Receptor Binding and Mitogenic Properties of Mouse Fibroblast Growth Factor 3

MODULATION OF RESPONSE BY HEPARIN*

(Received for publication, June 9, 1995, and in revised form, August 3, 1995)

Marc Mathieu, Eric Chatelaint, David Ornitz, Janine Bresnick, Ivor Mason, Paul Kiefer, and Clive Dickson

From the Imperial Cancer Research Fund, 44 Lincoln's Inn Fields, London, United Kingdom WC2A 3PX

The fibroblast growth factors constitute a family of nine proteins that share 35–55% amino acid identity over a core region (Refs. 1 and 2; reviewed in Refs. 3 and 4). The prototypic members, Fgf1 and Fgf2, have been ascribed a number of properties including the induction of cell proliferation, differentiation, migration, and cell survival, consistent with roles as autocrine and paracrine signaling molecules. For Fgf2 and Fgf3 there is also good evidence for the translocation of the protein directly to the cell nucleus (5–8); however, the biological significance of this event is not known. The common route for Fgf signaling is through an interaction of an extracellular Fgf with cell surface receptors (reviewed in Refs. 9 and 10). Two classes of Fgf receptor have been identified: a low affinity receptor typified by heparan sulfate proteoglycans that bind Fgfs to high capacity but seem not to signal (11) and a high affinity typified by heparan sulfate proteoglycans that bind Fgfs to high affinity to the IIIb isoforms of Fgf receptor (FgfR)1 and FgfR2 (ID50 = −0.8 nm) and with a lower affinity to the IIIc variant of FgfR2 (ID50 = −9 nm). No competition for the binding of 125I-Fgf1 was observed for FgfR1 (IIIc), FgfR3 (IIIb and IIIc), or FgfR4. Mitogenicity assays using BaF3 cells containing individual Fgf receptors showed a pattern of response in agreement with the receptor binding results. A comparison of two mammary epithelial cell lines showed a marked difference of potency and dependence upon heparin in their response to mouse Fgf3, suggesting a complex interaction between the ligand and its low and high affinity receptors.

fgf3 has been implicated in the embryonic and fetal development of the mouse and as an oncogene in murine breast cancer. We describe a procedure to purify the product of the mouse fgf3 gene and show it to be a potent mitogen for some epithelial cell lines. Using a receptor binding competition assay, Fgf3 was shown to bind with high affinity to the IIIb isoforms of Fgf receptor (FgfR)1 and FgfR2 (ID50 = −0.8 nm) and with a lower affinity to the IIIc variant of FgfR2 (ID50 = −9 nm). No competition for the binding of 125I-Fgf1 was observed for FgfR1 (IIIc), FgfR3 (IIIb and IIIc), or FgfR4. Mitogenicity assays using BaF3 cells containing individual Fgf receptors showed a pattern of response in agreement with the receptor binding results. A comparison of two mammary epithelial cell lines showed a marked difference of potency and dependence upon heparin in their response to mouse Fgf3, suggesting a complex interaction between the ligand and its low and high affinity receptors.

The fibroblast growth factors constitute a family of nine proteins that share 35–55% amino acid identity over a core region (Refs. 1 and 2; reviewed in Refs. 3 and 4). The prototypic members, Fgf1 and Fgf2, have been ascribed a number of properties including the induction of cell proliferation, differentiation, migration, and cell survival, consistent with roles as autocrine and paracrine signaling molecules. For Fgf2 and Fgf3 there is also good evidence for the translocation of the protein directly to the cell nucleus (5–8); however, the biological significance of this event is not known. The common route for Fgf signaling is through an interaction of an extracellular Fgf with cell surface receptors (reviewed in Refs. 9 and 10). Two classes of Fgf receptor have been identified: a low affinity receptor typified by heparan sulfate proteoglycans that bind Fgfs to high capacity but seem not to signal (11) and a high affinity receptor with intrinsic tyrosine kinase activity (reviewed in Refs. 9 and 10). Four tyrosine kinase receptor genes (fgfr1 to fgfr4) have been identified in mammals that encode an extra-

© 1995 by The American Society for Biochemistry and Molecular Biology, Inc.

THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 270, No. 41, Issue of October 13, pp. 24197–24203, 1995
Printed in U.S.A.
Receptor Activating Properites of Mouse Fgf3

After 48-72 h, the conditioned medium was harvested, and the following were added at the final concentrations indicated: 1 μg/ml aprotinin, 1 μg/ml leupeptin, 100 μg/ml phenylmethylsulfonyl fluoride, 1 mM dithiothreitol, and 1 mM EDTA. The conditioned medium (400 ml) was gently mixed overnight at 4°C with 80 μg of heparin-Sepharose beads (Pharmacia). The mixture was batch washed with 400 ml of phosphate-buffered saline and pooled. The conditioned medium was then subcloned into the expression vector MIRB. MIRB contains the Molony murine leukemia virus long terminal repeat, unique EcoRI, BamHI, and SpeI sites followed by the IRES-NEO gene in the Bluescript KS plasmid (described in Ref. 13). The plasmid pSVFGfr1 IIIb (31) was cloned as a 2.9-kbp BamHI-SpeI fragment into the corresponding sites of MIRB. Fgfr1 IIIc (11b) (18) was cloned as a 3.2-kbp EcoRI fragment into MIRB by converting a 5' Asp718 site into an EcoRI site and then excising with EcoRI. Fgfr2 IIIb (12) was cloned as a 2.9-kbp BamHI fragment into MIRB by converting a 5' SpeI site into a BamHI site and then excising with BamHI. Fgfr1 IIIc (34) was cloned into MIRB as a 3.6-kbp SpeI fragment by converting unique NarI and XbaI sites into SpeI sites.

MIRB-Fgfr plasmids were transfected into Balb/3 cells and selected in the presence of 600 μg/ml G418 (Life Technologies, Inc.). Individual clonal cell lines were isolated by limiting dilution and screened for responsiveness to Fgf1. These cell lines were used in quantitative proliferation assays, measuring [3H]thymidine incorporation into DNA as described previously (13, 33, 35). Recombinant Fgf (provided by K. Thomas, Merck) was used as a positive control in each experiment because Fgf1 is the only Fgf ligand that can activate all splice variants of all Fgf receptors.2

BAlb/MK and NIH3T3 cells were transferred to 48-well tissue culture plates (2 x 10^5 cells/well) in 0.5 ml of growth medium and left for 9 or 7 days, respectively, to become confluent and quiescent (29). The culture medium was then replaced with serum-free medium containing the test samples and processed as described previously (36). C57MG and HC11 cells were made quiescent by replacing the medium after 24 h of growth with DMEM containing 0.1% serum for 72 h. The cells were treated with the test samples in fresh medium containing 0.1% serum for 22 h. [3H]Thymidine incorporation assays were performed as described previously (36).

RESULTS

Purification of Mouse Fgf3—As a source of mouse Fgf3, we have used the conditioned culture medium from DMI-1 cells (27). These transformed NIH3T3 cells emerged from a selection procedure in which colonies transfected with fgf3 cDNA in a murine leukemia virus-based vector were tested for their ability to grow as anchorage-independent colonies. Mouse Fgf3 was checked by Western blot using a polyclonal anti-Fgf3 antibody. A single band was visualized by silver staining or immunoblotting, the preparation corresponding to the IRES-NEO gene in the Bluescript KS plasmid (described in Ref. 13). The plasmid pSVFGFR1 IIIb (31) was cloned as a 2.9-kbp BamHI-SpeI fragment into the corresponding sites of MIRB. MIRB contains the Molony murine leukemia virus long terminal repeat, unique EcoRI, BamHI, and SpeI sites followed by the IRES-NEO gene in the Bluescript KS plasmid (described in Ref. 13). The plasmid pSVFGFR1 IIIb (31) was cloned as a 2.9-kbp BamHI-SpeI fragment into the corresponding sites of MIRB. Fgfr1 IIIc (11b) (18) was cloned as a 3.2-kbp EcoRI fragment into MIRB by converting a 5' Asp718 site into an EcoRI site and then excising with EcoRI. Fgfr2 IIIb (12) was cloned as a 2.9-kbp BamHI fragment into MIRB by converting a 5' SpeI site into a BamHI site and then excising with BamHI. Fgfr1 IIIc (34) was cloned into MIRB as a 3.6-kbp SpeI fragment by converting unique NarI and XbaI sites into SpeI sites.

