimeric Synthetic Tyr–Ile–Gly–Ser–Arg (YIGSR)-containing Polypeptide from Laminin

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ABSTRACT

The YIGSR (Tyr–Ile–Gly–Ser–Arg) peptide, derived from the laminin \(\beta_1\) chain, decreases tumor metastasis and growth in experimental animals. The mechanism responsible for this inhibition is not known. We now report that a 16-mer branched form of YIGSR, synthesized by the multimeric antigen peptide system, induced the apoptosis of HT-1080 cells in vitro at 30 \(\mu\)g/ml (approximately 3 \(\mu\)M). Tumor cells treated with this peptide showed the expected morphological changes associated with apoptosis, acridine orange staining of nuclei, increased numbers of 3'-OH ends of DNA in nuclei, a DNA ladder pattern on agarose gels, and increased transforming growth factor \(\beta\) mRNA by Northern blot. The specificity of this peptide was confirmed by inhibition of apoptosis with a neutralizing antibody to the peptide. In addition, the branched 16-mer peptides of scrambled sequence did not induce apoptosis. Our in vitro results suggest that apoptosis may play a role in the antimetastatic and antitumor effects associated with the YIGSR peptide.

INTRODUCTION

Laminin, a large basement membrane-derived trimeric glycoprotein (1, 2), has multiple biological activities. Isolated from the Engelbreth-Holm-Swarm tumor and other sources, it is composed of three chains designated \(\alpha_1\) (M, 400,000), \(\beta_1\) (M, 210,000), and \(\gamma_1\) (M, 200,000), and functions to promote cell attachment, growth, differentiation, angiogenesis, neurite outgrowth, and tumor metastasis (3). Among the several active sites of laminin, the YIGSR\(^2\) sequence comprising residues 929–933 on the \(\beta_1\) chain has been most extensively studied because of its inhibitory effect on tumor metastasis (4–11) and growth (12, 13). YIGSR has been found to reduce the formation of osteolytic bone metastases by human melanoma cells in nude mice (14). When sarcoma 180 cells are grown as a solid tumor, daily i.p. injections of YIGSR block tumor growth; however, this peptide has no effect when the same cells are grown in suspension as ascites (10).

YIGSR has several additional biological activities other than metastasis inhibition. It affects the in vitro behavior of both normal and malignant cells. For example, it can promote adhesion and migration (4, 15). Differentiation of some cells, such as Sertoli cell cord formation on laminin, is blocked by the YIGSR peptide (16). Neural crest migration on laminin is also blocked by YIGSR but not by an RGD-containing synthetic peptide (17). These data demonstrate that YIGSR is a functional site in laminin for a variety of both malignant and normal cells.

Several modifications of YIGSR peptides have been made in an attempt to potentiate its biological activities; specifically, the objective has been to obtain a more potent antimetastatic agent. Cyclized YIGSR peptide was shown to have increased effectiveness (18) and conformational studies by nuclear magnetic resonance (19) suggest that the turn structure of the peptide may be important for its activity. Polymerized YIGSR was more effective as an antimetastatic agent than was the YIGSR monomer (6). Coupling the peptide to either polyethylene glycol (20) or bovine serum albumin (4) further increased the activity of YIGSR. Recently, we reported that the multimeric YIGSR peptide, using the multiple antigenic peptide system (21, 22), greatly increased the inhibition of tumor metastasis and growth (23). It is still not clear why such types of structural modifications resulted in increased activity. In the experiments described here, we analyzed the effect of multimeric YIGSR in vitro and showed that in micromolar concentrations it induces the apoptosis of HT-1080 human fibrosarcoma cells.

MATERIALS AND METHODS

Synthesis of Peptides. The method of synthesis of multimeric YIGSR (Ac-Y16) was described in a previous report (23). This peptide has 16 YIGSR sequences on a branched lysine tree and an approximate \(M\_\text{r}\) of 10,000. Two scrambled multimeric peptides, (Ac-GRISYG)\(_6\)K\(_8\)K\(_@\)K\(_2\)K\(_\text{G}\) and (Ac-GYSRIG)\(_6\)K\(_8\)K\(_2\)K\(_\text{G}\), and a multimeric form of RGD adhesive peptide, (Ac-GRGDS)\(_6\)K\(_8\)K\(_2\)K\(_\text{G}\), were also synthesized. The linear peptides Ac-YIGSRYIGSRYIGSR-NH\(_2\) (Ac-Y3) and Ac-YIGSR-NH\(_2\) were synthesized using an Applied Biosystem 431 automatic peptide synthesizer. The peptides were purified by reverse phase high performance liquid chromatography. Rabbit polyclonal antisera to the YIGSR peptide were generated after peptide polymerization (24).

