The keratinocyte growth factor (KGF or FGF-7) is unique among its family members both in its target cell specificity and its inhibition by the addition of heparin and the native heparan-sulfate proteoglycan (HSPG), glypican-1 in cells expressing endogenous HSPGs. FGF-1, which binds the FGF-7 receptor with a similar affinity as FGF-7, is stimulated by both molecules. In the present study, we investigated the modulation of FGF-7 activities by heparin and glypican-1 in IIS-free background utilizing either HS-deficient cells expressing the FGF-7 receptor (designated BaF/KGFR cells) or soluble extracellular domain of the receptor. At physiological concentrations of FGF-7, heparin was required for high affinity receptor binding and for signaling in BaF/KGFR cells. In contrast, binding of FGF-7 to the soluble form of the receptor did not require heparin. However, high concentrations of heparin inhibited the binding of FGF-7 to both the cell surface and the soluble receptor, similar to the reported effect of heparin in cells expressing endogenous HSPGs. The difference in heparin dependence for high affinity interaction between the cell surface and soluble receptor may be due to other molecule(s) present on cell surfaces. Glypican-1 differed from heparin in that it stimulated FGF-1 but not FGF-7 activities in BaF/KGFR cells. Glypican-1 abrogated the stimulatory effect of heparin, and heparin reversed the inhibitory effect of glypican-1, indicating that this HSPG inhibits FGF-7 activities by acting, most likely, as a competitive inhibitor of stimulatory HSPG species for FGF-7. The regulatory effect of glypican-1 is mediated at the level of interaction with the growth factor as glypican-1 did not bind the KGFR. The effect of heparin and glypican-1 on FGF-1 and FGF-7 oligomerization was studied employing high and physiological concentrations of growth factors. We did not find a correlation between the effects of these glycosaminoglycans on FGFs biological activity and oligomerization. Altogether, our findings argue against the heparin-linked dimer presentation model as key in KGFR activation, and support the notion that HSPGs primarily affect high affinity interaction of FGFs with their receptors.

The fibroblast growth factor (FGF) family constitute at present 19 structurally related polypeptides mitogens. Acidic-FGF (aFGF or FGF-1) and basic-FGF (bFGF or FGF-2) are the first isolated and best studied members of the FGF family (1, 2). They act on a wide spectrum of tissues and cell types, and play important roles in a multitude of physiological and pathological processes including embryonal development, neuronal survival, angiogenesis, wound repair, and tumor growth (1). The keratinocyte growth factor (KGF or FGF-7) is unique among FGFs in its specificity toward cells of epithelial origin (3). FGF-7 is secreted by stromal cells and it stimulates the differentiation and proliferation of a large variety of epithelial cells, acting as a paracrine mediator of mesenchymal-epithelial communication (3). FGF-7 is implicated in tissue development and repair, and in a number of pathological conditions such as prostate and breast cancer and inflammatory bowel disease (3–6).

The biological activities of FGFs are mediated by four distinct but highly related cell surface tyrosine kinase receptors (designated FGFR1-FGFR4). FGF receptors (FGFRs) display overlapping ligand binding properties and alternative splicing mechanism generates receptor isoforms with altered ligand binding properties (2, 7). FGF-1 interacts with the four FGFRs and the FGFR isoforms that have been characterized so far, whereas FGF-7 interacts only with an isoform of FGFR2 known as FGFR2IIIb form or the KGFR (8–13). The KGFR is expressed predominantly in cells of epithelial origin and it binds FGF-1 with an affinity similar to that observed for FGF-7 (14–16).

Beside interacting with FGFRs, FGFs bind to heparin and to heparan sulfate moieties of cell surface and extracellular matrix heparan sulfate proteoglycans (HSPG) (1, 17, 18). HSPGs and heparin are potent modulators of FGF activity. They can protect FGFs from thermal denaturation and proteolytic degradation, and binding of FGF to extracellular matrix HSPGs provides a reservoir from which FGFs can be rapidly released in response to specific triggering events (19–21). In the absence of cell surface HSPGs, cellular responses to FGFs are attenuated but can be restored by the addition of heparin or HS, indicating that these GAGs can enhance FGF receptor binding and signaling (22, 23). Studies with FGF-2 and FGF-1 suggested that heparin interacts with both the growth factor and its receptor to stimulate cellular responses to FGF (24, 25).
This issue is controversial because different groups reported conflicting results (26, 27). Others suggested that interaction of FGFs with HSPGs increases their receptor binding affinity by stabilizing growth factor-receptor complexes (28, 29). HSPGs and heparin may also facilitate FGFR dimerization and subsequent activation (26). The mechanism by which HSPGs and heparin stimulate receptor dimerization is controversial. Two models were proposed. In the first, the signal transducing complex is composed of a 1:1 FGF/FGFR complexes cross-bridged via HS or heparin, giving rise to a 2:2 molar ratio of FGF to FGFR (26). The second model suggests that FGF induces receptor dimerization as a monomeric ligand and HSPGs stabilize the complex (30, 31).