MIRB-Fgfr plasmids were transfected into Balb/3 cells and selected in the presence of 600 μg/ml G418 (Life Technologies, Inc.). Individual clonal cell lines were isolated by limiting dilution and screened for responsiveness to Fgf1. These cell lines were used in quantitative proliferation assays, measuring [3H]thymidine incorporation into DNA as described previously (13, 33, 35). Recombinant Fgf (provided by K. Thomas, Merck) was used as a positive control in each experiment because Fgf1 is the only Fgf ligand that can activate all splice variants of all Fgf receptors.2

BAlb/MK and NIH3T3 cells were transferred to 48-well tissue culture plates (2 x 10^5 cells/well) in 0.5 ml of growth medium and left for 9 or 7 days, respectively, to become confluent and quiescent (29). The culture medium was then replaced with serum-free medium containing the test samples and processed as described previously (36). C57MG and HC11 cells were made quiescent by replacing the medium after 24 h of growth with DMEM containing 0.1% serum for 72 h. The cells were treated with the test samples in fresh medium containing 0.1% serum for 22 h. [3H]Thymidine incorporation assays were performed as described previously (36).

RESULTS

Purification of Mouse Fgf3—As a source of mouse Fgf3, we have used the conditioned culture medium from DMI-1 cells (27). These transformed NIH3T3 cells emerged from a selection procedure in which colonies transfected with fgf3 cDNA in a murine leukemia virus-based vector were tested for their ability to grow as anchorage-independent colonies. Mouse Fgf3 was checked by Western blot using a polyclonal anti-Fgf3 antibody. A single band was visualized by silver staining or immunoblotting, the preparation corresponding to the IRES-NEO gene in the Bluescript KS plasmid (described in Ref. 13). The plasmid pSVFGFR1 IIIb (31) was cloned as a 2.9-kbp BamHI-SpeI fragment into the corresponding sites of MIRB. MIRB contains the Molony murine leukemia virus long terminal repeat, unique EcoRI, BamHI, and SpeI sites followed by the IRES-NEO gene in the Bluescript KS plasmid (described in Ref. 13). The plasmid pSVFGFR1 IIIb (31) was cloned as a 2.9-kbp BamHI-SpeI fragment into the corresponding sites of MIRB. MIRB contains the Molony murine leukemia virus long terminal repeat, unique EcoRI, BamHI, and SpeI sites followed by the IRES-NEO gene in the Bluescript KS plasmid (described in Ref. 13). The plasmid pSVFGFR1 IIIb (31) was cloned as a 2.9-kbp BamHI-SpeI fragment into the corresponding sites of MIRB. MIRB contains the Molony murine leukemia virus long terminal repeat, unique EcoRI, BamHI, and SpeI sites followed by the IRES-NEO gene in the Bluescript KS plasmid (described in Ref. 13). The plasmid pSVFGFR1 IIIb (31) was cloned as a 2.9-kbp BamHI-SpeI fragment into the corresponding sites of MIRB. MIRB contains the Molony murine leukemia virus long terminal repeat, unique EcoRI, BamHI, and SpeI sites followed by the IRES-NEO gene in the Bluescript KS plasmid (described in Ref. 13). The plasmid pSVFGFR1 IIIb (31) was cloned as a 2.9-kbp BamHI-SpeI fragment into the corresponding sites of MIRB. MIRB contains the Molony murine leukemia virus long terminal repeat, unique EcoRI, BamHI, and SpeI sites followed by the IRES-NEO gene in the Bluescript KS plasmid (described in Ref. 13). The plasmid pSVFGFR1 IIIb (31) was cloned as a 2.9-kbp BamHI-SpeI fragment into the corresponding sites of MIRB. MIRB contains the Molony murine leukemia virus long terminal repeat, unique EcoRI, BamHI, and SpeI sites followed by the IRES-NEO gene in the Bluescript KS plasmid (described in Ref. 13). The plasmid pSVFGFR1 IIIb (31) was cloned as a 2.9-kbp BamHI-SpeI fragment into the corresponding sites of MIRB. MIRB contains the Molony murine leukemia virus long terminal repeat, unique EcoRI, BamHI, and SpeI sites followed by the IRES-NEO gene in the Bluescript KS plasmid (described in Ref. 13). The plasmid pSVFGFR1 IIIb (31) was cloned as a 2.9-kbp BamHI-SpeI fragment into the corresponding sites of MIRB. MIRB contains the Molony murine leukemia virus long terminal repeat, unique EcoRI, BamHI, and SpeI sites followed by the IRES-NEO gene in the Bluescript KS plasmid (described in Ref. 13).
amounts of Fgf3. ID\textsubscript{50} values were calculated from competition binding curves as presented in Fig. 2. The results show that mouse Fgf3 effectively competes with 125\textsuperscript{I}-Fgf1 for binding to the IIIb and IIIc isoforms of FgfR2 and the IIIb isoform of FgfR1. Over the concentration range tested, which gave a molar excess of up to 133-fold, there was no competition for FgfR1 (IIIc), FgfR3 (IIIb and IIIc), or FgfR4. Surprisingly, the apparent affinity of mouse Fgf3 for the FgfR2 receptor isoforms were approximately 10-fold lower than those previously found using the Xenopus homologue of Fgf3 (Table I and Ref. 29). This result indicated that either mouse Fgf3 has a lower intrinsic affinity for these receptors or that its binding site for the receptor diverges from that of Fgf1. To distinguish between these two possibilities, the affinity of mouse Fgf3 was determined in a homologous system using 125\textsuperscript{I}-Fgf3 instead of 125\textsuperscript{I}-Fgf1 (Fig. 3). The ID\textsubscript{50} values using 125\textsuperscript{I}-Fgf3 as tracer were found to be 10-fold lower for FgfR1 (IIIb) and for FgfR2 (IIIb) but not for FgfR2 (IIIc). Hence, mouse Fgf3 binds with a high affinity to the IIIb variants of FgfR1 and FgfR2 (ID\textsubscript{50} = 0.8 nM) and binds with a lower affinity to FgfR2 (IIIc) (ID\textsubscript{50} = 9 nM) (see Table I).

