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Isolation of peptides that inhibit binding of basic fibroblast growth factor to its receptor from a random phage-epitope library

(fibroblast growth factor receptor)

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ABSTRACT **Basic fibroblast growth factor (bFGF) is known to bind to its cell-surface receptors with high affinity and in a heparin-dependent manner. In an attempt to predict the receptor recognition site on bFGF we screened phage-epitope libraries with monoclonal antibodies DG2 and DE6, which inhibit bFGF binding to its receptor. On the affinity-isolated phages, we identified several peptide sequences as the putative antibody-binding epitopes on bFGF. The identified library epitopes shared the consensus sequence Pro-(Pro/Ser)-Gly-His-(Tyr/Phe)-Lys, corresponding to two continuous protein sequences of bFGF: Pro-Pro-Gly-His-Phe-Lys and Arg-Thr-Gly-Gln-Tyr-Lys at amino acids 13–18 and 120–125 of bFGF, respectively. Synthetic peptides of the corresponding phage epitopes or of the above bFGF sequences specifically inhibited binding of the antibodies to bFGF, blocked binding of bFGF to its high-affinity receptor, and inhibited basal and bFGF-induced proliferation of vascular endothelial cells at submicromolar peptide concentrations. The potent inhibition of bFGF binding and biological activity by peptides recognized by the antibodies suggests that these sequences are functionally involved in receptor binding and may constitute part of the receptor-binding determinants on bFGF.**

Basic fibroblast growth factor (bFGF) is a potent heparin-binding growth factor that stimulates proliferation, migration, and differentiation of cells of mesenchymal and neuroectodermal origin (1–4). bFGF participates as an autocrine modulator of cell growth and transformation and is abundant in malignantly transformed cells (5, 6). Recent studies demonstrate that local *in vivo* expression of acidic FGF (aFGF) promotes intimal hyperplasia (7) and that exogenous administration of bFGF stimulates tumor angiogenesis and progression (8). Accumulating evidence suggests that blocking the interactions of bFGF with its receptors leads to inhibition of cell proliferation and tumor progression *in vivo*. For example, inhibition of FGF receptor (FGFR) 1 gene expression in human melanocytes and malignant melanomas by antisense oligonucleotides led to the inhibition of cell proliferation and the induction of differentiation (9). Targeting of FGFR-1 by a toxic FGF–saporin complex eliminated intimal smooth-muscle-cell proliferation in an experimental restenosis model (10).

The biological response of cells to bFGF is mediated through specific cell-surface receptors that possess intrinsic tyrosine kinase activity and are phosphorylated upon binding of bFGF (for reviews, see refs. 2 and 3). High-affinity binding and activation of FGFRs require cell-surface heparan sulfates, which in turn modulate FGF–receptor interactions (11, 12). Four FGFRs (denoted FGFR-1 to -4) have recently been identified, and their cDNA encode a multitude of structural and functional receptor variants (for reviews, see refs. 13–15). FGFR-1 (*flg*) is widely expressed in a variety of tumor-derived cells and tissues and is the major FGFR of vascular endothelial cells (16). Blocking of bFGF–FGFR-1 interactions may, therefore, have important implications for inhibition of pathological angiogenesis and attenuation of unrestrained cell proliferation and cancer.

Two monoclonal antibodies (mAbs) raised against human recombinant bFGF were found to efficiently block binding of bFGF to its cell-surface receptors (17). These two mAbs, designated DG2 and DE6, inhibited bFGF-stimulated proliferation of cultured human glioma cells and retarded rat C6 glioma growth in nude mice (18). A recent study showed that the administration of neutralizing mAbs to bFGF caused significant alterations in tumor growth *in vivo* and that these changes were specific for tumor type and bFGF characteristics (8). The inhibitory effects of the antibodies on bFGF binding and biological activities suggest that the bFGF epitopes recognized by the antibodies may coincide with sequences recognized by the receptor. To identify these sequences we used these antibodies to screen a phage-epitope library (19).

