Peptides Derived from the Histidine-Proline Domain of the Histidine-Proline-Rich Glycoprotein Bind to Tropomyosin and Have Antiangiogenic and Antitumor Activities

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ABSTRACT

The antiangiogenic activity of the multidomain plasma protein histidine-proline-rich glycoprotein (HPRG) is localized to its histidine-proline-rich (H/P) domain and has recently been shown to be mediated, at least partially, through binding to cell-surface tropomyosin in fibroblast growth factor-2-activated endothelial cells (X. Guan et al., Thromb Haemost, in press). HPRG and its H/P domain, but not the other domains of HPRG, bind specifically and with high affinity to tropomyosin. In this study, we characterize the interaction of the H/P domain with tropomyosin and delineate the region within the H/P domain responsible for that interaction. The H/P domain of HPRG consists mostly of repetitions of the consensus sequence [H/P][H/P]PHG. Applying an in vitro tropomyosin binding assay, we demonstrate that the synthetic peptide HHPHG binds to tropomyosin in vitro and inhibits angiogenesis and tumor growth in vivo. The affinity for tropomyosin increases exponentially upon multimerization of the HPHHG sequence, with a concurrent increase in antiangiogenic activity. Specifically, the tetramer (HHPHG)4 has significant antiangiogenic activity. HPRG and is also able to inhibit tumor growth, suggesting that cell surface tropomyosin may represent a novel antiangiogenic target for the treatment of cancer.

INTRODUCTION

We have described the antiangiogenic properties of the abundant plasma protein histidine-proline-rich glycoprotein (HPRG) and localized these properties to the His-Pro-rich (H/P) domain (1), a result that has recently been confirmed (2). In this regard, HPRG is functionally similar to an evolutionarily related protein, activated high molecular weight kininogen (HKa), which has also been demonstrated to inhibit angiogenesis (3–5). Rabbit and human HPRG are highly homologous and demonstrate similar biological activity (1, 6, 7). Thus, we have used rabbit HPRG (rbHPRG) for our studies rather than the human protein because it is more straightforward to isolate intact individual domains from rbHPRG (6, 7). Tropomyosin, which forms part of the actin cytoskeleton (8), has been identified on the surface of fibroblast (H/P) domain of HPRG, bind specifically and with high affinity to tropomyosin. In this study, we define the basis for the interaction of the H/P domain of HPRG with tropomyosin and delineate the region within the H/P domain responsible for its interaction with tropomyosin and its antiangiogenic activity. We show here that the peptide HPHHG has the highest affinity toward tropomyosin as well as the most potent antiangiogenic activity of the 5-mer peptides tested and that the His residues are essential for both binding to tropomyosin as well as for the inhibition of angiogenesis. Furthermore, we demonstrate that multimers of the HPHHG sequence have increased antiangiogenic activity and affinity for tropomyosin with progressively increasing multimer size. Finally, the largest multimer tested, the tetramer (HPHHG)4, also demonstrates substantial antitumor activity in two syngeneic [Lewis lung carcinoma (3LL) and B16F1] models of tumor growth in mice.

MATERIALS AND METHODS

Proteins, Peptides, and Materials. FGF-2 and vascular endothelial growth factor were purchased from Research Diagnostics, Inc. (Flanders, NJ). HKa was obtained from Enzyme Research Laboratories (South Bend, IN). Heparin was acquired from Sigma (St. Louis, MO). Intact protein and domains of rabbit HPRG were obtained as described previously (1). Peptides were synthesized and purified by PepTisyntha (Torrance, CA). HKa and HPRG were biotinylated using EZ-Link Sulfo-NHS-LC-biotin (Pierce, Rockford, IL) following the manufacturer’s instructions.