The modulation of ligand-receptor interaction by HS/heparin was mainly studied with FGFR1. Less is known about how these glycosaminoglycans modulate interaction of FGFs with other FGFRs. Previous studies revealed that heparin and the native HSPG glypic-an-1 are potent inhibitors of FGF-7 activities when added to cells that respond to FGF-7 and express endogenous HSPGs (32–34). This inhibitory effect was intriguing because both enhanced the interaction of FGF-1 with the KGFR and FGFR1 (34). The present study was undertaken to further characterize the involvement of heparin and glypic-an-1 in FGF-7 and FGF-1 receptor binding and signaling by utilizing both heparan sulfate-deficient cells expressing the KGFR and soluble extracellular domain of the receptor.

**EXPERIMENTAL PROCEDURES**

**Materials—** Human recombinant FGF-7 was produced in bacteria and purified as described previously (32). For the preparation of heparin-free FGF-7 we utilized a heparin-sti-gated FGF-7. The gene encoding human FGF-7 (residues Ala36 to Thr185) was cloned into plasmid pMAX260 and expressed as His-tagged products in Escherichia coli (49, 51). Expression of the recombinant proteins and preparation of the soluble fraction was performed as previously described (50). Purification was carried out by nickel nitrioli-triacetic acid affinity chromatography essentially as recommended by the manufacturer (Qiagen). The receptor binding affinity and mitogenic activity of the His-tagged FGF-7 are identical to those of the parental molecule lacking the tag. The expressed His-tagged FGF-7 were described elsewhere (37). Bovine brain FGF-1 was purchased from R & D Systems. Bovine lung-derived heparin was from Sigma. Heparin-described elsewhere (37). Bovine brain FGF-1 was purchased from R & D Systems. Bovine lung-derived heparin was from Sigma. Heparin-described elsewhere (37). Bovine brain FGF-1 was purchased from R & D Systems. Bovine lung-derived heparin was from Sigma. Heparin-described elsewhere (37). Bovine brain FGF-1 was purchased from R & D Systems. Bovine lung-derived heparin was from Sigma. Heparin-described elsewhere (37). Bovine brain FGF-1 was purchased from R & D Systems. Bovine lung-derived heparin was from Sigma. Heparin-described elsewhere (37). Bovine brain FGF-1 was purchased from R & D Systems. Bovine lung-derived heparin was from Sigma. Heparin-described elsewhere (37). Bovine brain FGF-1 was purchased from R & D Systems. Bovine lung-derived heparin was from Sigma. Heparin-described elsewhere (37). Bovine brain FGF-1 was purchased from R & D Systems. Bovine lung-derived heparin was from Sigma. Heparin-described elsewhere (37). HSPG glypican-1, 6–12 £ 10^6 cpm/µg of HS.

**Cell Surface Receptor Binding—** FGF binding to BaF3/KGFR cells were performed essentially as described (11) except that the assay was performed in suspension. Briefly, 2 £ 10^4 BaF/KGFR cells in 0.4 ml of binding buffer were incubated for 2 h at 4 °C in the presence of 8 ng/ml radioiodinated growth factors. Determination of specific binding and cross-linking were done as described previously (11). Ligand-receptor complexes were resolved on 6% SDS-PAGE. Equal amounts of total cell lysates were loaded onto each lane.