The library consists of phages bearing random hexapeptides fused to the amino terminus of the coat protein PIII. Screening of the library is accomplished by the use of a monospecific binding protein, such as an antibody, to affinity-purified phages that display a high-avidity binding peptide. The amino acid sequences of the hexapeptides displayed on the phage are then determined by sequencing the corresponding coding region in the phage DNA (19). Here we describe the characterization of a series of hexapeptides synthesized according to the epitope sequences identified in the phage-epitope library by screening with mAbs DG2 and DE6. These peptides show a significant ability to inhibit high-affinity receptor binding and biological activity of bFGF.

MATERIALS AND METHODS

Materials. Human recombinant bFGF was from Takeda (Osaka). The mAbs DG2 and DE6 have been described (17). Rabbit antibodies against human placenta alkaline phosphatase (AP) or against phage M13 were generated by immunization using a standard procedure (20). Heparin was from Hepar (Franklin, OH). The hexapeptide phage-epitope library was from G. Smith (University of Missouri, Columbia) (19).

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Abbreviations: FGF, fibroblast growth factor; aFGF, acidic FGF; bFGF, basic FGF; FGFR, FGF receptor; mAb, monoclonal antibody; AP, alkaline phosphatase; BSA, bovine serum albumin.

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Cell Lines. NIH 3T3 cells and primary cultures of bovine aortic endothelial cells were maintained in Dulbecco's modified Eagle's medium (DMEM)/10% fetal calf serum (HyClone)/1% glutamine.

Panning of the Phage-Epitope Library. Antibodies were biotinylated by incubation of 100 μg of antibody with 5 μg of biotinamidocaproate *N*-hydroxysuccinimide ester (Sigma B 2643) in 100 μl of 0.1 M $NaHCO_3$ for 2 hr at room temperature followed by dialysis against Tris-buffered saline (TBS, 50 mM Tris, pH 7.4/0.15 M NaCl). For panning, Petri dishes were coated with streptavidin at 10 μg/ml overnight at 4°C and then blocked with bovine serum albumin (BSA) at 1 mg/ml in TBS for 1 hr at room temperature. The free and phage-bound biotinylated antibodies were adsorbed onto the streptavidin-coated plates, and affinity-isolation of phages from the hexapeptide epitope library by three cycles of panning was carried out as described in ref. 19.

ELISA Assay for Affinity-Purified Phages. Microtiter plates were coated overnight with rabbit anti-M13 antibodies. After the plates were washed and blocked with BSA, 100 μl of *E. coli* supernatant from individual colonies obtained by panning was added to the wells, and plates were incubated for 2 hr at 37°C. After the plates were further washed, 100 μl of mAb DG2 or DE6 (1 μg/ml) was added, and the plates were incubated for 2 hr at 37°C. Binding of the antibodies to the phage was identified by the use of AP-conjugated rabbit anti-mouse immunoglobulins. Color was developed by adding 100 μl of 5 mM *p*-nitrophenylphosphate, and the OD at 405 nm was measured.

Binding of ^{125}I-Labeled FGF to Soluble FGFR. Iodination of bFGF was done as described (21). Conditioned medium from cells secreting FGFR-1–AP fusion protein (21, 22) was incubated for 45 min at room temperature with rabbit anti-human placental AP antibodies prebound to agarose-protein A beads (Pierce). The FGFR-1-coupled beads were washed three times with 1 ml of 20 mM Hepes, pH 7.5/150 mM NaCl/1% Triton X-100/10% (vol/vol) glycerol (HNTG medium) and incubated with ^{125}I-labeled FGF at 2 ng/ml, heparin at 1 μg/ml, and peptides at different concentrations for 1–2 hr at room temperature. High-affinity-bound ^{125}I-labeled bFGF was determined after three cycles of washing with HNTG medium and counting the radioactivity in the tubes with a γ counter.