Tropomyosin Competition Binding Assay. Chicken gizzard tropomyosin (Sigma) was dissolved in TBS [50 mM Tris, 140 mM NaCl (pH 7.5)]. Two hundred ng of chicken gizzard tropomyosin were added/well of a 96-well high-binding assay plate and incubated for 2 h at room temperature. The plate was washed with TBS-T [TBS with 0.05% Tween 80 (v/v)] and blocked with
2% fish gelatin (Sigma). Biotin-HKa (10 μM) in TBS containing 10 μM ZnCl2 was added to the plate together with the peptides. The plates were incubated at room temperature for 2 h and extensively washed with TBS-T buffer. Bound biotin-HKa was detected using avidin-horseradish peroxidase and the chromogenic substrate 3,3’-5,5’-tetramethylbenzidine (Roche, Indianapolis, IN). The indicated IC50 values were calculated by nonlinear fitting of experimental data using Prism.

Matrigel Plug Model. Cold Matrigel (500 μl) was mixed with heparin (50 μg/ml), FGF-2 (400 ng/ml), and the peptides to be tested. Positive control plugs did not contain the test peptides, and negative control plugs did not contain the pro-angiogenic FGF-2. In some experiments, 800 ng/ml FGF-2, 300 ng/ml vascular endothelial growth factor and heparin were used. The Matrigel mixture was injected s.c. into 4–8-week-old female BALB/c nude mice, and the animals were sacrificed and the plugs recovered 5 days after injection. The plugs were then minced and homogenized with a tissue homogenizer, and hemoglobin levels in the plugs determined using Drabkin’s solution according to the manufacturer’s instructions (Sigma). Alternatively, the Matrigel plug vascularization was determined after i.v. injection of 100 μl of FITC-dextran (M, 250,000; Sigma) into the tail vein followed by fluorometric analysis of the plug after removal. Both methods of detection yielded similar results.

Modified Matrigel Plug Model. MatLyLu rat prostate cancer cells (2 × 106 cells) were mixed with Matrigel and the peptide to be tested and then injected into the flank of a mouse following the protocol described for the Matrigel plug model. After 7 days, the animals were euthanized and the plugs removed and weighed. The plugs were then minced and homogenized with a tissue homogenizer, and hemoglobin levels were determined using Drabkin’s solution according to the manufacturer’s instructions.

Endothelial Cell Binding Assay. To assess protein binding, 30,000 HUVEC were plated in a 24-well plate and incubated overnight in M200 containing 2% serum. Medium was then changed to basal medium (M200) containing 2% fetal bovine serum (FBS) with or without 10 ng/ml FGF-2 and incubated for 4 h at 37°C. The medium was then aspirated, the plate placed at 4°C, and the desired amounts of biotin-rbHPRG and the peptide to be tested were added in 200 μl of HBS (pH 7.5) and 10 μM ZnCl2. Nonspecific binding was determined by measuring binding in the absence of Zn2+.

Endothelial Cell Proliferation Assay. HUVEC were cultured overnight in M200 containing 2% FBS. The following day, 3000 cells were plated in each well of a gelatin-coated 96-well plate. The cells were allowed to adhere and spread for 4–6 h, at which time, the medium was replaced with fresh medium containing 2% FBS, 1 ng/ml FGF-2, and varying concentrations of specific test compounds in the presence and absence of 10 μM ZnCl2. Cells were then cultured for an additional 48 h, and relative cell numbers in each well were determined using the Cell Titer Aqueous Cell Proliferation Assay (Promega, Madison, WI).

Tumor Growth Inhibition Experiment and Cell Culture. The 3LL cells were a gift from the laboratory of Dr. Judah Folkman (Children’s Hospital, Harvard Medical School). The cells were grown in DMEM, 10% FBS containing 80 μM t-glutamine, and 0.15% sodium bicarbonate at 37°C in a humidified 5% CO2 incubator. B16F1 cells were obtained from the laboratory of Dr. Ronald Goldfarb (University of North Texas Health Science Center) and were maintained in RPMI 1640 containing 10% FBS and 1% MEM nonessential amino acids at 37°C in a humidified 5% CO2 incubator. For the tumor growth inhibition experiments, C57B1/6 female mice were inoculated s.c. with 2.5 × 106 3LL cells or 7.5 × 106 B16F1 cells. After 4 days, peptide (dissolved in PBS) was administered by injection (3 mg/kg, once/week) and metronomically dosed (170 mg/kg, once/week) cyclophosphamide (Sigma) was used as a positive control (14).