**Heparin-Free Binding—** Solid-phase binding assays were performed in 96-well ELISA dishes as recently described (37). For binding of [125]I-glypic-an-1 to FGF-7, 0.2 µg of FGF-7 in coating buffer was adsorbed to each well. For binding of radioiodinated FGF-1, FGF-7, or glypic-an-1 to KGFR/AP, wells were first coated with a monoclonal antibody against alkaline phosphatase, then conditioned medium from NIH3T3 cells expressing KGFR/A (0.15 AP OD units/min) was added to antibody-coated wells. Specific binding was determined by subtracting counts/ min of samples incubated with 1 µg/ml unlabeled ligand from the counts/min bound in the absence of unlabeled ligand. Negative controls included wells coated with bovine serum albumin alone, without AP antibody or KGFR/AP. Nonspecific binding was less than 10% of the total binding. Cross-linking experiments in solution were performed with 2 ng/ml radioiodinated FGF-7 or FGF-1 using affinity purified KGFR/AP, as described (34).

**[3H]Thymidine Incorporation and Cell Proliferation Assays—** Mitogenic assays were performed in 96-well microtiter plates. BaF/KGFR cells were seeded at 2 £ 10^3 cells/well in RPMI plus 10% fetal calf serum and the desired concentrations of growth factors, heparin, or glypic-an-1. [3H]Thymidine incorporation was assayed as described previously (34). For proliferation assays, the cells were seeded into 24-well plates (5 £ 10^5 cells/well). Fresh growth factor and heparin were added every other day and viable cells were counted on day 5 after seeding. Each data point was performed in duplicates or triplicates and each experiment was repeated at least 3 times. The variation between different experiments did not exceed 10%.

**Cross-linking of FGFs—** Each reaction contained either a mixture of radiolabeled and unlabeled growth factor or radiolabeled growth factor alone (see text), and increasing concentrations of glypic-an-1 or heparin. The binding and cross-linking experiments were carried out in a volume of 20 µl essentially as described by Ornitz et al. (27). Samples were separated on 12% SDS-PAGE and cross-linked FGFs were visualized by autoradiography.

**RESULTS**

**Effect of Heparin on Receptor Binding and Mitogenic Activity of FGF-7 and FGF-1 in HS-deficient Cells Expressing the KGFR—** Previous studies showed that heparin and cell surface HSPGs differentially modulate the activity of FGF-1 and FGF-7, enhancing the activity of FGF-1 but inhibiting that of FGF-7 (34, 38). To further investigate this differential effect of HSPGs on FGF-1 and FGF-7 receptor binding and activation, and to determine whether heparin stimulates or modulates the activities of FGF-7 we coexpressed the KGFR in BaF3 cells (designated BaF/KGFR cells). BaF3 cells do not express HSPGs and FGFRs and therefore provide a useful model system to study effects of heparin-like molecules (17, 27). We then compared the effect of heparin on receptor binding and mitogenic activity of FGF-7 and FGF-1 over a wide range of heparin concentrations (0.05–100 µg/ml). Binding of the radiiodinated ligands to the KGFR was evaluated by covalent cross-linking.
As shown in Fig. 1, heparin at concentrations ranging from 0.05 to 10 μg/ml dramatically enhanced the binding of both FGF-1 and FGF-7 to the KGFR. Binding of FGF-1 reached a maximal level at 1 μg/ml heparin (Fig. 1A). Quantitation of the intensity of radioactivity in each band, in several different experiments, revealed that 1 μg/ml heparin increases specific binding of FGF-1 by 12–15-fold compared with the binding in the absence of heparin. Binding of 125I-FGF-7 to KGFR reached a maximal level (about 12-fold increase) at 10 μg/ml (Fig. 1B). Higher concentrations of heparin differentially affected binding of each ligand to the KGFR, stimulating that of FGF-1 but strongly inhibiting that of FGF-7. Similar results were obtained using several different preparations of commercial heparin (data not shown). In addition, the effect of heparin on the mitogenic activity of each growth factor correlated very well with the observed effects on receptor binding (Fig. 1C).

**Heparin Enhances but Is Not Essential for FGF-7 Activity**—The results presented in Fig. 1 showed that at low concentration and in the absence of heparin, FGF-7 did not stimulate a mitogenic response in BaF/KGFR cells. To further characterize the effect of heparin, we examined whether higher concentrations of FGF-7 can stimulate cell proliferation in the absence of heparin, utilizing FGF-7 preparation that was not exposed to heparin-Sepharose (see “Experimental Procedures” and Ref. 51). The assay was performed with increasing concentrations of FGF-7 (5–1000 ng/ml) and in the absence or presence of 1 μg/ml heparin. Physiological concentrations of FGF-7 had little or no effect on cell proliferation in the absence of heparin. By contrast, added heparin supported proliferation to a level equivalent to that observed with IL-3 (Fig. 2). In the absence of heparin, cells were capable of responding to FGF-7 to a similar maximal extent, but significantly higher concentrations of growth factor were required. These findings suggest that although FGF-7 can stimulate cell proliferation in the absence of heparin, heparin is required to enhance the efficacy of the growth factor.