Mitogenic Activity on Cells Expressing Individual Fgf Receptors—Mouse Fgf3 was compared with Fgf1 and Fgf7 over a range of ligand concentrations for their ability to stimulate DNA synthesis in BaF3 cells expressing individual Fgf receptors. The results show that cells expressing FgfR1 (IIIb) and FgfR2 (IIIb) gave the best response to Fgf3, whereas FgfR2 (IIIc)-expressing cells were only weakly stimulated by this factor (Fig. 4). No growth stimulation occurred on BaF3 cells expressing FgfR1 (IIIc) (Fig. 4), FgfR3 (IIIb), FgfR3 (IIIc), and FgfR4 (data not shown). These findings confirmed the binding specificity of Fgf3 and indicated that its interactions with FgfR1 (IIIb) and FgfR2 were functional. However, it is not clear at present why the mitogenic potential of Fgf3 on BaF3 cells expressing the IIIb isoforms of FgfR1 or FgfR2 is lower than that of Fgf1 (and Fgf7 in the latter case), because these factors have a similar affinity for these receptors (data not shown and Ref. 13). Furthermore, on a mammary epithelial cell line that naturally expresses the same receptors, Fgf3 is a more efficient mitogen than Fgf1 or Fgf7, suggesting that factors other than receptor binding affinity influence the activity of Fgfs (see below).

Modulation of Mitogenic Activity by Heparin on Mammary Epithelial Cells—During the development of the purification procedure, we found that mouse Fgf3 is a potent mitogen for the keratinocyte cell line BALB/MK (Fig. 1a). However, because of its involvement in breast cancer, we were particularly interested to assess the activity of Fgf3 on mammary epithelial

| Receptor | 125\textsuperscript{I}-Fgf1 | 125\textsuperscript{I}-mouse Fgf3 |
|----------|----------------|------------------|
| FgfR1 (IIIb) | 6.0–8.1 \textsuperscript{a} | 0.7–1.3 |
| FgfR1 (IIIc) | > 40 | ND \textsuperscript{b} |
| FgfR2 (IIIb) | 3.4–5.8 | 0.6–0.9 |
| FgfR2 (IIIc) | 7.4–11.8 | 9.0 |
| FgfR3 (IIIb) | > 40 | ND \textsuperscript{b} |
| FgfR3 (IIIc) | > 40 | ND \textsuperscript{b} |
| FgfR4 | > 40 | ND \textsuperscript{b} |

\textsuperscript{a} ID\textsubscript{50} values were determined by competition binding analyses as described under “Materials and Methods” and shown in Figs. 2 and 3. The range is given when more than one determination was made.