Binding of ^{125}I-Labeled bFGF to mAb DG2. Maxisorb plates (Nunc) were coated with 100 μl of rabbit anti-mouse IgG (Jackson ImmunoResearch) diluted 1:500 in 0.1 M $NaHCO_3$. After 16 hr at 4°C the plates were washed five times with phosphate-buffered saline (PBS)/0.05% Tween 20, and mAb DG2 (100 μl containing 20 ng) was then added to the wells and incubated for 16 hr. After being washed as described above and being blocked with 10% low-fat milk in PBS for 1 hr, the plates were washed again, and 50 μl of ^{125}I-labeled bFGF (0.2 ng/ml) was added in the presence of increased concentrations of various peptides. After 2 hr of incubation the plates were washed with HNTG medium, and the bound radioactivity was dissolved in 0.1 M NaOH and counted in a γ counter.

Binding and Cross-Linking of ^{125}I-Labeled bFGF to Cells. Confluent cultures of NIH 3T3 cells in 24-well plates (Falcon) were precooled and washed twice with cold DMEM/20 mM Hepes, pH 7.5/0.1% BSA (DMEM/BSA). They were then incubated for 1.5 hr at 4°C with ^{125}I-labeled bFGF at 2 ng/ml in DMEM/BSA and different concentrations of peptides. The binding medium was discarded, and the cells were washed once with ice-cold DMEM/BSA and twice with cold PBS, pH 7.5, containing 1.6 M NaCl. High-affinity receptor-bound bFGF was determined by extraction of the cells with 20 mM $NaC_2H_3O_2$, pH 4.0/2.0 M NaCl. Nonspecific binding was estimated as the residual counts in the presence of a 100-fold excess of unlabeled bFGF. For cross-linking the beads were resuspended in 0.4 ml of 0.15 mM disuccinylimidyl suberate (Pierce) in PBS for 30 min, washed with PBS, and boiled for 5 min in sample buffer [50 mM Tris, pH 6.8/25% (vol/vol) glycerol/6% (vol/vol) 2-mercaptoethanol/4% (wt/vol) SDS/1 mM EDTA/10% (wt/vol) bromophenol blue]. The protein complexes were run on a SDS/7.5% polyacrylamide gel. The gel was dried and exposed to x-ray film (Agfa).

Cell Proliferation Assay. Bovine aortic endothelial cells were seeded in 24-well plates (5000 cells per well) in DMEM/10% bovine calf serum. Peptide, dissolved in PBS and filtered, was added every day for 1–6 days, according to the experimental design. Cells were removed by trypsin, and radioactivity was counted after 6 days or every other day with a Coulter Counter.

DNA Sequencing. Phages from the supernatants of positive colonies were precipitated with polyethyleneglycol, and their DNA was prepared by phenol extraction. DNA was sequenced by the chain-termination method with the aid of a described primer (19).

RESULTS

Isolation of Phages Displaying bFGF Epitopes from the Hexapeptide Epitope Library. Our approach is based on the assumption that antibodies that neutralize bFGF and block receptor binding may recognize bFGF epitopes that participate in the binding of bFGF to the FGFR. To identify such epitopes on bFGF we screened the hexapeptide phage-epitope library with the two mAbs DG2 and DE6, shown previously to potently inhibit bFGF binding *in vitro* (17) and *in vivo* (18). After three cycles of panning and phage amplification, the individual phages derived from each mAb screen were grown, and their epitope-encoding regions were sequenced. The results shown in Table 1 indicate that mAbs DG2 and DE6 bind to similar epitopes, most of which share the consensus epitope sequence P(P/S)GH(Y/F)K (single-letter amino acid code). Sequence comparison with bFGF revealed two possible homologous sequences that may represent the authentic epitopes on bFGF—namely, PPGHFK and RTGQYK at aa 13–18 and 120–125, respectively. ELISA analysis of the binding of the two mAbs to their corresponding epitope-presenting phages showed that phages isolated by one antibody cross-react with the other antibody, suggesting that the two antibodies may recognize a similar epitope (data not shown, see also Table 1). Competitive binding-inhibition studies with synthetic peptides corresponding to either the phage-displayed epitope or the putative authentic epitopes from bFGF demonstrated high-affinity binding of these peptides to both mAbs. Fig. 1*A* depicts the inhibition pattern of several peptides on the binding of mAb DG2 to phages displaying the epitope PSGHYK. Interestingly, the peptide APSGHYKG, which corresponds to the phage-epitope sequence PSGHYK and includes the two flanking amino acids of the phage coat protein PIII (19), had a 50-fold higher

