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Recombinant kringle 5 from plasminogen antagonises hepatocyte growth factor-mediated signalling

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ARTICLE INFO

Article history:
Received 4 June 2009
Received in revised form 15 December 2009
Accepted 17 December 2009
Available online 12 January 2010

Keywords:
Angiogenesis modulators
Hepatocyte growth factor
Cancer

ABSTRACT

The blood protein plasminogen is proteolytically cleaved to produce angiostatin and kringle 5 (K5), both of which are known angiogenesis inhibitors. A common structural element between K5, angiostatin and other endogenous angiogenesis inhibitors is the presence of the kringle protein-interacting domain. Another kringle domain-containing protein, hepatocyte growth factor (HGF), promotes angiogenesis by binding to and stimulating the tyrosine kinase receptor Met. HGF binding to Met is dependent on the kringle domains of HGF. Because both K5 and HGF contain kringle motifs and because these proteins have opposite effects on angiogenesis, we hypothesised that K5 can antagonise HGF-mediated signalling in a Met-dependent manner. We determined that K5 binding to H1299 cells is competed by HGF suggesting that these two proteins bind to the same protein. Purified K5 immunoprecipitates with Met and this interaction is abolished by increasing doses of HGF. Using proliferation, phosphorylation of Met and Akt as markers of HGF activity, we determined that K5 inhibits HGF-mediated signalling. Taken together, these data support a model by which K5 binds to Met and functions as a competitive antagonist of HGF signalling and presents a novel mechanism of action of K5.

1. Introduction

The inhibition of angiogenesis offers a promising therapeutic approach to inhibit tumour growth. Several endogenous angiogenesis inhibitors are protein fragments derived from extracellular matrix[1] or haemostatic system proteins.[2] The blood protein plasminogen is proteolytically cleaved into angiostatin (kringles 1–4) and kringle 5 (K5), both of which are potent angiogenesis inhibitors.[3–5] A hallmark of several endogenous antiangiogenesis inhibitors, including K5, is the presence of a protein–protein interaction domain termed the kringle domain.[6]

Hepatocyte growth factor (HGF)/scatter factor is a member of the plasminogen-related growth factor family. Despite the presence of four kringle domains, HGF promotes angiogenesis.[7,8] Full length HGF is synthesised as a single chain protein that is cleaved to form a heterodimer consisting of a 69 kDa α-chain and a 34 kDa β-chain.[9] The kringle domains located in the α-chain of HGF are required for high affinity binding to the HGF receptor Met.[10,11] Two naturally occurring HGF

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doi:10.1016/j.ejca.2009.12.026

isoforms, NK1 and NK2, exist and both are capable of binding to Met.[12–14] The ability of NK1, which contains the N-terminus and kringle domain 1, to bind Met indicates that a peptide consisting of a single kringle domain is sufficient to facilitate binding to Met.

Activation of Met by HGF induces phosphorylation of the C-terminal kinase domain of Met and creates a docking site for many downstream effectors which promote the HGF-stimulated events including proliferation, migration and invasion.[15] Ligand binding to Met does not necessarily stimulate both the mitogenic and the motogenic phenotypes of the HGF/Met-signalling axis. For example, the naturally occurring HGF isoform NK2 binds to Met and promotes the motogenic but not the mitogenic signal transduction cascades.[16] Furthermore NK4, a protein consisting of all four kringle domains of HGF, binds to Met and functions as a competitive antagonist of HGF signalling and is a potent inhibitor of angiogenesis.[6,17–20] The kringle-domain containing protein Des-γ-carboxy prothrombin acts as a Met agonist whereas angiostatin acts as an HGF-Met antagonist indicating that the ability of Met to bind kringle domains is not specific to those derived from HGF.[21,22]

Although K5 and HGF share a high degree of sequence similarity,[23] they have opposite effects on tumour growth and angiogenesis. Because both proteins contain kringle motifs and because K5 can antagonise HGF-mediated functions,[24] we hypothesised that K5 can antagonise HGF-mediated signalling in a Met-dependent manner. We demonstrate in this paper that K5 binding to tumour cells can be competed by HGF, K5 immunoprecipitates with Met and that pretreatment of cells with K5 inhibits subsequent HGF-mediated signalling and proliferation. Taken together, these data support a model by which K5 binds to Met and functions as a competitive antagonist of HGF signalling and represents a novel mechanism of action of K5.