Cell Culture. HT-1080 cells [human fibrosarcoma cells (25)], were cultured in DMEM (GIBCO, Grand Island, NY) containing 10% fetal bovine serum (Hyclone, Logan, UT), 100 units/ml penicillin, and 100 \(\mu\)g/ml streptomycin (GIBCO, Grand Island, NY). SW480 human colon adenocarcinoma cells (26) were cultured in RPMI 1640 (GIBCO) containing 10% fetal bovine serum, antibiotics, and insulin (5 \(\mu\)g/ml). Cell cultures were performed on tissue culture plates (Nunc, Inc., Naperville, IL). In some experiments, polyHEMA coated plates (50 \(\mu\)l of 10 mg/ml poly-HEMA solution in each 96-well plate) were used to maintain cells in suspension (27).

Assessment of Cell Numbers. Cell attachment was assayed in 96-well round bottomed plates of culture plastic coated with either laminin or synthetic peptides. Various amounts of either laminin or peptides were dissolved in Milli-Q water and 50 \(\mu\)l was added to each well, followed by drying overnight. The wells were blocked by the addition of 100 \(\mu\)l of 3% BSA in DMEM for 1 h and washed three times with DMEM containing 0.1% BSA. Cells (2 \(\times\) 10\(^4\)) in 0.2 ml of DMEM were added to each well and cultured for 1 h at 37°C in 5% CO\(_2\). After washing to remove the unattached cells, the attached cells were stained with 0.2 ml of 0.2% crystal violet aqueous solution in 20% methanol for 10 min. After washing and drying, 50 \(\mu\)l of 1% sodium dodecyl sulfate was used to dissolve the cells and the absorbance at 560 nm was measured by a Titertek Multiscan. Peptide-coated wells without cells were processed simultaneously to subtract background because peptides at higher concentrations were stained with crystal violet. The absorbance of attached cells in the presence of 20% fetal bovine serum was designated as 100%.

Inhibition of attachment was assayed in the 96-well plates, each coated with 1 \(\mu\)g of laminin by incubating the cells in the presence of various concentrations of peptides. The number of attached cells was measured as described above.
For the proliferation assay, $1 \times 10^4$ cells were plated in each well of a 96-well culture dish. After 6 h, varying amounts of Ac-Y16 were added. The cell numbers were assessed by crystal violet staining at 6, 12, 24, 36, 48, and 60 h after peptide treatment. Each condition was tested in triplicate and all assays were repeated at least twice.

The effect of scrambled peptides on cell viability was assessed as described above, except that $2 \times 10^4$ cells were used in each well and the incubation was 16 h. Five replicate wells were used in these tests.

**Metabolic Labeling of Proteins.** Thirty thousand cells were plated on 3.5-cm diameter plates and incubated for 12 h with Ac-Y16. The medium was changed into methionine-free DMEM containing $50 \mu$Ci/ml $^{[35}S\text{]}$methionine (specific activity, 1135 Ci/mmol). The supernates were harvested 4 h later and separated by 7.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by fluorography.

**Histology of Apoptotic Cells.** After various treatments, the floating cells were collected by centrifugation. Acridine orange (Sigma Chemical Co., St. Louis, MO) was added at a final concentration of 5 $\mu$g/ml and the wet mount slides were examined with a Nikon fluorescent microscope with V-type filters. The attached cells were directly stained with an acridine orange solution (5 $\mu$g/ml) in phosphate-buffered saline. For the detection of cleaved DNA in situ, either attached cells or cytospin-prepared floating cells were fixed in 10% neutral formalin/phosphate-buffered saline for 10 min followed by postfixation with an ethanol:acetic acid (3:1) solution at $-20^\circ$C for 5 min. Digoxigenin-dUTP was used to label the 3'-OH ends by TdT and digoxigenin was detected by anti-digoxigenin-peroxidase labeling, followed by dianisobenzidine reaction (28) using the Apoptag kit (Oncor, Gaithersburg, MD). Immunohistochemistry for transglutaminase C was performed on the floating cells collected by a cytospin using goat anti-rabbit transglutaminase C (gift from Dr. S. I. Chung, National Institute of Dental Research) (29) and then anti-goat IgG-horseradish peroxidase. Diaminobenzidine/H$_2$O$_2$ was used for detection.