Table 1. Epitope sequence of phages isolated by panning with anti-bFGF antibodies and their corresponding bFGF sequences

Antibody	Epitope (*n*)
mAb DG2	PSGHYK (2)
	PPGHFK (3)
mAb DE6	PSGHYK
	PSGHFK
	PPGHFK
	PWGHFK
bFGF-(13–18)	PPGHFK
bFGF-(120–125)	RTGQYK

Single numbers in parentheses (*n*) represent number of phage sequenced. The phage epitope (single-letter amino acid code) was deduced from the DNA sequence.

affinity toward mAb DG2 than did the shorter hexapeptide PSGHYK. This result suggests that the amino acids flanking the phage epitope may contribute to its binding affinity. Fig. 1*B* shows the inhibition pattern of the same peptides on binding of mAb DG2 to bFGF. This pattern resembles that shown by the inhibition to the epitope-displaying phage (Fig. 1*A*). It is noted that peptide PPGHFK (residues 13–18 of bFGF) is a better inhibitor than peptide KRTGQYKL (residues 119–126 of bFGF).

Epitope-Library-Derived Peptides Inhibit Binding of bFGF to Its Receptor. To analyze the capacity of the peptide epitopes derived from the phage library to inhibit bFGF binding to its receptor, we examined their effect on the binding of ^{125}I-labeled bFGF to FGFR-1, using the secreted FGFR-1–AP fusion protein, as described (21, 22). Fig. 2 shows an example of such an analysis: peptides identified by screening of the phage library with the anti-bFGF mAbs DG2 and DE6, as well as their corresponding authentic peptides derived from the bFGF sequence, competitively inhibit binding of ^{125}I-labeled bFGF to its receptor. The bFGF-derived peptides KRTGQYKL and PPGHFK, which correspond to the antibody-identified epitopes (Table 1), are ≈100- and 10-fold better inhibitors, respectively, than the phage epitope PSGHYK (Fig. 2 *A* and *B*). It is therefore likely that the antibody-identified epitopes represent a sequential determinant of bFGF that may be involved in both antibody and FGFR-1 binding. In this system the peptide KRTGQYKL (residues 119–126 of bFGF) is a better inhibitor than peptide PPGHFK (residues 13–18 of bFGF), in contrast to the inhibition pattern of the two peptides when tested on antibody binding to the phages (Fig. 1).