2. Materials and methods

2.1. Materials, solutions and buffers

Pichia-expressed K5 was expressed as described previously.[25] Briefly, the human K5 gene was expressed in the methylotrophic yeast Pichia pastoris (Invitrogen). Genetic transcription of rK5 was under the control of the alcohol oxidase (AOX1) promoter. The AOX1 promoter permits high-level expression of heterologous proteins in Pichia. The K5 expression construct also includes a secretion signal sequence to direct transport of the protein to the medium. The plasmid construct was a hybrid of commercially available plasmid sequences from Invitrogen, designated pHIL-S1 and pHILD2. The expressed rK5 was purified by SP-Sepharose fast flow cation exchange chromatography, Hexyl Toyopearl 650C hydrophobic interaction chromatography and Q-Sepharose Fast Flow anion exchange chromatography. Correct folding of purified K5 was determined by NMR characterisation.

Cloning and expression of recombinant kringle 5 used for ALEXA labelling: in order to produce kringle 5 (K5) with the desired serine at the N-terminus, a modified form of the pET32a/K5 expression vector previously described was used.[24] Briefly, the K5 SLLPD was amplified from plasmid pEt32a/K5 using primers K5-EK-SLLPD-BglII-S: (5′-ccagatctgggtaccgacgacgacgacaagtccctgcttccagatgtagagac) and K5-3′-AS (5′-Pi-tattaggccgcacacggagggacatcacagtag), and subcloned into pETa vector using standard techniques to make pET32a/SLLPD-K5. After sequence confirmation, the pET32a/SLLPD-K5 vector was retransformed into *Escherichia coli* BL21 cells (DE3, Novagen) for expression as per the manufacturer's instructions. The cells were recovered from the media by centrifugation and stored frozen at –80 °C until use.

2.1.1. Purification

The frozen cells were resuspended in lysis buffer (50 mM Tris pH 8.0, 300 mM NaCl, 1 mM NaN_3, 2 mM $MgCl_2$) containing 0.1 mg/ml lysozyme and 20 units/ml benzonase at a ratio of approximately 4:1 (ml buffer:gram cell paste). The suspended cells were lysed using a Micro Fluidizer (Microfluidics International, Newton, MA). The his-tagged Trx-fusion protein was purified over Probond nickel resin (Invitrogen), and the resultant protein was dialysed in 50 mM Tris pH 8.0, 1 mM NaN_3. The Trx-fusion protein and his-tag were cleaved from the rK5 molecule by treatment with enterokinase (Invitrogen), yielding an SLLPD N-terminus on the rK5. The his-tagged Trx-fusion partner was removed by passing through a second Probond nickel column. The resultant SLLPD-K5 was further purified using Q-Sepharose ff (GE Healthcare, Piscataway, NJ) in 50 mM Tris pH 8.0 with a linear gradient of 0–300 mM NaCl.

2.1.2. ALEXA labelling

The SLLPD-K5 was dialysed in 50 mM sodium bicarbonate pH 7.8. Methione was added to reach 5 mM, and the pH was adjusted to 8.8 with 50% ammonium hydroxide in water. The SLLPD-K5 was then mildly oxidised by the addition of 10 equiv. of sodium periodate in two aliquots, generating a glyoxal form from the N-terminal serine side chain. This material was then dialysed in 50 mM sodium acetate, pH 5.0. The material was then allowed to react with ALEXA-488 hydrazide (Invitrogen). This reaction results in the addition of ALEXA-488 to only the N-terminus of the protein. The resultant labelled K5 was dialysed in 10 mM Bicine, pH 8.0, and purified using Q-Sepharose ff (GE Healthcare, Piscataway, NJ) in 10 mM Bicine, pH 8.0, with a linear gradient of 0–500 mM NaCl. The fractions were examined by mass spectrometry and those with the expected modification pooled. The pool was then dialysed in phosphate-buffered saline (PBS, Sigma, St. Louis, MO). Endotoxin levels of the final material were examined by Limulus Amebocyte Lysate (LAL) assay (Charles River Laboratories, Charleston, SC) and found to be less than 1 EU/mg.