Statistical Analysis. Results were analyzed using the Student’s t test. Data are presented as mean ± SD or mean ± SE (Fig. 7).

RESULTS

Identification of the Pentapeptide from the H/P Domain of HPRG with the Highest Affinity for Tropomyosin. To identify the peptide from the consensus sequence [H/P][H/P]PHG required for the binding of HPRG to tropomyosin, three peptides, PPHGP, HPHPG, and HHPHG, having free or capped termini (acetylated and amidated at the COOH- and NH2-termini, respectively) were synthesized. We have previously demonstrated that the binding of HPRG and HKa to immobilized tropomyosin in a 96-well solid phase assay correlates with the binding to FGF-2-stimulated HUVECs (5, 9). HKa and HPRG could be used interchangeably in this assay, but HKA provides a superior signal-to-noise ratio and was thus used in all subsequent experiments. This assay was used to study the interaction of the H/P-derived peptides with tropomyosin. The six peptides (three capped, three uncapped) were screened for their ability to compete with biotin-HKa for binding to tropomyosin at concentrations of 50 and 200 μM (Fig. 1A). Only the uncapped forms of peptides HHPHG and HPPHG substantially inhibited binding in this assay, and all subsequent studies were carried out using these two peptides. The difference in affinity of HHPHG as a capped or uncapped peptide is shown as a dose titration curve in Fig. 1B. The IC50 for the uncapped peptide is ~150 μM, whereas the capped peptide competes with the binding of biotin-HKa with an IC50 ~1 mM, indicating that a free NH2- and/or COOH-terminus are important for the interaction with immobilized tropomyosin. Similar results were found for capped and uncapped HPPHG (data not shown).

Ala Scanning Replacement of HHPHG and HPPHG. Ala scanning replacement of both peptides was carried out with the objective of assessing the relative contribution of each residue to tropomyosin
by the peptides was not due to stripping Zn$^{2+}$ from the assay. Because the affinity of the HHPHG peptide ($IC_{50} = 154 \pm 49 \mu M$) is somewhat higher than that of the HPPHG peptide ($IC_{50} = 242 \pm 144 \mu M$) in the tropomyosin binding assay, the HHPHG peptide was chosen for additional studies.

To test whether binding to tropomyosin in vitro correlated with antiangiogenic activity in vivo, pairs of peptides with low and high $IC_{50}$ values in the tropomyosin binding assay were compared in both the Matrigel plug model and the modified Matrigel plug model in which the angiogenic stimulus is provided by tumor cells that are mixed with the Matrigel (1). The HHPHG peptide had significant inhibitory activity in both assays (Fig. 4), suggesting activity against both FGF-2 and tumor-driven angiogenesis. In contrast, the activity of the AHPPG peptide, which had the lowest affinity (highest $IC_{50}$) of all of the Ala mutants of the HHPHG series for tropomyosin, was significantly lower than that of HHPHG in both in vitro models (Fig. 4). Similar results were obtained with HPPHG (low $IC_{50}$, high antiangiogenic activity) and APPHG (high $IC_{50}$, low antiangiogenic activity; data not shown). These results demonstrate the correlation between tropomyosin binding in vitro and antiangiogenic activity in vivo. Furthermore, the fact that HHPHG and HPPHG have antiangiogenic activity is consistent with our hypothesis that the antiangiogenic activity of the H/P domain resides in the consensus sequence [H/P]$_n$ [H/P]PHG.