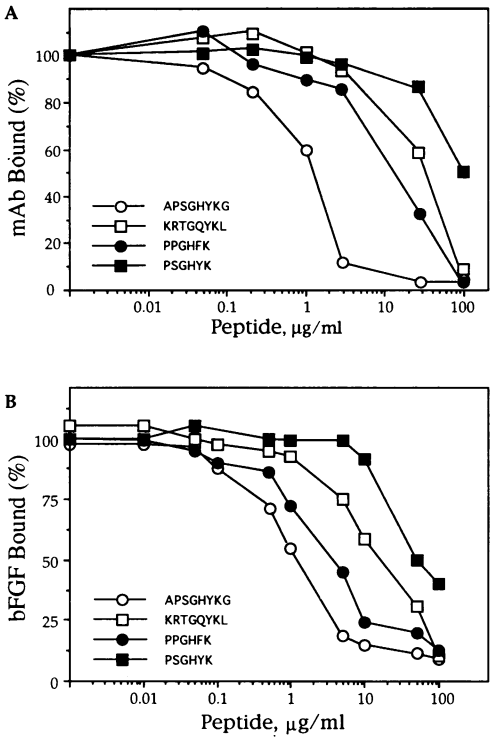

FIG. 1. Inhibition by peptides of mAb DG2 binding. (*A*) ELISA of the inhibition by various peptides of mAb DG2 binding to phages displaying the epitope PSGHYK; the assay was done as described, with 100 ng of mAb DG2 per well and increased concentrations of the indicated peptide. Binding of mAb to phages was monitored by AP-conjugated anti-mouse immunoglobulin, followed by the addition of *p*-nitrophenylphosphate and reading absorbance values at 405 nm. The identified peptides are listed in Table 1. The control bound mAb had an OD value of 0.60. (*B*) Inhibition of mAb DG2 binding to ^{125}I-labeled bFGF by peptides derived from the epitope library or from bFGF; the assay was done as described, using 96-well plates coated with rabbit anti-mouse immunoglobulin. After binding of mAb DG2 to the coated wells, ^{125}I-labeled FGF (0.2 ng in 50 μl) was added in the presence of increased concentrations of the indicated peptides. After 2 hr of incubation the plates were washed, and radioactivity was counted in a γ counter. The control bound bFGF yielded 4000 cpm.

We next tested the capacities of different peptides to inhibit high-affinity binding of ^{125}I-labeled bFGF to NIH 3T3 cells, which are known to express FGFR-1 and respond to bFGF (23). An inhibition pattern similar to that shown with the soluble receptor was observed (Fig. 3), except that the IC_{50} for inhibition of bFGF binding to cells by the different peptides was 10- to 20-fold higher than that required for comparable inhibition of bFGF binding to the soluble receptor FGFR-1–AP. This result can probably be explained by the ≈20-fold higher affinity of bFGF to cell-bound receptors than to the soluble receptors (21, 22).

Affinity Labeling of Soluble FGFR-1 with ^{125}I-Labeled bFGF Is Inhibited by the Epitope-Library-Derived Peptides. To further demonstrate the capacity of the epitope-library-derived peptides and their corresponding bFGF epitopes to block bFGF–receptor interactions, we performed binding and chemical cross-linking of ^{125}I-labeled bFGF to soluble FGFR-1 in the absence or presence of the different peptides. Cross-linking of labeled bFGF and FGFR-1–AP in the presence of heparin at 200 ng/ml to allow high-affinity binding (21) resulted in a typical labeled complex of ≈150 kDa (Fig. 4). Preincubation of the iodinated bFGF with mAb DG2 at 1 μg/ml abolished the binding and cross-linking of bFGF to soluble FGFR-1. The antibody-derived phage epitope APSGHYKG, as well as its corresponding bFGF peptide KRT-