**DNA Electrophoresis.** The cells were incubated in 80-cm$^2$ culture dishes with various concentrations of Ac-Y16. The floating cells were collected by centrifuging the supernatant at $200 \times g$ for 5 min. The collected cells were lysed with 500 $\mu$l of 10 mM Tris-HCl (pH 6.0) containing 1 mM EDTA and 0.2% Triton X-100. After centrifugation at 13,000 $\times g$ for 10 min, 100 $\mu$l of 5 M NaCl and 700 $\mu$l of ice-cold n-propyl alcohol were added to the supernatant. The DNA was precipitated at $-20^\circ$C for 16 h, collected by centrifugation, and separated in 1.5% agarose gel. Ethidium bromide staining was used to visualize the DNA.

**Northern Blot.** Total RNA was extracted from the cells cultured in the presence of various concentrations of Ac-Y16 (0, 10, 30, and 60 $\mu$g/ml) for 16 h. Cells were scraped into a solution of guanidine isothiocyanate and purified by ultracentrifugation on a CsCl cushion. Ten $\mu$g of total RNA were run on a denaturing 1% agarose-formaldehyde gel and transferred into Nytran (Schleicher & Schuell, Keene, NH) by capillary action. The human TGF-$\beta_1$ complementary DNA obtained from R. Derynck (Genetech) (30) was used as a probe. The radiolabeled probe was prepared with an ethanol:acetic acid (3:1) solution at $-20^\circ$C for 5 min. Digoxigenin-dUTP was used to label the 3'-OH ends by TdT and digoxigenin was detected by anti-digoxigenin-peroxidase labeling, followed by dianisobenzidine reaction (28) using the Apoptag kit (Oncor, Gaithersburg, MD). Immunohistochemistry for transglutaminase C was performed on the floating cells collected by a cytospin using goat anti-rabbit transglutaminase C (gift from Dr. S. I. Chung, National Institute of Dental Research) (29) and then anti-goat IgG-horseradish peroxidase. Diaminobenzidine/H$_2$O$_2$ was used for detection.

**RESULTS**

Attachment assays were carried out to determine the relative biological activities of various YIGSR-containing peptides (Fig. 1). When compared to the monomer, Ac-Y16 showed similar activity at 100-fold less concentration and Ac-Y3 at 10-fold less concentration for HT-1080 cell attachment. The attachment of HT-1080 cells to Ac-Y16 was almost equal to that observed with native laminin, although the Ac-Y16 has about 640 times more YIGSR sites than the equivalent $\mu$g amount of laminin. The attachment of cells to Ac-Y16 was decreased at concentrations higher than 1 $\mu$g/well, while laminin did not exhibit the same pattern (data not shown). Ac-Y16 was effective in blocking cell attachment to laminin (49% inhibition at 500 $\mu$g/ml concentration) but other less complex YIGSR peptides did not show any significant inhibition of attachment (Fig. 2).

The YIGSR-containing peptides were next tested for their effect on blocking invasion of cells in vitro (31). The addition of Ac-Y16 to the upper compartment of Boyden chambers dramatically decreased the invasion of HT-1080 cells through the Matrigel in a dose-dependent manner, with approximately 40% inhibition at 60 $\mu$g/ml of peptide and more than 90% inhibition at 100 $\mu$g/ml (data not shown). By zymography using culture supernates (32), the secretions of both the gelatinase A ($M_r$ 72,000) and gelatinase B ($M_r$ 92,000) were decreased in HT-1080 cells in the presence of Ac-Y16 at 30 and 100 $\mu$g/ml (data not shown). In contrast, SW480 cells did not show down-regulation of either gelatinase A or B. These data suggest that reduced gelatinases secretion may account, at least in part, for decreased HT-1080 cell invasion in the presence of Ac-Y16.