**Tropomyosin Binding Affinity and Antiangiogenic Activity of Multimers of HHPHG.** Although truncation of the last Gly residue in the HPPHG peptide and replacement of Pro residues with Ala had only minor effects on binding, structural roles for these two residues might not be observed when they are part of a small peptide but could become important if that sequence was contained in a larger peptide or protein. The H/P domain is known to adopt a poly-Pro type II helix (7). Thus, Gly residues may act as spacer residues, and Pro residues may promote the poly-Pro secondary structure in larger peptides. Therefore, these two residues were retained in each HHPHG subunit in our investigation of the effect of multimerization on binding and antiangiogenic activity. Capped and uncapped multimers (two to four repeats) of the HHPHG peptide were synthesized and tested in the tropomyosin binding and Matrigel plug assays (Table 1). A direct correlation between the number of repeats of the consensus sequence and the affinity for tropomyosin and antiangiogenic activity was

![Fig. 2. Ala scanning replacement of HPPHG. A. peptides containing Ala replacement in each position of the peptide HPPHG were tested in the tropomyosin binding assay as described in Fig. 1A, at 100, 1000 or 10,000 \(\mu M\). The absorbance in the absence of peptide is considered no inhibition. B. 10\( \mu \)M biotin-HKa are mixed with increasing concentrations of the HPPHG (●) or the APPHG (□) peptides as described in Fig. 1B.](image-url)

binding. Replacement of either His residue in HPPHG with Ala significantly decreased the ability of the peptide to compete for binding to tropomyosin (Fig. 2A) and substituting the NH$_2$-terminal histidine completely abrogated the ability of the peptide to compete for binding to tropomyosin (Fig. 2B). A more detailed Ala replacement study was carried out with the HHPHG peptide (Fig. 3). The $IC_{50}$ values of the variant peptides in the tropomyosin binding assay were normalized to the activity of HHPHG, $IC_{50} = 155 \pm 49 \mu M$. As observed with the HPPHG peptide, the first His residue provides the largest contribution to binding. Substitution of the other His residues also decreased binding, but replacement at the non-His positions had no significant effect (Fig. 3). Truncation of the last Gly residue (HHPH) and even combining the truncation with the Ala substitution (HHAH) had only a small effect on the binding affinity.

These results indicate that the activity of the peptides in the tropomyosin binding assay is His-residue dependent. His residues also bind to divalent cations (15), and thus, a trivial explanation for the inhibition of binding of biotin-HKa to tropomyosin is that the peptides could require for this binding. To assess this possibility, we preincubated the peptides at three different concentrations close to their $IC_{50}$ values with the same concentration of $ZnCl_2$, used in the assay (data not shown). If the peptides were prebound with $Zn^{2+}$, their capacity to inhibit biotin-HKa binding by competing for $Zn^{2+}$ should be diminished. However, even after preincubation with $Zn^{2+}$, the peptides retained full inhibitory capacity, indicating that the inhibition of binding of biotin-HKa to tropomyosin

![Fig. 3. Alanine scanning replacement of HHPHG. Peptides containing Ala replacement in each position of the peptide HHPHG were tested in the tropomyosin binding assay as described in Fig. 1. The data are the mean and SD of \(n = 4\) experiments. The calculated $IC_{50}$ values were normalized to the activity of HHPHG, $IC_{50} = 155 \pm 49 \mu M$.](image-url)
and the number of repeats for the uncapped multimers is determined by nonlinear regression analysis and is defined as IC₅₀. Carcinoma MatLyLu were mixed with Matrigel, together with 700 or 900 /H9262