2.1.3. Purification of rabbit anti-K5 polyclonal antibody

The total IgG fraction of pooled rabbit anti-K5 antiserum was purified by protein A affinity chromatography (BioSepra) according to the manufacturer's recommendation. The resultant IgG pool was dialysed against PBS and the resultant pool filtered through a 0.2 mm filter. Biotinylated K5 was made using biotin hydrazide (Pierce Chemicals, Rockford, IL) and following the procedure outlined for ALEXA-488 labelling above. The biotinylated K5 was bound to immobilised NeutrAvidin Beads (Pierce Chemicals, Rockford, IL). The total IgG fraction was then incubated with this K5 affinity resin,

washed to baseline with PBS and affinity purified rabbit anti-K5 polyclonal antibody eluted with pH 3.0 citrate. The eluted pool was neutralised by the addition of 1/10th volume of 1 M Tris pH 8.0 and dialysed in PBS.

HGF was purchased from US Biological (Swampscott, MA). Goat IgG, goat anti-Met (H9786), the PI3 kinase (PI3K) inhibitor LY294,002, IGF-1, HIS-select nickel affinity gel and the Met and prolactin receptor 6Xhis fusion proteins were purchased from Sigma. Phospho-Met Tyr1234/1235, β-Tubulin, β-Actin and Phospho Ser473 Akt antibodies were purchased from Cell Signalling (Danvers, MA). Odyssey blocking buffer, goat anti rabbit 680 (p-Akt) and goat anti mouse 800 (β-tubulin) secondary antibodies were obtained from Li-Cor Biosciences (Lincoln NE).

2.2. Cell culture

NCI-H1299 (H1299) and HT1080 cells were obtained from American Tissue Type Collections (Manassas, VA). H1299 cells were maintained in RPMI + 10% FBS. HT1080 cells were maintained in DMEM + 10% and incubated at 37 °C, 95% relative humidity and 5% CO_2.

2.3. Binding assay

The cells (100,000) were suspended in the presence of listed compounds along with 5 μM alexa K5. The cells were incubated in the dark for 60 min, washed and analysed by flow cytometry using a BD LRII flow cytometer (BD Biosciences). The events (5000) were recorded.

2.4. Western blot to detect phospho-Met

H1299 cells were cultured in serum-free DMEM overnight. The cells were treated with listed doses of K5 and incubated 60 min. The cells were treated with 10 ng/ml HGF, incubated 15 min. The cells were lysed in 2X SDS sample buffer and western blotting performed to detect Phospho-Met $Tyr^{1234/1235}$ and β-actin.

2.5. In cell western blot to detect phospho-Akt

The cells (20,000) were plated into each well of a 96-well tissue culture treated plate and incubated approximately 6 h, then the cells were starved in DMEM + 0.1% BSA overnight. For experiments using LY294,002 and the anti-met antibody, the cells were treated with these inhibitors for 30 min, then treated with HGF for 12 min and fixed in a final concentration of 4% formaldehyde. For experiments involving K5, the cells were pretreated with K5 as indicated, treated with the indicated growth factors for 12 min and then fixed in a final concentration of 4% formaldehyde. After fixing, the cells were permeabilised by washing 5 times, 5 min each wash with a solution of 0.1% triton-X100 in PBS, blocked for 90 min at room temperature in Odyssey blocking buffer and then incubated overnight at 4 °C with primary antibody. The primary antibodies were washed off using 0.1% tween-20 in PBS for 5 min, 5 times each. The cells were incubated in the dark with secondary antibodies for 1 h at room temperature, re-washed and scanned using an Odyssey imager (Li-Cor Biosciences). Statistical significance was determined using a Student's *t*-test.

2.6. Proliferation assay

HUVEC cells (3000) were plated into each well of a 96-well tissue culture treated plate and incubated overnight. The cells were starved in DMEM + 0.1% BSA for 4 h, treated with K5 for 90 min and then treated with HGF. The cells were incubated overnight and cell count measured using MTS proliferation reagent (Promega).