To determine whether decreased secretion of gelatinases is specific to this enzyme or whether it results from a general decrease in the synthesis of total proteins, the amount of secreted protein was determined by $^{[35}S\text{]}$methionine labeling (Fig. 3). Protein secretion by HT-1080 cells was slightly decreased at a concentration of 10 $\mu$g/ml, further decreased at 30 $\mu$g/ml, and almost completely lost at 100 $\mu$g/ml of Ac-Y16, although all of the bands did not show the same decreasing pattern. The amount of labeled proteins present in the cell lysate also showed a decrease in the presence of Ac-Y16 (data not shown).

The proliferation of HT-1080 cells was measured in the presence of various concentrations of Ac-Y16 (Fig. 4). Proliferation was markedly decreased at 60 $\mu$g/ml and 100 $\mu$g/ml, while only a small effect was observed at 30 $\mu$g/ml. SW480 cells were found to have only slightly reduced proliferation at 100 $\mu$g/ml, with no effect at 30 $\mu$g/ml (data not shown). These data suggest that the Ac-Y16 peptide may have different effects on different cell types.

Treated cells showed surface blebbing or protrusions as observed by inverted microscopy and some of the cells were detached from the plastic. These unattached cells were smaller than the attached cells and contained pyknotic nuclei. The treated cells were analyzed by fluorescent microscopy after treatment with acridine orange, which specifically stains apoptotic cells (Fig. 5a). After 16 h in the presence of 30 $\mu$g/ml Ac-Y16, 12% of the cells were detached from the culture plastic and about 30% of the floating cells were apoptotic based on either chromatin condensation or fragmented nuclei detected by acridine orange staining. The apoptotic cells were detectable by labeling

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Fig. 1. Attachment of HT-1080 cells to various YIGSR peptides and to laminin. Twenty thousands cells were incubated on the peptide-coated 96-well plates and the number of the attached cells were assessed by crystal violet staining. Data are expressed as mean of triplicate results. •, laminin; ○, Ac-Y16; □, Ac-Y3; ▲, YIGSR.
DNA electrophoresis was performed since DNA fragmentation into oligonucleosome-length fragments is one of the most reliable biochemical markers for many cell types undergoing apoptosis (34). After culturing HT-1080 cells for 16 h in the presence of Ac-Y16 at a dose of 30 μg/ml, DNA isolated from floating cells exhibited the ladder pattern characteristics of apoptosis (Fig. 6).

The specificity of the YIGSR sequence involved in the process of apoptosis was confirmed using peptide antibodies and control peptides. Using a rabbit polyclonal anti-YIGSR antisera, we tested whether apoptosis could be blocked. As assessed by phase contrast microscopy, anti-YIGSR antibody (1:10 dilution) could inhibit the morphological derangement of the cells, while preimmune serum did not block the cell damage (data not shown). We next compared the effect on cell viability of Ac-Y16 to that of scrambled 16-mer peptides with the same star burst structure. After 16-h treatment at 60 μg/ml, Ac-Y16 decreased the number of attached cells by 43.5% compared to the control, while scrambled peptides did not affect the number of attached cells (Table 1). By the trypan blue exclusion test, more than 99% of the attached cells were alive after scrambled peptide treatment. On the basis of this result, scrambled 16-mer peptides did not promote cell death. In addition, we tested the same 16-mer structure of RGD peptide, a known biologically active sequence, and it did not induce cell death up to 100 μg/ml. These data demonstrate the specificity of the YIGSR sequence in inducing apoptosis.

To test whether Ac-Y16-induced apoptosis is due to its inhibitory effect on cell attachment, the cells were cultured on poly-HEMA coated plates. No cell attachment was observed. Only 0.5% of the cells cultured on poly-HEMA-coated plates for 16 h showed apoptotic nuclear change on acridine orange staining, although they were maintained in suspension. The addition of 60 μg/ml of Ac-Y16 to the poly-HEMA-coated plate induced apoptosis in 2.6% of the cells; this amount increased to 5.0% at 100 μg/ml. We therefore conclude that the inhibition of attachment by Ac-Y16 is not a cause of apoptosis.