The plugs removed and weighed. All treatments were tested in triplicate and means described for the modified Matrigel Plug assay. After 7 days, the animals were sacrificed; AHPHG, respectively, and then injected into the flank of a mouse following the protocol described for the Matrigel plug assay as described in Fig. 4. The HHPHG peptide has antiangiogenic activity, which is significantly decreased in the modified AHPHG peptide. A, 300 μM of both peptides, HHPHG and AHPHG, were added into a mixture containing FGF-2, heparin, and Matrigel. This was then injected into the flank of a mouse following the protocol described for the Matrigel Plug assay. After 3 days in the mice, the plugs were removed and analyzed. Full (100%) angiogenesis is defined as the hemoglobin level determined by Drabkin’s method in the FGF-2 containing plug minus the plug lacking FGF-2. B, 2 × 10⁶ cells of the rat prostate carcinoma MatLyLu were mixed with Matrigel, together with 700 or 900 μM HHPHG and AHPHG, respectively, and then injected into the flank of a mouse following the protocol described for the modified Matrigel Plug assay. After 7 days, the animals were sacrificed; the plugs removed and weighed. All treatments were tested in triplicate and means ± SD are given.

Table 1

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Tropomyosin binding IC₅₀ (μM)</th>
<th>SD</th>
<th>Matrigel plug % inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>HHPHG</td>
<td>92</td>
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<td>68*</td>
</tr>
<tr>
<td>(HHPHG)₆</td>
<td>11.6</td>
<td>2.8</td>
<td>32</td>
</tr>
<tr>
<td>Ac-(HHPHG)₆-NH₂</td>
<td>26.9</td>
<td>2.5</td>
<td>41</td>
</tr>
<tr>
<td>(HHPHG)₄</td>
<td>1.25</td>
<td>0.31</td>
<td>41</td>
</tr>
<tr>
<td>Ac-(HHPHG)₄-NH₂</td>
<td>1.58</td>
<td>0.53</td>
<td>53</td>
</tr>
<tr>
<td>(HHPHG)₃</td>
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<td>0.07</td>
<td>69</td>
</tr>
<tr>
<td>Ac-(HHPHG)₃-NH₂</td>
<td>0.245</td>
<td>0.12</td>
<td>84</td>
</tr>
</tbody>
</table>

NOTE. The peptides were tested in the tropomyosin binding assay as described in Fig. 1. The data are the mean and SD of n = 3 experiments. The peptides were also tested in the Matrigel plug assay as described in Fig. 4A. The data shown are from a representative experiment. HHPHG was tested at a concentration of 300 μM and the other peptides at 0.5 μM. The relationship between the number of repeats and the IC₅₀ is exponential as determined by nonlinear regression analysis of the data (Table 1). Similar results were obtained with the capped peptides (data not shown). The activity of Ac-(HHPHG)₄-NH₂ was characterized further in the Matrigel plug assay, and the titration curve generated (Fig. 5) showed an IC₅₀ for Ac-(HHPHG)₄-NH₂ of ~600 nM, only 6-fold higher than that measured for the full-length rabbit H/P domain (1). Because Ac-(HHPHG)₄-NH₂ can also bind zinc, we tested for the possibility that Ac-(HHPHG)₄-NH₂ manifested its antiangiogenic activity by chelating zinc and inhibiting zinc-dependent processes, e.g., enzymes, such as metalloproteinases, that are important for angiogenesis. To that end, Ac-(HHPHG)₄-NH₂ (0.5 μM) was tested in the Matrigel plug model alone or after preincubating the peptide with a 20-fold molar excess of ZnCl₂. If the antiangiogenic mechanism of action of Ac-(HHPHG)₄-NH₂ involves zinc sequestration, then saturating all possible binding zinc sites within the peptide should abrogate or decrease the antiangiogenic activity of Ac-(HHPHG)₄-NH₂. However, the magnitude of angiogenesis inhibition was similar regardless of whether the peptide had been preincubated with a 20-fold excess of zinc (~62 ± 22 versus ~60 ± 15% in the plugs where no zinc was added). ZnCl₂ alone, at an excess of 20-fold to the peptide concentration, did not have any significant activity in this assay (inhibition of ~5%). These results indicate that the antiangiogenic activity of Ac-(HHPHG)₄-NH₂ observed in the Matrigel plug model in vivo was most likely not due to chelation of zinc and inhibition of zinc-dependent processes.