\textsuperscript{b} ND, not determined.
cells. Fgf3 was indeed found to be mitogenic for the two mouse mammary cell lines, C57MG and HC11 (Fig. 5 top panels). On HC11 cells, Fgf3 was a potent inducer of DNA synthesis with an ED50 (concentration eliciting 50% of the maximum response) of \(0.1\) nM, whereas on the C57MG cell line the ED50 was above 1 nM. Interestingly, heparin, a glycosaminoglycan that can mimic the Fgf low affinity receptors (see introduction) enhanced by more than 10-fold the potency of Fgf3 on HC11 cells (Fig. 5 right panels) while diminishing significantly the response of C57MG cells to this factor at concentrations above \(2\) \(\mu\)g/ml (Fig. 5 left panels). We also found that heparin differentially modulated the mitogenic effect of various Fgfs on the same cell line (Fig. 5 lower panels). On HC11, for instance, heparin enhanced the response to Fgf3 at all the doses tested but with a maximum effect at around \(2\) \(\mu\)g/ml. Higher concentrations of heparin were needed to increase the mitogenic activity of Fgf1, with a 3-fold enhancement at \(100\) \(\mu\)g/ml. In contrast, the response to Fgf7 was progressively inhibited at concentrations of heparin exceeding 0.5 \(\mu\)g/ml.

The differential sensitivity of the two mammary cell lines toward Fgf3 could reflect a difference in the expression of its high affinity receptors. To gain some insight into the Fgf receptor repertoire of these and other Fgf3-responsive cell lines, total cell RNA was prepared and used for RT-PCR analyses with receptor-specific oligonucleotide primers. The entire ex-
tracellular domain of each Fgf receptor was amplified, and different splice variants (α and β forms) were detected by ethidium bromide staining of the PCR products fractionated on agarose gels (Fig. 6, a and b). IIIb and IIIc variants of FgfR2 were distinguished by digesting the PCR products with restriction endonucleases, which cleave exclusively one or the other variant (Fig. 6b and Ref. 30). For the corresponding isoforms of FgfR1, a different approach was needed that relied on one primer hybridizing specifically to the IIIb or IIIc spliced isoform (Fig. 6a). Three size variants of FgfR1 and FgfR2 were detected in all the cell lines tested. Previous sequence analysis of FgfR2 PCR products has shown these forms represent the α, β, and γ plus acid box receptor variants (29). The results show that HC11 and BALB/MK express the IIIb and IIIc isoforms of FgfR1 and FgfR2 (IIIb), whereas C57MG and NIH3T3 express only the IIIc variant of these receptors. Thus, the greater sensitivity of HC11 toward Fgf3 in the proliferation assay can be explained by the presence of two receptor isoforms with an approximately 10-fold greater affinity for Fgf3 (see Table I).

The mitogenic potential of Fgf3 was then compared with that of Fgf1 and Fgf7 on the mammary cell lines (Fig. 7). Fgf1 gave a similar response on both cell lines (ED50 = 0.1 nM), whereas Fgf7 induced DNA synthesis only in HC11 cells (ED50 = 0.2 nM), as expected from the previous receptor expression study. Fgf1 showed a higher efficiency than Fgf3 in triggering a mitogenic response in C57MG cells (ED50 = 0.9 nM versus 1 nM), probably as a result of having a higher affinity than Fgf3 for FgfR2 (IIIc) (ID50 = 2 nM versus 9 nM; Table I and data not shown), of having a broader range of Fgf receptor interactions, and of being potentiated by heparin, which was included in this assay. On the contrary, the dose-response curves obtained with HC11 cells indicated that Fgf3 was more efficient than the other Fgfs in stimulating DNA synthesis (ED50 = 0.01 nM). As HC11 cells express FgfR2 (IIIb), which has a similar affinity for the three Fgfs tested (Table I and Ref. 13), the variation in potency can be explained in this case by the use of heparin at a concentration of 2 μg/ml, which was found to be optimal for Fgf3 but not for Fgf1 and Fgf7 (see Fig. 5). However, the maximum responses obtained with these Fgfs were in the same range.

**DISCUSSION**

The results described herein show that biologically active mouse Fgf3 can be purified from culture supernatants of NIH3T3 cells engineered to secrete high levels of this factor. Immunological and bioactivity criteria were used to show that differential salt elution from a heparin-Sepharose column was effective in separating a mixture of Fgf7 and Fgf3 that occurs in the conditioned medium of these cells. In previous studies, recombinant Fgf3 made in bacterial or baculovirus expression
systems was found to be mostly insoluble. However, the small amount of soluble recombinant Fgf3 showed no mitogenic activity on cells that we showed here to be responsive to Fgf3. Using a cell-free translation system we were able to make biologically active Fgf1, Fgf4, and Fgf7, but Fgf3 gave equivocal results (39–41). This could have resulted from the use of inappropriate assay cells, or alternatively the Fgf3 was inactive; the studies reported here suggest the latter explanation.