3. Results

3.1. Binding of rK5 to H1299 cells can be competed by HGF

Potential crosstalk between K5 and HGF was assessed by determining if HGF could inhibit K5 binding to cells. Incubation of H1299 cells with 5 μM ALEXA-488-labelled K5 (aK5) resulted in an increase in fluorescence on the cells indicating these cells bind K5 (Fig. 1A). This binding could be competed away with 50 and 100 μM concentrations of HGF likely suggesting that HGF and K5 bind to the same protein. The aK5 binding was also competed away by unlabelled K5 (data not shown).

Because Met is the receptor for HGF,[10] and because other non-HGF kringle-domain containing proteins are known to bind to Met,[21,22] we hypothesised that both HGF and K5 are binding to Met. To determine if K5 is binding to Met, a 6X histidine-tagged chimera of the extracellular domain of Met was immunoprecipitated in the absence or presence of 50 μM K5 and western blotting was performed to detect K5 (Fig. 1B). The results show that K5 can be immunoprecipitated with Met. Increasing concentrations of HGF were able to inhibit the amount of K5 that immunoprecipitated with Met. In contrast to what was observed with Met, K5 did not immunoprecipitate with a control protein, 6X histidine-tagged chimera of the extracellular domain of the prolactin receptor, in either the presence or the absence of HGF.

3.2. rK5 inhibits HGF-mediated increase in phospho-met in H1299 cells

Upon HGF binding, Met becomes phosphorylated and activates downstream signalling cascades.[15] Because both K5 and HGF bind Met, we were interested in determining if K5 could antagonise HGF-mediated signalling through the Met axis. Starved H1299 cells were pretreated with 100 μM K5 or vehicle control for 1 h and then HGF was added at a final concentration of 10 ng/ml. The cells were incubated for an additional 15 min and then phospho-Met was measured using western blot analysis (Fig. 2). HGF treatment alone resulted in a robust increase in the levels of phospho-Met but 100 μM K5 greatly diminished the ability of HGF to induce Met phosphorylation.

3.3. HGF-mediated increase in phospho-Akt is inhibited by K5 in H1299 cells

Phosphorylation of Met creates a docking site for many downstream effectors which promote the HGF-stimulated downstream events.[15] Because K5 inhibits the HGF-mediated increase in phospho-Met, we next determined if K5 antagonises HGF-mediated downstream signalling. A map of

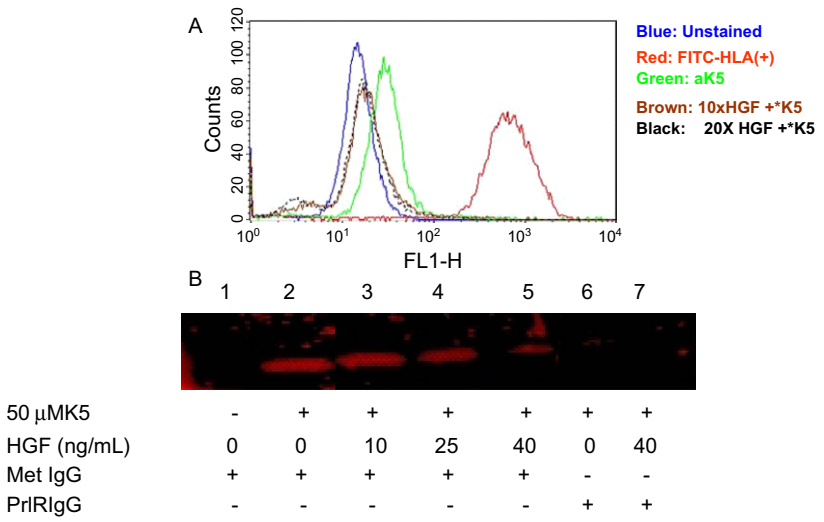

Fig. 1 – K5 binding to Met is inhibited by HGF. (A) H1299 cells were incubated with 5 μM alexa488-labelled K5 (aK5) alone (green), or in the presence of 10- (brown) or 20-fold (black) molar excess of HGF. Cellular fluorescence was measured using flow cytometry. As a positive control, the cells were stained with FITC-labelled HLA (red). As a negative control, unlabelled cells were analysed (blue). (B) The his-tagged extracellular domain of Met was incubated with no K5 control or with 50 μM K5 in the presence of increasing concentrations of HGF. Met was immunoprecipitated and co-precipitated K5 was detected by western blotting. As a negative control, the his-tagged extracellular domain of the prolactin receptor (PrlR) was used. (For interpretation of the references in colour in this figure legend, the reader is referred to the web version of this article.)