**Glypican-1 Does Not Enhance the Activity of FGF-7**—Because glypican-1 and heparin exert a similar inhibitory effect on FGF-7 activities in cells that naturally express KGFR and HSPGs, we investigated whether glypican-1 can also stimulate the activities of FGF-7 in BaF/KGFR cells. Glypican-1, failed to enhance the binding of 125I-FGF-7 to the KGFR over a wide concentration range (0.01–25 μg/ml) (Fig. 3A, bottom, and data not shown). Similarly, glypican-1 did not enhance the mitogenic activity of FGF-7 in BaF/KGFR cells (Fig. 3B). To ensure that glypican-1 is biologically active we tested its effect on receptor binding and mitogenic activity of FGF-1, as glypican-1 had a stimulatory effect on FGF-1 activities (34). Glypican-1 enhanced the binding of FGF-1 to KGFR in all the concentrations that were tested (0.1–25 μg/ml). An 8-fold increase in specific binding of FGF-1 was observed in the presence of 10 μg/ml glypican-1 compared with FGF-1 binding in the absence of glypican-1 (Fig. 3A, top). Similarly, glypican-1 enhanced the mitogenic activity of FGF-1 (Fig. 3B). These results suggest that the failure of glypican-1 to enhance FGF-7 activities is an intrinsic property of this HSPG and point to an important difference between the effect of glypican-1 and heparin on FGF-7 activities.

**Glypican-1 Antagonizes the Promoting Effect of Heparin on Binding of FGF-7 to KGFR**—The observed lack of stimulatory
binding of FGF-7 to KGFR. Binding of 125I-FGF-7 to BaF/KGFR cells in the presence of 1 μg/ml heparin and increasing concentrations of glypican-1. Binding and cross-linking were performed as described in the legend to Fig. 1, B, reversal of the inhibitory effect of glypican by heparin. Binding of 125I-FGF-7 to BaF/KGFR cells was performed in the presence of 7.5 μg/ml glypican-1 and increasing concentrations of heparin, as described under “Experimental Procedures.”

To examine whether glypican-1 can act as an antagonist of stimulatory species of HS toward FGF-7. To test this possibility, we examined whether glypican-1 can abrogate the stimulatory effect of heparin on binding of FGF-7 to the KGFR. Thus, 125I-FGF-7 was bound to BaF/KGFR cells in the presence of 1 μg/ml heparin and increasing concentrations of glypican-1. As shown in Fig. 4, glypican abrogated the stimulatory effect of heparin in a dose-dependent manner. Quantification of the amount of bound 125I-FGF-7 revealed that glypican-1, at a concentration of 5 μg/ml, inhibited the binding of FGF-7 to the KGFR by about 60%, and binding was almost completely abolished at 25 μg/ml. To find out whether heparin can reverse the inhibitory effect of glypican, we performed the assay in the presence of a fixed amount of glypican-1 and increasing concentrations of heparin. As shown in Fig. 4B, addition of heparin reversed the inhibitory effect of glypican-1. These findings suggest that glypican-1 acts most likely as a competitive inhibitor of heparin.

Soluble KGFR Binds to FGF-1 and FGF-7 but Not to Glypican-1—To examine whether apart from binding to FGF-7, the inhibitory effect of glypican-1 is mediated also via binding to the KGFR, we studied its interaction with the KGFR in a cell-free system. The soluble extracellular domain of KGFR was produced as a fusion protein with secreted human placental alkaline phosphatase (designated KGFR/AP). We first tested if KGFR/AP is functional by measuring its binding and cross-linking to FGF-1 and FGF-7 in both solid phase and solution. KGFR/AP was adsorbed to ELISA dishes coated with an antibody to alkaline phosphatase and used in a quantitative binding assay. KGFR/AP binds efficiently to both FGF-1 and FGF-7. Binding was specific, dose-dependent, and saturable (Fig. 5A). However, unlike the situation with the cell surface receptor, high affinity binding was readily detected in the absence of heparin. Similar results were obtained following heparinase treatment or high salt extraction to remove contaminating HS PGs, and in cross-linking experiments performed in solution with affinity purified KGFR/AP (Fig. 5A, inset, and data not shown). Low concentrations of heparin slightly enhanced the binding of FGF-1 and FGF-7 to the receptor, and high concentrations of heparin inhibited binding of FGF-7 to the KGFR, similar to the situation in intact cells (Table I).