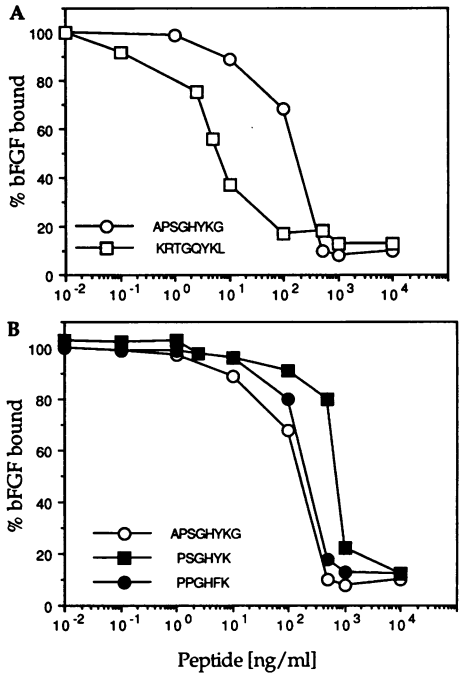

FIG. 2. Inhibition by synthetic peptides of bFGF binding to FGFR-1. Immobilized FGFR-1 was prepared by the binding of conditioned medium from cells secreting soluble FGFR-1–AP to rabbit anti-AP prebound to Sepharose-protein A (21). The washed beads were incubated in 200 μl of DMEM/20 mM Hepes/0.1% BSA containing ^{125}I-labeled bFGF (2 ng/ml), heparin (200 ng/ml), and the indicated peptides at increased concentrations. After incubation with shaking at room temperature, the beads were washed with HNTG medium three times, and radioactivity was counted. (*A*) Inhibitory peptides derived from the epitope-presenting phage (Table 1) and the corresponding bFGF sequence (aa 119–126). (*B*) Inhibitory peptides derived from phage epitope with and without phage-flanking amino acids and the corresponding bFGF peptide (aa 13–18).

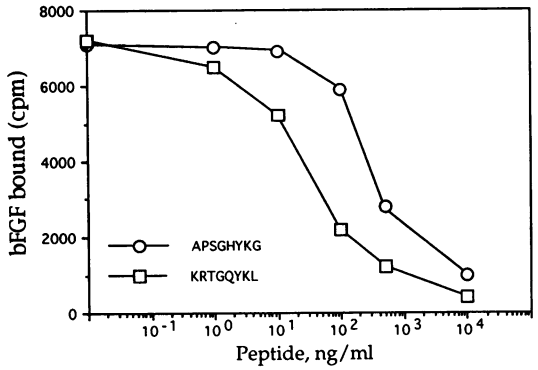

FIG. 3. Inhibition by synthetic peptides of bFGF binding to high-affinity receptors on NIH 3T3 cells. NIH 3T3 cells were incubated with a constant concentration of ^{125}I-labeled bFGF (2 ng/ml) in 250 μl of DMEM/0.1% BSA/heparin at 200 ng/ml with increased concentrations of epitope-library-derived peptide APSGHYKG or bFGF-derived peptide KRTGQYKL for 90 min at 4°C. Cell-associated radioactivity was determined after washing, as described.

GQYKL, efficiently blocked cross-linking of ^{125}I-labeled bFGF to its receptor at peptide concentrations of 20 ng/ml.

Epitope-Library-Derived Peptides Inhibit Endothelial Cell Proliferation. Fibroblast growth factors stimulate endothelial cell proliferation and migration *in vitro* and are potent angiogenic factors *in vivo* (1). We therefore tested the effects of the epitope-library-derived peptides on the proliferation of bovine aortic endothelial cells. As can be seen in Fig. 5*A*, daily additions of either APSGHYKG or KRTGQYKL at 1 μg/ml completely inhibit the basal growth of aortic endothelial cells under conditions in which the untreated cells multiplied by >10-fold. Moreover, in the presence of either phage or bFGF-epitope-derived peptides, the number of viable endothelial cells decreased with time, suggesting that the peptides interfere with endogenous bFGF–FGFR interactions in these cells, thereby affecting their survival. To determine whether the antiproliferative effect of these peptides is, indeed, due to inhibition of bFGF activity, the cells were incubated with submaximal concentrations (0.2 μg/ml) of the phage-derived peptide APSGHYKG with or without human recombinant bFGF at 10 ng/ml. Fig. 5*B* shows that the peptide alone inhibits cell growth by ≈70%, whereas in the presence of bFGF the inhibitory effect is significantly