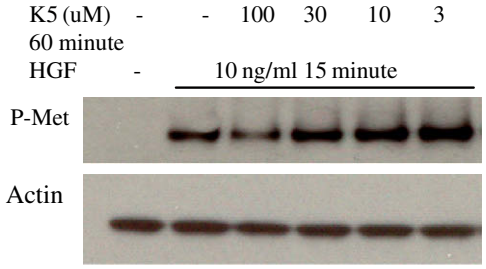

Fig. 2 – K5 treatment inhibits HGF-mediated phosphorylation of Met. H1299 cells in serum-free media were incubated for 60 min with the listed concentrations of K5 and then stimulated with 10 ng/ml HGF for 15 min (total K5 treatment of 75 min). Cell lysates were prepared and western blotting was performed to detect phospho-Met Tyr$^{1234/1235}$ as well as β-actin.

Met-regulated signalling pathways was generated using the genego software programme (Fig. 3). HGF treatment increases phosphorylation of Akt through a phosphatidylinositol 3-kinase-dependent (PI3 K) pathway.

To demonstrate that HGF increases phospho-Akt in H1299 cells, starved H1299 cells were treated with 2.5 ng/ml HGF or vehicle control for 12 min and Phospho-Akt was measured using in-cell western blot analysis (Fig. 4). HGF treatment resulted in an approximately fourfold increase in the levels of phospho-Akt. To determine if the HGF-mediated increase in phospho-Akt was Met-dependent, a Met antibody that blocks HGF binding was employed. The HGF-mediated increase in phospho-Akt was not inhibited by pretreatment with a non-specific IgG but was abolished by pretreatment with an anti-Met antibody (Fig. 4). Pretreatment with the PI3 K inhibitor LY294,002 inhibited the HGF-mediated increase in phospho-Akt in a dose responsive manner indicating that the PI3 K pathway is mediating the increase in phospho-Akt (Fig. 4).

To determine if pretreatment with K5 antagonises HGF-mediated signalling, H1299 cells were preincubated with varying concentrations of K5 for 90 min, stimulated with 2.5 or 5 ng/ml HGF for 12 min and phospho-Akt was quantitated (Fig. 5A). A K5 dose response curve indicated that at 2.5 ng/ml HGF, the IC_{50} was ~15 μM, at 5 ng/ml HGF, the IC_{50} was ~30 μM. Increasing doses of HGF were able to overcome the K5-mediated inhibition of phospho-Akt induction indicating that antagonism is likely competitive (Fig. 5B).

3.4. *rK5-mediated block in phospho-Akt is selective for HGF*

To determine if the K5-mediated inhibition of phospho-Akt activation was specific for HGF treatment, we interrogated if K5 could block an IGF-1-mediated increase in phosho-Akt. The cells were pretreated with 50 or 500 μM K5 for 90 min and then treated with 5 ng/ml of either HGF or IGF-1. The HGF-mediated increase in phospho-Akt was significantly inhibited by pretreatment with 50 μM K5 and 500 μM completely abolished HGF-mediated signalling (Fig. 6). In contrast, 50 μM K5 had no effect and 500 μM K5 had only a modest effect on the IGF-1-induced increase in phospho-Akt.