The competition for receptor binding experiments (Figs. 2 and 3) clearly show that mouse Fgf3 has the highest affinity for the IIb isoforms of Fgfr1 and Fgfr2 and a 10-fold lower affinity for Fgfr2 (IIIc). There was no detectable competition for 125I-Fgf1 binding to Fgfr1 (IIic), Fgf3 isoforms, or Fgfr4. It is also worth noting that we detected no significant differences between the binding of Fgf3 to the α and β isoforms of Fgfr2 (data not shown). The lower apparent affinity of mouse Fgf3 for Fgfr2 (IIIc) was observed using either 125I-Fgf1 or 125I-Fgf3 as tracer, indicating that mouse Fgf3 has indeed a lower intrinsic affinity for this receptor isoform. The relative binding affinities correlated well with the mitogenic activity of Fgf3 on BaF3 cells expressing single isoforms of the various Fgfs receptors (Fig. 4) and are consistent with the mitogenic potential of Fgf3 for different cell lines that express combinations of these Fgf receptors (Figs. 5 and 7). In contrast to mouse Fgf3, the Xenopus homologue was previously shown to have a similar high affinity for the IIb and IIIC isoforms of Fgfr2 and, as expected, is a more potent mitogen for cells expressing Fgfr2 (IIic) (Refs. 29 and 36 and data not shown).

Analysis of the mitogenic potential of Fgf3 on two mammary epithelial cell lines showed that C57MG cultures required at least 10 times more ligand compared with those of HC11 to give a half-maximum response (Fig. 5). This difference in sensitivity can be largely explained by the expression on HC11 cells of the IIb variants of Fgfr1 and Fgfr2, which show a higher affinity toward Fgf3 than that of the IIIC variants found in C57MG cells (Fig. 6). An unexpected observation was the opposite effect of heparin on the mitogenic response elicited by Fgf3 on the two cell lines: heparin progressively increased the potency of Fgf3 on HC11 cells but decreased it on C57MG cells (Fig. 5). This differential modulation by heparin does not simply correlate with the presence or absence of the IIb or IIIC variants of the Fgfr1 and Fgfr2 receptors. For instance, NIH3T3 cells are similar to C57MG in that they express the Fgf receptor IIIC (Refs. 29 and 36 and data not shown). The lower apparent affinity of mouse Fgf3 for Fgfr2 (IIIc) was observed using either 125I-Fgf1 or 125I-Fgf3 as tracer, indicating that mouse Fgf3 has indeed a lower intrinsic affinity for this receptor isoform. The relative binding affinities correlated well with the mitogenic activity of Fgf3 on BaF3 cells expressing single isoforms of the various Fgf receptors (Fig. 4) and are consistent with the mitogenic potential of Fgf3 for different cell lines that express combinations of these Fgf receptors (Figs. 5 and 7). In contrast to mouse Fgf3, the Xenopus homologue was previously shown to have a similar high affinity for the IIb and IIIC isoforms of Fgfr2 and, as expected, is a more potent mitogen for cells expressing Fgfr2 (IIic) (Refs. 29 and 36 and data not shown).

Analysis of the mitogenic potential of Fgf3 on two mammary epithelial cell lines showed that C57MG cultures required at least 10 times more ligand compared with those of HC11 to give a half-maximum response (Fig. 5). This difference in sensitivity can be largely explained by the expression on HC11 cells of the IIb variants of Fgfr1 and Fgfr2, which show a higher affinity toward Fgf3 than that of the IIIC variants found in C57MG cells (Fig. 6). An unexpected observation was the opposite effect of heparin on the mitogenic response elicited by Fgf3 on the two cell lines: heparin progressively increased the potency of Fgf3 on HC11 cells but decreased it on C57MG cells (Fig. 5). This differential modulation by heparin does not simply correlate with the presence or absence of the IIb or IIIC variants of the Fgfr1 and Fgfr2 receptors. For instance, NIH3T3 cells are similar to C57MG in that they express the Fgf receptor IIIC (Refs. 29 and 36 and data not shown). The lower apparent affinity of mouse Fgf3 for Fgfr2 (IIIc) was observed using either 125I-Fgf1 or 125I-Fgf3 as tracer, indicating that mouse Fgf3 has indeed a lower intrinsic affinity for this receptor isoform. The relative binding affinities correlated well with the mitogenic activity of Fgf3 on BaF3 cells expressing single isoforms of the various Fgf receptors (Fig. 4) and are consistent with the mitogenic potential of Fgf3 for different cell lines that express combinations of these Fgf receptors (Figs. 5 and 7). In contrast to mouse Fgf3, the Xenopus homologue was previously shown to have a similar high affinity for the IIb and IIIC isoforms of Fgfr2 and, as expected, is a more potent mitogen for cells expressing Fgfr2 (IIic) (Refs. 29 and 36 and data not shown).