TGF-β1 mRNA expression was measured by Northern blot because this message has been shown to be elevated in both normal and tumor cells during apoptosis (35). The 2.5-kilobase TGF-β1 transcript was more than 2-fold elevated after Ac-Y16 treatment (30 μg/ml) for 16

Fig. 4. Effect of Ac-Y16 on HT-1080 cell proliferation. Ten thousands cells were plated in 96-well culture plates and varying amounts of Ac-Y16 were added after 6 h. The cell numbers were assessed at 6, 12, 24, 36, 48, and 60 h after peptide treatment. Data are expressed as mean of triplicate results. +, no peptide; ●, 5 μg/ml of Ac-Y16; ■, 10 μg/ml; ▲, 20 μg/ml; ○, 30 μg/ml; ◆, 60 μg/ml; X, 100 μg/ml.

Fig. 3. Protein secretion of the HT-1080 cells treated with Ac-Y16. The conditioned media from the cells cultured with [35S]methionine (50 μCi/ml) were collected and separated by 7.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by fluorography. Ordinate, molecular weight in thousands. Lane 1, no peptide; Lane 2, 3 μg/ml of Ac-Y16 in the culture media; Lane 3, 10 μg/ml; Lane 4, 30 μg/ml; Lane 5, 100 μg/ml.

3'-OH ends of DNA with digoxigenin-UTP using the TdT enzyme (Fig. 5b). The percentage of apoptotic cells labeled by TdT enzyme was less than 0.01% in control cells or cells treated with 10 μg/ml of Ac-Y16, 0.1% at 30 μg/ml, 3.2% at 60 μg/ml, and 9.2% at 100 μg/ml after 16 h of treatment. Not all of the cells stained by acridine orange showed positive labeling with TdT enzyme, and vice versa, although most of the cell populations were stained by both methods.

Since an increase in transglutaminase is associated with apoptosis in many cell types (33), immunohistochemical staining of transglutaminase C on the cytospin-prepared floating cells was carried out. Small cells with condensed chromatin showed positive cytoplasmic staining, whereas normal cells did not stain (Fig. 5c). The small transglutaminase-positive cells were considered apoptotic based on their nuclear morphology.
Table 1: Effect of Y1GSR peptides and scrambled 16-nter peptides on the survival of the cells

<table>
<thead>
<tr>
<th>Peptides</th>
<th>Absorbance*</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no peptide)</td>
<td>1.698 ± 0.029</td>
<td>100.0</td>
</tr>
<tr>
<td>Ac-Y16</td>
<td>0.738 ± 0.032</td>
<td>43.5b</td>
</tr>
<tr>
<td>Ac-Y3</td>
<td>1.644 ± 0.024</td>
<td>97.3</td>
</tr>
<tr>
<td>Ac-YIGSR-NH2</td>
<td>1.734 ± 0.049</td>
<td>102.1</td>
</tr>
<tr>
<td>(Ac-GYSRIG)K7K2KG</td>
<td>1.747 ± 0.040</td>
<td>102.8</td>
</tr>
<tr>
<td>(Ac-GYSRIG)K2K2KG</td>
<td>1.705 ± 0.033</td>
<td>100.4</td>
</tr>
</tbody>
</table>

DISCUSSION

With a few exceptions, cell death occurs by one of two categories: necrosis or apoptosis. Necrosis occurs in nonphysiological conditions and evokes a sustained immune response. In contrast, apoptosis is a regulated self-destruction that functions in the normal control of development and requires specific gene transcription and protein synthesis. Morphological features of apoptosis include focal cell surface protrusion, nuclear and cytoplasmic condensation, and dense aggregation of chromatin. The biochemical hallmark of apoptosis is internucleosomal cleavage of DNA into oligonucleosomal length fragments. About 30% of the chromatin becomes fragmented by cleavage at linker DNA sites between the nucleosomes, generating a population of DNA fragments of varying size. The DNA fragments are multimers of approximately 180-base pair nucleosome units, resulting in the appearance of a ladder pattern in agarose gels (34). This cleavage of DNA produces many free 3'–OH ends of DNA, which can be labeled by TdT enzyme, resulting in a direct method to detect apoptotic cells in situ.

Here we have found that a multimeric form of an adhesion se-
sequence of laminin induced many changes characteristic of apoptosis, including (a) morphological changes demonstrated by microscopy, (b) a characteristic chromatin pattern demonstrated by the DNA-binding dye acridine orange, (c) in situ detection of intranuclear high concentrations of 3'-OH ends of DNA, (d) increased expression of transglutaminase by immunohistochemistry, (e) a DNA ladder pattern on agarose gels, and (f) up-regulation of TGF-β mRNA by Northern blot. By consensus, there is not a single test to confirm apoptosis but the above combined evidences reported here indicate that Ac-Y16 evokes apoptosis in HT-1080 cells.