3.5. *HGF-mediated increase in phospho-Akt is inhibited by K5 in other cell lines*

The ability of K5 to inhibit HGF signalling was tested in HUVEC and HT1080 cell lines to determine if this action is cell-line specific. H1299, HT1080 and HUVEC cells in starvation media were preincubated for 90 min with varying

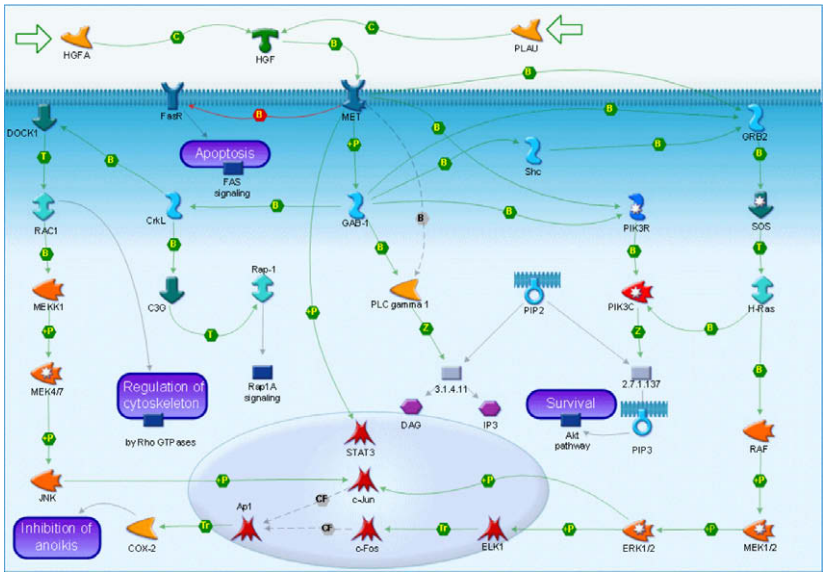

Fig. 3 – Signalling pathways regulated by HGF binding to Met. Using GeneGo software, signalling pathways identified by HGF binding to Met were elucidated.

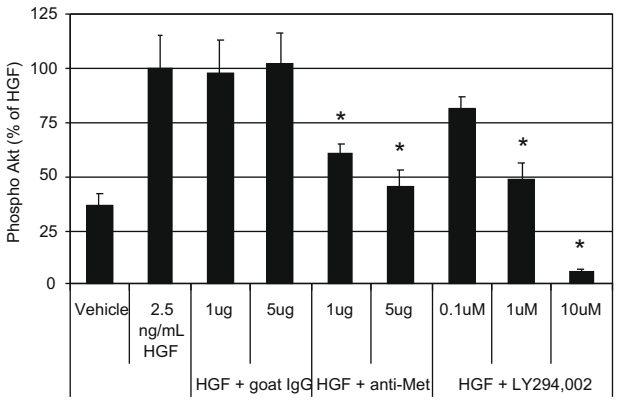

Fig. 4 – HGF-mediated phosphorylation of Akt is Met and phosphatidylinositol 3-kinase-dependent. H1299 cells in serum-free media were incubated for 30 min with goat IgG, goat anti-met IgG that blocks HGF binding or the phosphatidylinositol 3-kinase inhibitor LY294,002. After 30 min, the cells were stimulated with 2.5 ng/ml HGF for 12 min, fixed and in cell western blotting was performed to quantitate phospho-Akt. Asterisks denote $p \leqslant 0.05$.

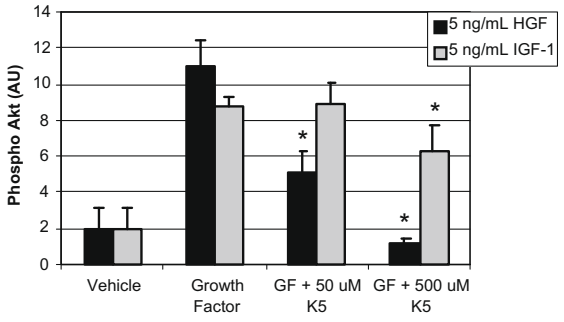

Fig. 6 – K5 selectively inhibits HGF-mediated phosphorylation of Akt. H1299 cells in serum-free media were incubated with listed doses of K5 for 90 min, stimulated with 5 ng/ml of HGF or IGF-1 for 12 min, fixed and in cell western blotting was performed to quantitate phospho-Akt. Asterisks denote $p \leqslant 0.05$.

concentrations of K5 followed by treatment with 2.5 ng/ml HGF for 12 min. Similar to what is observed in H1299 cells, K5 inhibits HGF-mediated signalling in a dose-dependent manner in HT1080 and HUVEC cell lines (Fig. 7).