To demonstrate that peptides derived from the H/P domain of HPRG could directly affect endothelial cells, we investigated the HUVEC binding and antiangiogenic activities of the peptide Ac-(HHPHG)₄-NH₂. We recently reported that biotin-rbHPRG, in the presence of zinc, binds with high affinity to FGF-2-stimulated HUVECs at two binding sites: cell surface-exposed tropomyosin (high affinity) and heparan sulfate proteoglycans (moderate affinity) (9). It was also demonstrated that the isolated H/P domain of HPRG, but not the remainder of protein (N/C fragment), can compete HPRG binding to HUVEC (9). Thus, we tested Ac-(HHPHG)₄-NH₂ for its ability to compete biotin-rbHPRG binding to HUVEC. Ac-(HHPHG)₄-NH₂, but not the N/C fragment, was able to compete the binding of biotin-rbHPRG to HUVEC in vitro (Fig. 6A) with an IC₅₀ ~0.5–1.0 μM. Ac-(HHPHG)₄-NH₂ also inhibited FGF-2-driven HUVEC proliferation in vitro in a zinc-dependent manner, as has previously been demonstrated for HPRG (Ref. 1; Fig. 6B). In summary, Ac-(HHPHG)₄-

![Fig. 4](image1)

![Fig. 5](image2)

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NH$_2$ appears to bind to HUVEC and inhibits the proliferative effects of FGF-2.

Antitumor Activity of (HHPHG)$_4$. The H/P domain of rbHPRG inhibits tumor-cell-driven angiogenesis but has no direct effect on tumor cells (1). This domain was not tested in a murine model of cancer because of the difficulty in obtaining sufficient amounts of the pure H/P domain. However, because sufficient quantities of peptides could be synthesized for animal studies, we evaluated the antitumor activity of the Ac-(HHPHG)$_4$-NH$_2$ peptide in two fast-growing syngeneic tumor models: the 3LL and the B16F1 melanoma. The capped peptide, which has activity similar to that of uncapped (HHPHG)$_4$ peptide or the N/C fragment, was used in this study to minimize exoproteolytic degradation after injection into mice. We first determined the best route of administration at several doses of peptide using the Matrigel plug model. Ac-(HHPHG)$_4$-NH$_2$ was administered i.p., i.v., or s.c. into mice that had previously been implanted with Matrigel plugs containing FGF-2 and vascular endothelial growth factor (Fig. 7). The peptide demonstrated antiangiogenic activity in this model regardless of the route of administration (Fig. 7). However, angiogenesis was most effectively blocked when the peptide was administered i.v. On the basis of these preliminary results, we decided to deliver the peptide daily at two doses (25 and 50 mg/kg) by i.v. bolus Monday-Friday in the 3LL model and at a dose of 75 mg/kg using the same schedule in the B16F1 model. Treatment was initiated in both models 4 days after tumor cell inoculation. The 50 mg/kg dose in the 3LL model significantly inhibited tumor growth by 48% ($P = 0.031$) at the end of the experiment (Fig. 8A). Similar results were observed at the 75 mg/kg dose in the B16F1 model (Fig. 8B) where tumor growth was inhibited by 47% ($P = 0.053$). We could not absolutely rule out an immune response against Ac-(HHPHG)$_4$-NH$_2$ in these studies, which could have the effect of neutralizing the antitumor activity of the peptide. However, because of rapid progression of tumor growth in these studies, it is unlikely that the animals had sufficient time to mount a robust immune response. In addition, mouse HPRG has eight repeats of HHPHG, making generation of an immune response against this sequence less likely. In any event, significant inhibition of tumor growth was observed in both models regardless of any possible immune response demonstrating the antitumor activity of the Ac-(HHPHG)$_4$-NH$_2$ peptide.