Transglutaminases are a family of Ca$^{2+}$-dependent enzymes which catalyze an acyl-transfer reaction among polypeptide chains, leading to the formation of protein cross-links including ε-(γ-glutamyllysine) and N,N-bis-(γ-glutamyl) polyamine isodipeptide linkages (29). Such covalent bonds between polypeptide chains result in the formation of insoluble proteins, which are found in apoptotic cells. Recent reports suggest that one of the genes specifically induced during the apoptotic program is the gene that codes for transglutaminase C (33). It was proposed that transglutaminase-dependent formation of an insoluble protein scaffold in apoptotic cells prevents the leakage of intracellular components, resulting in a lack of inflammatory processes in the surrounding tissue (36). In our studies, immunostaining of transglutaminase on the floating cells revealed that only the small apoptotic cells were positive. However, total transglutaminase activity in the cells, assayed by the incorporation of $^{3}H$putrescine into N,N'-di-methylcasein (36), did not increase with Ac-Y16 treatment (data not shown).

TGF-β1 is a potent growth regulator of several normal and neoplastic cells and its inhibitory effect is important in the process of apoptosis in vitro (37, 38) and in vivo (39). TGF-β1 mRNA levels are elevated in the apoptotic process as determined by Northern blot (35) and the protein level is increased as determined by immunohistochemistry (39). Our data revealed that the mRNA expression of TGF-β1 is increased by multimeric YIGSR peptide treatment.

Although many nonphysiological drugs, especially chemotherapeutic agents, can induce apoptosis in vitro, only a few proteins or peptides which are normally found in the body are known to induce apoptosis. Loo et al. (40) induced the apoptosis of cultured neuronal cells by a synthetic fragment of β-amyloid protein, which is accumulated in Alzheimer’s disease. Neurons treated with β-amyloid protein exhibited membrane blebbing, compaction of nuclear chromatin, and DNA fragmentation on agarose gels. Another example is a partial peptide sequence of cellular prion, a sialoglycoprotein in neurons, which was shown to induce apoptosis in primary neuronal culture (41).

There is also suggestive evidence that the extracellular matrix might be involved in the regulation of apoptosis of the prostate gland, a hormone-dependent tissue. Apoptosis is readily induced by hormonal ablation. Although involution of the prostate after castration results in the loss of approximately 90% of the epithelial cells (42), not all of the epithelial cells in the glands are equally sensitive to the induction of apoptosis. The secretory epithelial cells that are sensitive to castration are localized in the distal region of the glands and are in direct contact with the underlying matrix. In contrast, the epithelial cells in the proximal part of the duct lack contact with the matrix due to underlying basal cells and are more resistant to death after castration, although both the proximal and distal cells have similar amounts of androgen receptor. It has been suggested that changes in cell-matrix interactions are important in the process of apoptosis (43).

Our data provide the initial evidence that the laminin-derived adhesion peptide sequence YIGSR is involved in cell death through an apoptotic pathway. A recent paper revealed that cells detached by the RGD peptide undergo apoptosis (44). The mechanism of YIGSR-induced apoptosis might not be simply the inhibition of attachment, based on our finding that some of the attached cells demonstrated the morphology of apoptosis before detachment and that inhibition of attachment by poly-HEMA coating did not evoke apoptosis. Whether this apoptotic signal is delivered by a specific YIGSR-receptor interaction or blocking of receptor binding to the ligand is not clear.

The mechanism by which YIGSR inhibits tumor growth and metastasis is still unknown. The peptide does not affect tumor cell arrest in the lungs as determined by injection of radiolabeled B16F10 cells in the presence of YIGSR (45). Because angiogenesis is necessary for tumor growth, an antiangiogenic effect of this peptide partly explains the mechanism of inhibition of tumor growth (10). Our data suggest another mechanism of YIGSR peptide inhibition of tumor growth, which involves apoptosis. An apoptotic effect could explain how the peptide reduces both tumor growth and metastasis particularly when given as a single dose in the experimental metastasis model.

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