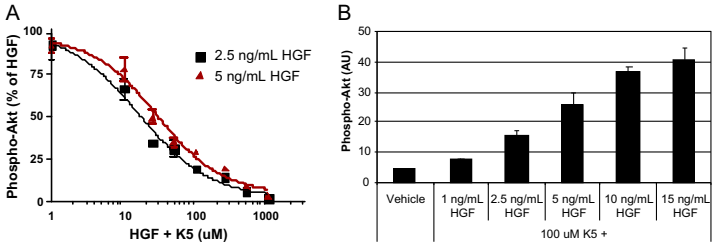

Fig. 5 – K5 inhibits HGF-mediated induction of phospho-Akt. (A) H1299 cells were incubated in serum-free media overnight. (A) The cells were treated with listed doses of K5 for 90 min, stimulated with 2.5 or 5 ng/ml HGF for 12 min, fixed and in cell western blotting was performed to quantitate phospho-Akt. (B) The cells were treated with 100 μM K5 for 90 min, stimulated with listed doses of HGF for 12 min, fixed and in cell western blotting was performed to quantitate phospho-Akt.

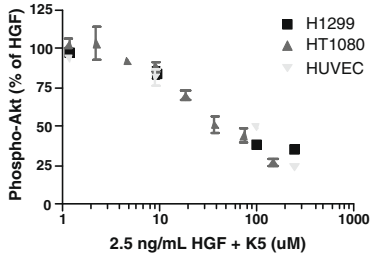

Fig. 7 – K5 inhibits HGF signalling in other cell lines. H1299, HUVEC and HT1080 cells in serum-free media were incubated with listed doses of K5 for 90 min, stimulated with 2.5 ng/ml HGF for 12 min, fixed and in cell western blotting was performed to quantitate phospho-Akt.

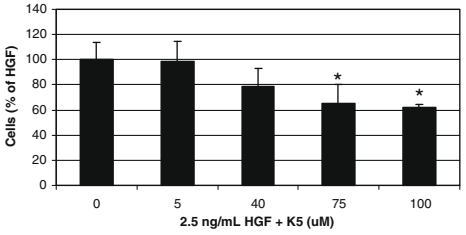

Fig. 8 – K5 inhibits HGF-induced Proliferation of HUVEC Cells. HUVEC cells in serum-free media were incubated with the listed doses of K5 for 90 min, stimulated with 2.5 ng/ml HGF, incubated overnight and proliferation was measured using MTS reagent. Asterisks denote $p \leqslant 0.05$.

3.6. *K5 inhibits HGF-mediated proliferation in HUVEC cells*

The ability of K5 to inhibit of HGF-mediated proliferation was tested in HUVEC cell lines. HUVEC cells in starvation media were preincubated for 90 min with varying concentrations of K5 followed by treatment with 2.5 ng/ml HGF. K5 inhibits HGF-mediated proliferation in a dose-dependent manner (Fig. 8).

4. Discussion

Previous studies of K5 activity have focused on its role as a potent inhibitor of angiogenesis demonstrating inhibition of endothelial cell migration,[24,26–28] proliferation[4,24,28,29] and induction of apoptosis.[24,28] Although less sensitive to K5 than endothelial cells,[30] a growing body of evidence indicates K5 treatment can directly induce apoptosis of tumour cells directly.[24,30,31] Because of the dual roles of K5 affecting tumour vasculature and tumour cells directly, there is significant interest in its potential application as a cancer therapeutic.

Our results demonstrate that K5 binds to H1299 non-small cell lung cancer cells and this binding can be competed by HGF. Inhibition of K5 binding by HGF suggests the two proteins bind to the same receptor. K5 immunoprecipitates with the HGF receptor Met and this interaction is abolished by increasing doses of HGF. Using Met and Akt phosphorylation as markers of HGF activity, we demonstrate that K5 inhibits HGF-mediated signalling. Taken together, these data support a model by which K5 binds to Met and functions as a competitive antagonist of HGF signalling. These results define a mechanism by which K5 can act directly upon tumour cells to antagonise HGF-mediated activation of pro-proliferative and anti-apoptotic survival cascades of importance in tumour biology.