Analysis of the mitogenic potential of Fgf3 on two mammary epithelial cell lines showed that C57MG cultures required at least 10 times more ligand compared with those of HC11 to give a half-maximum response (Fig. 5). This difference in sensitivity can be largely explained by the expression on HC11 cells of the IIb variants of Fgfr1 and Fgfr2, which show a higher affinity toward Fgf3 than that of the IIIC variants found in C57MG cells (Fig. 6). An unexpected observation was the opposite effect of heparin on the mitogenic response elicited by Fgf3 on the two cell lines: heparin progressively increased the potency of Fgf3 on HC11 cells but decreased it on C57MG cells (Fig. 5). This differential modulation by heparin does not simply correlate with the presence or absence of the IIb or IIIC variants of the Fgfr1 and Fgfr2 receptors. For instance, NIH3T3 cells are similar to C57MG in that they express the Fgf receptor IIIC isoforms; however, they show an enhanced response to Fgf3 in the presence of heparin (data not shown). Furthermore, we found that heparin also differentially modulated the effect of other Fgfs on the same cell line. For example, heparin decreased Fgf7 mitogenic activity on HC11 cells, whereas it potentiated Fgf1 and Fgf3, albeit at different concentrations. Recently, a similar observation was reported by others using BALB/MK cells (42). Thus, different Fgfs may require distinct combinations and/or concentrations of low and high affinity receptors to achieve an optimal signaling effect. Response potentiation by heparin has been proposed to occur through the stabilization of the Fgf molecule and of Fgf-F-gf receptor complexes (18, 43–46). Oligomerization of Fgs by heparin may further increase the affinity of Fgf for the Fgfr receptor and results in dimerization and activation of the Fgfr receptor (16, 17, 35). However, the mechanism of inhibition by heparin as shown here and by others (42) suggests a more complex role for the low affinity receptors in modulating Fgf responses.

The receptor specificity of Fgf3 encompasses that found for Fgf7 (13–15). It is therefore interesting that in some cases where Fgf7 has been implicated as a mesenchyme-secreted growth/differentiation factor acting on the adjacent epithelium, such as the prostate and seminal vesicle, Fgf3 ectopically expressed as a transgene has been shown to act as an oncogene (22, 47–49). A similar situation may also exist for the mammary gland where the high affinity receptors for Fgf3 are present and provide an explanation for its role in virally induced breast cancer. Thus, virally mediated activation of Fgf3 directly in the epithelial cells could result in autocrine growth stimulation (reviewed in Ref. 21). Additionally, recent evidence suggests that growth factors can protect cells from undergoing apoptotic death, a process that occurs throughout the mammary gland after lactation. Therefore, Fgf3 may act in a dual capacity to facilitate the retention and accumulation of cells susceptible to further somatic mutation. The presence of Fgfs and Fgf receptors in the mammary gland (50) also implies that the normal development and differentiation of the mammary gland during pregnancy and lactation may involve steps mediated by members of the Fgf family. We are presently investigating this possibility to gain further insight into the role of Fgfs in normal breast development.

Acknowledgments—We thank Jeffrey Rubin and Stuart Aaronson for the kind gift of the 1G4 murine monoclonal antibody and Peter Parker and Julian Downward for critical reading of the manuscript.