Next we utilized the solid phase binding assay to examine whether the receptor can bind 125I-glypican-1. As shown in Fig. 5B, glypican-1 at concentrations of 5–200 ng/ml did not bind KGFR. Binding was not detected at concentrations of up to 2 μg/ml 125I-glypican before and after high salt wash (data not shown). By contrast, as expected, glypican-1 binding to FGF-7 was readily detected and heparin displaced bound glypican-1 from FGF-7 (Fig. 5B). The binding of 125I-glypican-1 to FGF-7 was saturable in the range of 10–20 ng/well and Scatchard analysis of several different experiments revealed a dissociation constant of 0.6–1 × 10^−7 M.

Effect of Glypican-1 and Heparin on Oligomerization of FGF-1 and FGF-7—HS-induced oligomerization of FGFs is thought to play a central role in increasing the affinity of FGFs for their signaling receptors and in facilitating FGF receptors dimerization and subsequent activation (39). Therefore, we investigated whether the inability of glypican-1 to stimulate FGF-7 activities correlates with its effect on FGF-7 oligomerization. Oligomerization was assessed by chemical cross-linking of FGF-7 to itself following incubation of fixed amounts of the growth factor and increasing concentrations of glypican-1 (0.01–50 μg/ml) or heparin (0.01–50 μg/ml). Similar to the report effect of heparin on FGF-2 oligomerization (27), dimers and trimers of FGF-7 were efficiently induced by heparin using a high concentration of FGF-7 (15 μg/ml) (Fig. 6A). Interestingly, glypican-1 similarly induced FGF-7 oligomerization, even though this HS PG does not stimulate FGF-7 activity (Fig. 6B). Because the FGF-7 concentration that gives rise to maximal biological response is lower by 3 orders of magnitude than that used in the experiment shown in Fig. 6, A and B, we repeated the experiment using a physiological FGF-7 concentration of 10 ng/ml. Under these conditions, neither heparin nor glypican-1 induced FGF7 oligomerization. In fact, a low

![Figure 4](image4.png)

**Figure 4.** A, glypican-1 antagonizes the stimulatory effect of heparin on binding of FGF-7 to KGFR. Binding of 125I-FGF-7 to BaF/KGFR cells in the presence of 1 μg/ml heparin and increasing concentrations of glypican-1. Binding and cross-linking were performed as described in the legend to Fig. 1. B, reversal of the inhibitory effect of glypican by heparin. Binding of 125I-FGF-7 to BaF/KGFR cells was performed in the presence of 7.5 μg/ml glypican-1 and increasing concentrations of heparin, as described under “Experimental Procedures.”

![Figure 5](image5.png)

**Figure 5.** Glypican-1 binds to FGF-7 but not to the KGFR. A, specific binding of 125I-glypican-1 to FGF-7 or 125I-FGF-7 to KGFR/AP-coated wells. B, specific binding of 125I-glypican-1 to FGF-7 or KGFR/AP. Binding was performed on ELISA dishes coated with FGF-7 or soluble KGFR (KGFR/AP). After 2 h incubation at room temperature, the wells were extensively washed, and the amount of specifically bound ligand was determined as described under “Experimental Procedures.” Binding of glypican-1 to immobilized FGF-7 was competed by 0.5 μg/ml heparin (FGF-7 + Hep). Inset, covalent cross-linking of 125I-FGF-7 to affinity purified KGFR/AP. Binding and cross-linking were performed in solution, and ligand receptor complexes were resolved on 6% SDS-PAGE. Lane 1, bound 125I-FGF-7. Lanes 2–4, bound 125I-FGF-7 in the presence of 1 μg/ml heparin, unlabeled FGF-7, or epidermal growth factor, respectively.