Our results indicate that K5 inhibits HGF signalling with an IC50 of approximately 15 μM K5 when 2.5 ng/ml HGF is used. The physiological concentration of HGF is 0.5 ng/ml in prostate cancer patients.[32] Supraphysiological, saturating concentrations of HGF were used in the experiments reported here to increase the dynamic range and to decrease the variability of the HGF-induced responses. Because the K5-mediated antagonism is competitive, at physiological HGF concentrations the IC50 amount of K5 needed in inhibiting HGF signalling should be greatly reduced. Furthermore, although multiple published reports have demonstrated the utility of K5 as a pharmacological agent in preclinical xenograft models,[33,34] little data have emerged to indicate what concentrations of K5 are required to achieve tumour inhibition *in vivo*. Therefore, it remains to be determined to what extent K5-mediated antagonism of HGF signalling contributes to the anti-tumour activity of pharmacologically administered K5.

Other kringle-domain containing protein fragments have been shown to bind to Met. For example, the naturally occurring HGF isoform HGF/NK2 binds to Met and promotes the motogenic but not the mitogenic signal transduction cascades.[16] Furthermore NK4, a protein consisting of all four kringle domains of HGF, binds to Met. NK4 has been shown to be a competitive antagonist of HGF signalling and is a potent inhibitor of angiogenesis.[6,17–20] In addition to kringle-domain protein fragments derived from HGF, non-HGF kringle domain-containing proteins also bind Met. For example, Des-γ-carboxyl prothrombin is a potent Met agonist[21] whereas angiostatin is a Met antagonist.[22] Collectively, these data demonstrate that kringle domain proteins can function as agonists, antagonists or partial antagonists of HGF/Met signalling. The data presented here demonstrate that K5 functions as an antagonist of HGF signalling. Furthermore, similar to what was observed for angiostatin,[22] higher doses of HGF were able to overcome inhibition by K5 indicating that both compounds are competitive antagonists of HGF.

Although both angiostatin and K5 antagonise HGF-mediated signalling, K5 is less potent. Angiostatin binds Met with a Kd of 0.75 μM,[22] whereas we report that K5 inhibits HGF signalling with an IC50 of approximately 15 μM K5 when 2.5 ng/ml HGF is used. Several possible explanations for the differences in K5 potency exist. First, angiostatin has four kringle domains whereas K5 only has one kringle domain. In an analogous situation, full length HGF which contains four kringle domains binds Met with approximately a 8–11-fold higher affinity than its naturally occurring isoform NK1 which only contains 1 kringle domain.[35] This may indicate that although a single kringle domain is capable of binding Met, the presence of additional kringle domains can increase the avidity of the interaction. Another potential explanation of the higher relative potency of angiostatin in inhibiting HGF signalling is that angiostatin may more closely resemble HGF and therefore bind Met with a higher affinity. Based on mutational analysis and molecular modelling, Glu159, Ser161, Glu195

and Arg197 have been strongly implicated as critical residues involved in Met binding.[36] For comparison, kringle domain 1 of angiostatin has identical residues at two of these locations, whereas K5 has identity at only one of these residues.[23] Similar to what was observed for angiostatin,[22] higher doses of HGF were able to overcome inhibition by K5 indicating that both compounds are competitive antagonists of HGF.

In summary, we have shown that K5 is an antagonist of the HGF-Met signalling axis. The ability of K5 to bind to and antagonise the HGF-met signalling axis represents a novel mechanism of K5.

Conflict of interest statement

None declared.

Acknowledgements

The authors would like to thank David Egan, Robert W. Johnson, Paul Richardson, Earl Gubbins Andrew Schneider, Jack Henkin and Karl Walter for the preparation and ALEXA-488 labelling K5, Jeanne Severin for the purification of the K5 antibody, Barbara Perilli-Palmer, Harold Staak, Dave Post and Doug Spaulding for the preparation of K5 from Pichia and Taro Fujimori for the preparation of a section of the manuscript.

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