REFERENCES
1. Miyamoto, M., Naruo, K., Seko, C., Matsumoto, S., Kondo, T., and Kurokawa, T. (1993) Mol. Cell Biol. 13, 4253–4259.
2. Tanaka, A., Miyamoto, K., Minamino, N., Takeda, M., Sato, B., Matsuo, H., and Matsumoto, K. (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 8928–8932.
3. Goldfarb, M. (1990) Cell Growth & Differ. 1, 439–445.
4. Burgess, W. H., and Maclag, T. (1989) Annu. Rev. Biochem. 58, 575–606.
5. Baldwin, V., Roman, A.-M., Bosc-Bierne, I., Amalric, F., and Bouche, G. (1990) EMBO J. 9, 1511–1517.
6. Bouche, G., Gas, N., Prats, H., Baland, V., Tabar, J.-P., Teissie, J., and Amalric, F. (1987) Proc. Natl. Acad. Sci. U.S.A. 84, 6770–6774.
7. Adand, P., Dixon, M., Peters, G., and Dickson, C. (1990) Nature 343, 662–665.
8. Kiefer, P., Adand, P., Pappin, D., Peters, G., and Dickson, C. (1994) EMBO J. 13, 4126–4136.
9. J. S. Bresnick, unpublished data.
31. Werner, S., Duan, D.-S. R., de Vries, C., Peters, K. G., Johnson, D. E., and Williams, L. T. (1992) Mol. Cell. Biol. **12**, 82–88
32. Dixon, M., Deed, R., Acland, P., Moore, R., Whyte, A., Peters, G., and Dickson, C. (1989) Mol. Cell. Biol. **9**, 4896–4902
33. Ornitz, D. M., and Leder, P. (1992) J. Biol. Chem. **267**, 16305–16311
34. Mansukhani, A., Delfera, P., Moscatelli, D., Kornbluth, S., Hanafusa, H., and Basilico, C. (1992) Proc. Natl. Acad. Sci. U. S. A. **89**, 3305–3309
35. Ornitz, D. M., Yayon, A., Flanagan, J. G., Svahe, C. M., Levi, E., and Leder, P. (1992) Mol. Cell. Biol. **12**, 240–247
36. Kiefer, P., Mathieu, M., Close, M., Peters, G., and Dickson, C. (1993) EMBO J. **12**, 4159–4168
37. Zhan, X., Culpepper, A., Reddy, M., Loveless, J., and Goldfarb, M. (1987) Oncogene **1**, 369–376
38. Kiefer, P., Peters, G., and Dickson, C. (1993) Mol. Cell. Biol. **11**, 5929–5936
39. Dickson, C., Fuller-Pace, F., Kiefer, P., Acland, P., MacAllan, D., and Peters, G. (1991) in The Fibroblast Growth Factor Family (Baird, A., and Klagsbrun, M., ed.) pp. 18–26, The New York Academy of Sciences, New York
40. Dickson, M., Deed, R., Acland, P., Moore, R., Whyte, A., Peters, G., and Dickson, C. (1989) Mol. Cell. Biol. **9**, 4896–4920
41. Paterno, G., Gillespie, L., Dixon, M., Slack, J., and Heath, J. (1989) Development **106**, 79–83
42. Ron, D., Bottaro, D., Finch, P., Morris, D., Rubin, J., and Aaronson, S. (1993) J. Biol. Chem. **268**, 2984–2988
43. Kaplow, J., Bellot, F., Crumley, G., Dionne, C., and Jaye, M. (1990) Biochem. Biophys. Res. Commun. **172**, 107–112
44. Rognani, M., Mansukhani, A., Dell’Era, P., Bellotta, P., Basilico, C., Rifkin, D. B., and Moscatelli, D. (1994) J. Biol. Chem. **269**, 3976–3984
45. Springer, B. A., Pantoliano, M. W., Barbera, F. A., Guzyulu, P. L., Thompson, L. D., Herblin, W. F., Rosenfeld, S. A., and Book, G. W. (1994) J. Biol. Chem. **269**, 26879–26884
46. Pantoliano, M. W., Horlick, R. A., Springer, B. A., Vandyk, D. E., Tobery, T., Wetmore, D. R., Lear, J. D., Nahapetian, A. T.,Bradley, J. D., and Sisk, W. P. (1994) Biochemistry **33**, 10239–10248
47. Mason, I. (1994) Cell **78**, 547–552
48. Mason, I., Fuller-Pace, F., Smith, R., and Dickson, C. (1994) Mech. Dev. **45**, 15–30
49. Alarid, E., Rubin, J., Young, P., Chedid, M., Ron, D., Aaronson, S., and Cunha, G. (1994) Proc. Natl. Acad. Sci. U. S. A. **91**, 1074–1078
50. Coleman-Kmacik, S., and Rosen, J. M. (1994) Mol. Endocrinol. **8**, 218–229
51. Kozak, M. (1991) J. Biol. Chem. **266**, 19867–19870