**DISCUSSION**

In this study, we have defined the consensus region within the H/P domain of HPRG responsible for its interaction with tropomyosin and for its antiangiogenic activity. The pentapeptides from the consensus sequence of the H/P domain have moderate affinity for tropomyosin that correlates with antiangiogenic activity *in vivo*. The potency of the binding and the antiangiogenic effect increases significantly in multimers of the consensus sequence, and an exponential relationship exists between the number of consensus sequence units and affinity for tropomyosin. A tetramer of the sequence HHPHG has significant antitumor activity in two syngeneic (3LL and B16F1) models of tumor growth in mice. This correlation between affinity for tropomyosin and antiangiogenic and antitumor activities provides additional support for endothelial cell surface tropomyosin as an antiangiogenic receptor. To date, tropomyosin has been shown to be involved in binding and relaying antiangiogenic signals for HKa (5), HPRG (9),...
interactions involving these repetitive units. Interestingly, a basic overall affinity may be the sum of several coordinated individual helical repetitive structures of opposite charge, suggesting that the H/P domain is a large electrostatic contribution to the binding process. This is consistent with the His residues of HPRG being essential for protonation of histidines induced under mild acidic conditions (9), suggesting that there is a large electrostatic contribution to the binding process. This is consistent with the His residues of HPRG being essential for the electrostatic nature of the interaction of the H/P domain and derived peptides with tropomyosin, it is tempting to speculate that HHRGK and other small antiangiogenic basic peptides (19) may be acting through binding to tropomyosin on the surface of endothelial cells.

The affinity of the peptides examined here for tropomyosin increases exponentially with the number of repeats of HPHPG, a corollary of this relationship is that a peptide comprised of 5 HHPGH units would have a predicted IC_{50} of 23 nM in the tropomyosin binding assay (using the equation derived in Table 1). This value is practically identical to that of the full H/P domain of HPRG (IC_{50} when competing with 10 nM HPRG ~14 nM). Therefore, it is possible that the affinity of the H/P domain for tropomyosin, and conceivably its full antiangiogenic activity, would be recapitulated by a pentamer of HPHPG units, nearly a 4-fold reduction in size from the H/P domain. The consequences of this possibility for understanding the interaction of the H/P domain with tropomyosin and for the development of an antiangiogenic agent are currently under investigation.

In summary, we have characterized some of the biochemical and biophysical aspects of the interaction of HPRG with tropomyosin that mediate the antiangiogenic activity of this relatively abundant plasma protein and have identified a 16-mer peptide with significant antiangiogenic and antitumor activity derived from a consensus sequence within the H/P domain of HPRG.

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18. Fazekas K, Janovics A, Dome B, Koska P, Albini A, Timar J. Effect of HGF-like factors on cell adhesion to tropomyosin and play a role in its antiangiogenic signaling. Structural tropomyosin is a parallel coiled coil containing repetitions of a 7-mer motif (17). The different tropomyosin isoforms are very similar in sequence and have an acidic isoelectric point of ~4.6 (8). The H/P domain is a poly-Pro type II helix (7) in which the His residues of the repeats of the consensus sequence protrude outward. The H/P domain appears to interact with negatively charged tropomyosin only when the H/P domain acquires a positive charge either through binding to Zn^{2+} via noncharged His residues or by direct protonation of histidines induced under mild acidic conditions (9), suggesting that there is a large electrostatic contribution to the binding. This is consistent with the His residues of HPRG being essential for affinity for tropomyosin and antiangiogenic activity as demonstrated in this study. Thus, both, the H/P domain and tropomyosin are helical repetitive structures of opposite charge, suggesting that the overall affinity may be the sum of several coordinated individual interactions involving these repetitive units. Interestingly, a basic pentapeptide from the beta chain of hepatocyte growth factor (HHRGK) has also been shown to have antiangiogenic activity (18). This peptide has sequence similarity and basic character analogous to HPHPG. In light of the electrostatic nature of the interaction of the H/P domain and derived peptides with tropomyosin, it is tempting to speculate that HHRGK and other small antiangiogenic basic peptides (19) may be acting through binding to tropomyosin on the surface of endothelial cells.
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