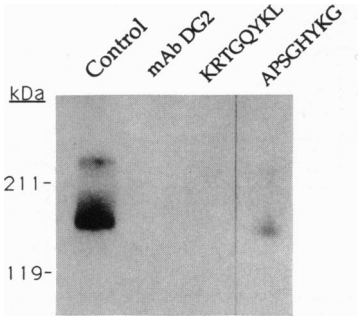

FIG. 4. Cross-linking of bFGF to FGFR-1 in the presence of synthetic peptides. Insoluble FGFR-1 was prepared as described (ref. 21, see also Fig. 2). Washed beads (50 μl) containing FGFR-1 were incubated with ^{125}I-labeled bFGF (2 ng/ml), heparin (200 ng/ml), and the indicated peptides (10 ng/ml) or antibody (1 μg/ml) in 1 ml of DMEM/0.1% BSA/20 mM Hepes, pH 7.4. After 45 min of shaking at 22°C, the beads were washed and resuspended in 0.4 ml of PBS/0.15 mM disuccinylimidyl suberate for 30 min at 22°C. After the beads were washed, they were suspended in sample buffer and run on a SDS/7.5% polyacrylamide gel. After the run, the gel was dried and exposed to x-ray film for several days.

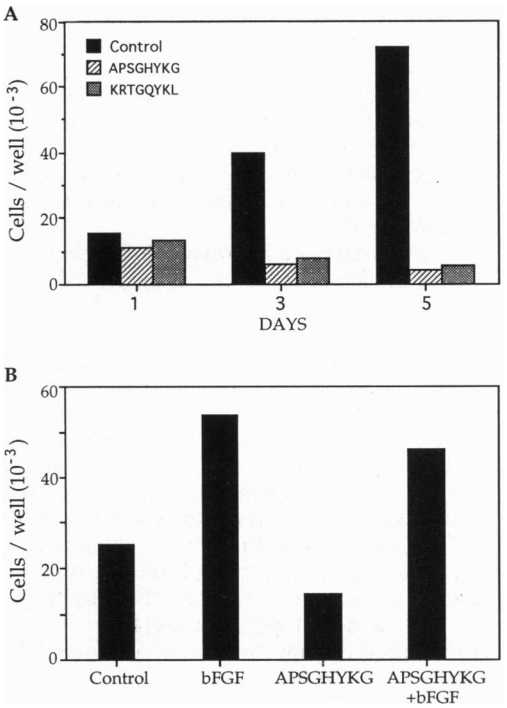

FIG. 5. Effects of epitope-derived peptides on endothelial cell proliferation. Bovine aortic endothelial cells were seeded at 5000 cells per well in complete growth medium in the absence and presence of the epitope-derived peptides at 1 μg/ml. (*A*) Peptides were added every day, and the cells were counted every other day for 5 days. (*B*) Comparison of the effect of peptide APSGHYKG without and with bFGF (10 ng/ml) on cell proliferation after 3 days. Peptide concentration was 0.2 μg/ml.

diminished to ≈15%, suggesting that an excess of exogenous bFGF can overcome the inhibitory effect of the peptide on endothelial cell proliferation and survival.

DISCUSSION

High-affinity ligand–receptor interaction is fundamental for the specific and diverse biological activities of cellular growth factors and a main target for the development of growth modulators and for therapeutic intervention. Binding of FGFs to their receptors involves a complex interaction, as multiple ligands and receptor variants exist (13–15) and several structural elements in the receptor determine ligand binding and specificity (24–26). Moreover, high-affinity receptor binding of FGF requires heparin or cell-surface heparan sulfates (11, 12). Several attempts have been made to identify functional domains in bFGF that participate in receptor binding and could serve as bFGF antagonists (27, 28). In an extensive survey of overlapping synthetic peptides spanning the entire primary sequence of bFGF, Baird and colleagues (27) identified two major regions suspected of involvement in ligand–receptor interaction. Peptides derived from either aa 24–68 or 103–146 of bFGF inhibited bFGF binding to the receptor and heparin, as well as bFGF-induced DNA synthesis in 3T3 fibroblasts. The carboxyl-terminal peptide includes our identified inhibitory sequence KRTGQYKL. The shortest peptide identified as a bFGF antagonist by this synthetic peptide approach was an 18-aa-long peptide corresponding to residues 103–120 (27). However, in spite of the capacity of these relatively long peptides to inhibit thymidine incorporation in 3T3 fibroblasts, they had no effect on either basal or bFGF-induced proliferation of endothelial cells. In a later report, Seno and colleagues (28) constructed a series of truncated bFGF molecules that lacked an amino or

a carboxyl terminus and confirmed the importance of carboxyl-terminal structures in bFGF for both receptor and heparin binding.