| Heparin | Specifically bound 125I-FGF-7 | Specifically bound 125I-FGF-1 |
|---------|-------------------------------|-------------------------------|
| μg/ml   | cpm                           | cpm                           |
| 0       | 39,000                        | 52,000                        |
| 1       | 49,000                        | 63,000                        |
| 10      | 36,160                        | 62,500                        |
| 25      | 22,000                        | 63,000                        |
amount of dimers was observed in the absence of added GAGs, and this amount decreased rather than increased when either heparin or glypican-1 was added (Fig. 6, C and D). Similarly, both heparin and glypican-1 induced oligomerization of FGF1 when high concentrations of this growth factor were employed (Fig. 6, E and F). At 10 ng/ml FGF-1, a very little amount of dimers was induced at GAGs concentrations between 5 and 100 ng/ml, and higher GAGs concentrations inhibited dimer formation (Fig. 6G, and data not shown). The biological activity of radioiodinated ligands was identical to that of the unlabeled ligands (data not shown).

**FIG. 6. Effect of glypican-1 and heparin on oligomerization of FGF-1 and FGF-7.** FGF-7 (panels A-D) and FGF-1 (panels E-G) were incubated in the presence of the indicated concentrations of heparin or glypican-1. Incubation was carried out for 1 h at room temperature and the growth factors were cross-linked with disuccinimidyl suberate as described under “Experimental Procedures.” Products were separated on 12% SDS-PAGE, and the gels were dried and exposed to an x-ray film. In panels A, B, E, and F, 13 μg/ml unlabeled growth factor were mixed with 33 ng/ml corresponding 125I-labeled factor. In panels C, D, and G, the experiments were performed with radio-labeled growth factor alone. The specific activity of radioiodinated factors was 75,000 and 330,000 cpm/ng for FGF-7 and FGF-1, respectively.

DISCUSSION

Previous studies on the modulation of FGF-1 and FGF-7 activities by heparin and HS/SGs in cells expressing endogenous HS/SGs revealed that heparin and HS/SGs potentiate the biological activity of FGF-1 but strongly inhibit the activity of FGF-7 (32–34). The findings that HS inhibit FGF-7 activities was surprising in view of the well-established heparin requirement of all other FGFs. However, the interpretation of these results was complicated due to possible effects of endogenous HS/SGs. We thus studied the heparin requirements of FGF-7 in a HS-free setting utilizing the HS and FGF-1-deficient cell line, BaF3, transfected with functional KGFR. We show that the interaction of FGF-7 with the KGFR and its mitogenic activity are facilitated by low concentrations of heparin. These findings suggest that FGF-7 does not inherently differ from other FGFs in its requirement for heparin and are in agreement with the conclusion of Jang et al. (40) based on studies with protamine sulfate.

Consistent with the results in cells expressing endogenous HS/SGs, high concentrations of heparin stimulated FGF-1 activities but strongly inhibited receptor binding and mitogenic activity of FGF-7 in BaF3KGFR cells, generating a biphasic dose-response curve. The biphasic effect of heparin on FGF-7 activities can explain why a stimulatory effect was not observed in cells expressing HS/SGs (34, 38). In these cells, endogenous HS/SGs may be present at a concentration that confines full activity on FGF-7. Therefore, the addition of heparin causes inhibition rather than stimulation by bringing the overall local concentration of HS at the cell surface to a level that falls into the inhibitory phase of the dose-response curve. The biphasic effect of heparin suggests that soluble heparin may interact with two distinct sites (high and low affinity sites) within the growth factor-receptor complex. The known binding affinity of FGF-7 to cell-associated HS/SGs (14, 38) suggests that the putative higher affinity site resides within the growth factor. The lower affinity site for heparin may reside either in the ligand or in the receptor. Two groups reported recently that the KGFR binds heparin with low affinity (41, 42). However, the relevance of this binding to the inhibition of FGF-7 remains unclear because the conclusions of the two studies were contradictory. Further characterization of heparin-binding domains in FGF-7 and the receptor are required to resolve this issue.

The soluble KGFR, unlike the cell surface receptor, did not exhibit a strong heparin dependence for high affinity interaction with FGF-7 or FGF-1. However, the effect of high concentrations of heparin was similar to that observed in intact cells. These findings suggest that high affinity ligand binding is an intrinsic property of the receptor, and that the difference between the HS/SG dependence of ligand binding to cell surface versus soluble KGFR may be due to other molecules present on cells. The apparent lack of heparin dependence for high affinity binding conflict the results of Hsu et al. (41) who reported that FGF-7 does not bind to soluble KGFR in the absence of heparin. It is unlikely that the lack of heparin dependence of our soluble receptor is due to contamination with HS/SGs, because binding in solution was performed with purified receptor and binding in solid phase was not affected following heparinase treatment or high salt wash of the immobilized receptor. Moreover, a bacterially expressed KGFR that was not exposed to heparin-Sepharose during purification also binds FGF-7 in the absence of heparin. Similar lack of heparin dependence for high affinity binding was recently reported for soluble KGFR produced in HS-negative cells (42).

The effects of heparin on FGFs activities and the mechanism by which heparin exerts these effects have been extensively studied. However, because cells express on their surfaces HS/SGs and not heparin, it is critical to investigate how these HS/SGs exerts their regulatory activity. Previous study indicated that glypican-1, similar to heparin, differentially modulates cellular responsiveness to FGF-1 and FGF-7 in cells expressing endogenous HS/SGs (34). Our effort to explore how glypican-1 inhibits FGF-7 activities revealed an important dif-
ference between the mode of action of heparin and glypicanc-1, as glypicanc-1 stimulated receptor binding and mitogenic activity of FGF-1, but not FGF-7 in BaF/KGFR cells. When added together with heparin, glypicanc-1 abrogated the stimulatory effect of heparin on binding of FGF-7 to BaF/KGFR cells, and heparin could reverse the inhibitory effect of glypicanc-1. These findings suggest that glypicanc-1 behaves as a competitive binding antagonist of endogenous stimulatory HSPG species for FGF-7. It appears that the modulatory effects of glypicanc-1 are mediated at the level of interaction with the growth factors, as glypicanc-1 did not bind the KGFR. This situation differs from that reported for the interaction of FGF-2 with FGFR1, in which heparin interacts with both the growth factor and the receptor to elicit a regulatory response (24).

Among models proposed to explain how HS, FGF, and FGFR interact to instigate a signal, the most prevalent is the dimer presentation model. This model was initially based on the observation that heparin can induce dimerization of FGF-2 and FGF-1, and in recent years it received further support from NMR and x-ray crystallography analysis (43, 44). The concentrations of growth factors and oligosaccharides employed in these studies far exceeded those required for biological activity. Thus, a key question is whether oligomers are formed at physiological concentrations of FGF and heparin. Different studies have demonstrated that maximal oligomerization is induced when the molar ratio of protein to heparin or HS is 1:1 (45, 46). In experiments performed in cultured cells, maximal biological response is observed at 1–10 ng/ml growth factor and 0.5–10 μg/ml heparin or HS. Under these conditions one would not expect the formation of oligomers because of the low protein to heparin molar ratio. Our results confirm this prediction, as oligomers were not induced at physiological concentrations of either FGF-7 or FGF-1 and stimulatory concentrations of heparin. Consistent with previously reported results, heparin efficiently induced oligomerization of the FGFs at high growth factor concentrations. However, even under these conditions we did not observe a good correlation between the HS dose dependence for oligomerization and for biological activity. Thus, high concentrations of heparin stimulated cellular responses to FGF-1 but inhibited its oligomerization, and glypicanc-1 that differentially modulates FGF-1 and FGF-7 activities, similarly induced their oligomerization. Together, these results argue against the dimer presentation model. While this article was in preparation, Pye and Gallagher (47) reported that a monomer of FGF-2 and HS is sufficient for biological activity, and Hsu et al. (41) showed that the stoichiometry of FGF-7/KGFR complex is 1:2. In addition, in a different study we found that certain mutations in FGF-7 did not affect affinity for heparin and receptor but reduced the biological activity, suggesting a sequential model for KGFR dimerization similar to that proposed for growth hormone and FGF-2 (31, 48, 51).

In summary, the present work indicates that heparin and the native HSPG glypicanc-1 modulate differently the interaction of FGF-7 with its receptor as only heparin but not glypicanc-1 stimulated FGF-7 receptor binding in HS-deficient cells expressing the KGFR. Altogether, the observed effects of glypicanc-1 and heparin on receptor binding of FGF-7 and FGF-1, on mitogenic activity and growth factor oligomerization argue against the heparin-linked dimer presentation model as key in FGF activation (39, 44), and support the notion that HSPGs primarily affect high affinity interaction of FGFs with their receptors (28, 29).

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