The phage-epitope-library technology has become a powerful tool for identifying determinants recognized by antibodies and other proteins (29). Of interest is the identification of peptide epitopes that may inhibit the binding of nonpeptidic ligands. For example, the consensus peptide Tyr-Pro-Tyr identified in a phage-epitope library by Con A can inhibit the binding of methyl α-D-mannopyranoside to Con A (30, 31). These and other results suggest that if the epitope is continuous (sequential determinant), the homology between phage-epitope sequences and the ligand sequence may be high, whereas in the case of a noncontiguous sequence there may be no homology at all with the sequence of the ligand.

To gain an insight into the complex and dynamic interactions of bFGF with FGFR-1 and identify structural elements that participate in FGF–receptor interactions, we screened an epitope library of hexapeptides presented on phages (19) with neutralizing antibodies to bFGF. We found that the peptides recognized by the mAbs DG2 and DE6 represent a continuous bFGF sequence. This result is supported by the ability of the peptides derived from bFGF sequences to inhibit the binding of bFGF to its receptor and by the homology between these peptides and the phage-displayed peptide.

Although the two mAbs cross-react, their relative affinities toward the various peptides and peptide-presenting phages suggest that they are unique and are directed against two distinct, though similar, epitopes on bFGF. Indirect evidence for their distinctive nature comes from a previous study demonstrating a different pattern of binding and neutralizing activity toward bFGF (17, 18). Nevertheless, the striking similarity of the epitopes identified by the mAbs (Table 1) strongly suggests that the two mAbs react, although in a somewhat different way, with the same or almost the same determinant on bFGF.

The localization of the bFGF epitope that corresponds to the phage peptide sequence is not finally conclusive because peptides from either the amino-terminal sequence (residues 13–18) or the carboxyl-terminal sequence (residues 119–126) showed inhibition of bFGF binding. It is more likely that the actual neutralizing activity of these antibodies is through binding to the carboxyl-terminal residues, in accordance with the previous localization of bFGF receptor-binding domains to either residues 24–68 or 103–146 (27), both of which are beyond the presently identified amino-terminal sequence. Moreover, a peptide consisting of aa 106–115 of bFGF was shown to bind heparin, interact with the bFGF receptor, and be active in mitogenic assays of bFGF (27). Recent crystallographic studies of bFGF indicate that fragment 106–115 forms an open loop on the surface of the bFGF molecule (32–34). It was suggested that this region, which also constitutes the most extended segment of structural similarity between bFGF and interleukin-1β, may form a conserved backbone conformation common to several members of this family of growth factors, whereas the amino acid residues in this backbone determine ligand-binding specificity (32). Chemical modification studies have shown that reductive methylation of Lys-118 in aFGF completely abolishes its mitogenic activity and substantially decreases the affinity of aFGF for heparin (35). Similar results were obtained by replacement of this lysine with glutamate (36). Lysine-118 in aFGF corresponds to Lys-125 in bFGF, which is penultimate to the carboxyl-terminal residue in the presently identified inhibitory peptide KRTGQYKL. This result further supports the notion that the neutralizing activity of these antibodies is through binding to the carboxyl-terminal residues of bFGF rather than to the similar amino-terminal residues, which in turn actively participate in bFGF–receptor binding. Nevertheless, as the sequences presented on the phages share higher homology with the amino-terminal domain of bFGF, one cannot exclude the possibility that the antibodies were formed against this part of bFGF.

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