FGF3 Attached to a Phospholipid Membrane Anchor Gains a High Transforming Capacity

IMPLICATIONS OF MICRODOMAINS FOR FGF3 CELL TRANSFORMATION*

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NIH3T3 cells transformed by mouse FGF3-cDNA (DMI cells) selected for their ability to grow as anchorage-independent colonies in soft agar and in defined medium lacking growth factors exhibit a highly transformed phenotype. We have used dominant negative (DN) fibroblast growth factor (FGF) receptor 2 (FGFR2) isoforms to block the FGF response in DMI cells. When the DN-FGFR was expressed in DMI cells, their transformed phenotype can be reverted. The truncated FGFR2(IIIb), the high affinity FGFR for FGF3, is significantly more efficient at reverting the transformed phenotype as the IIIc isoform, reaffirming the notion that the affinity of the ligand to the DN-FGFR2 isoform determines the effect. Heparin or heparan sulfate displaces FGF3 from binding sites on the cell surface inhibiting the growth of DMI cells and reverts the transformed phenotype (1). However, the presence of heparin is necessary to induce a mitogenic response in NIH3T3 cells when stimulated with soluble purified mouse FGF3. We have investigated the importance of cell surface binding of FGF3 for its ability to transform NIH3T3 cells by creating an FGF3 mutant anchored to the membrane via glycosylphosphatidylinositol (GPI). The GPI anchor renders the cell surface association of FGF3 independent from binding to heparan sulfate-proteoglycan of the cell surface membrane. Attachment of a GPI anchor to FGF3 also confers a much higher transforming potential to the growth factor. Even more, the purified GPI-attached FGF3 is as much transforming as the secreted protein acting in an autocrine mode. Because NIH3T3 cells do not express the high affinity tyrosine kinase FGF receptors for FGF3, these findings suggest that FGF3 attached to GPI-linked heparan sulfate-proteoglycan may have a broader biological activity as when bound to transmembrane or soluble heparan sulfate-proteoglycan.

The fibroblast growth factor (FGF) family presently constitutes 22 structurally related polypeptides that show a wide range of biological activities. They modulate growth, differentiation, migration, and survival depending on cell type and biological context (reviewed in Refs. 2–8). In vivo the FGFs have been implicated in embryonic development, neuronal survival, wound repair, and angiogenesis, but also in a number of pathological responses such as neovascularization and tumor growth. The mouse FGF3int-2 gene is one of several cellular oncogenes identified at sites of proviral integration in retrovirus-induced breast carcinomas associated with infection by mouse mammary tumor virus (MMTV). In cell culture it proved to be a weak transforming gene, but under selective pressures it causes the morphological transformation of NIH3T3 cells (1). FGFs signal by binding to and activating high affinity cell surface tyrosine kinase receptors. Four high affinity receptor genes (designated FGFR1–FGFR4) have been identified that encode a cytoplasmic tyrosine kinase domain, an extracellular region composed of two (β-form) or three (α-form) immunoglobulin-like domains, depending on the choice of splice sites. The ligand binding site involves the two Ig loops located adjacent to the cell membrane. Alternative splicing of the membrane proximal loop generates isoforms (termed IIIb and IIIc) that have different ligand binding specificities (9–15). FGF signaling can be blocked by dominant negative mutant FGFRs (DN-FGFR) that have a cytoplasmic domain with the kinase domain removed. The mutant receptors form non-functional heterodimers with the wild type receptors through binding to a common ligand. The effectiveness of DN-FGFRs has been used to demonstrate a requirement for FGF signaling in mesoderm formation in Xenopus embryos, as well as in skin wound repair, development of the mouse lung, and lobulo-alveolar development of the mammary gland. Furthermore, expression of a DN-FGFR mutant is able to revert the transformed phenotype of NIH3T3 cells transformed by FGF4 (16–21).

Recent reports have demonstrated that besides the FGF isoforms present on the cell surface, the binding of FGFs and FGFRs to heparan sulfate-proteoglycans (HSPGs) act as cofactors to regulate FGF signaling (22–24). FGF3 exhibits ~10× higher affinity for the IIIb isoforms of FGFR1 and FGFR2 compared with the IIIc variant of FGFR2. Previous studies (25) revealed that heparin progressively increased the potency of FGF3 on HC11 cells expressing the IIIb variants of FGFR1 and FGFR2 but decreased it on C57MG cells that express the corresponding IIIc variants. This different modulation of FGF3 activity is not easily explained by the expression of different FGFR variants because NIH3T3 cells express the same FGF isoforms as C57MG cells, but they show a dependence on heparin for FGF3-mediated mitogenic response. Although heparin is essential for FGF3-induced mitogenic activity on NIH3T3 cells, heparin inhibits the growth of FGF3-transformed NIH3T3 cells and reverts their transformed phenotype (1).
Secreted FGFR3 is preferentially associated with the cell surface and is displaced by heparin and soluble heparan sulfate (26, 27). Therefore, cell-surface-bound FGFR3 protein appears to be essential in the morphological transformation of NIH3T3 cells.

The present study was undertaken to characterize the dependence of the FGFR3 cell surface localization on its ability to transform NIH3T3 cells. In this report we were able to show that the phenotype of FGFR3 transformed cells could be reverted by expressing pFGFR2 mutants and that the truncated pFGFR2(IIIb) variant was significantly more efficient at reverting the phenotype than the IIC isoform. We created a GPI-anchored FGFR3 mutant to insert the ligand in the plasma membrane via a GPI anchor sequence. The GPI anchor renders the cell surface association of FGFR3 independent of binding to the cell surface membrane via HS PGs, suggesting that localization on the cell surface is crucial for FGFR3 transformation and presumably not a requirement for interacting with specific cell surface HS PGs.

EXPERIMENTAL PROCEDURES

Cell Culture—COS-1, NIH3T3, and DMI-1 cells were maintained as previously described (28). For transient DNA transfection, plasmid DNA as indicated was introduced into 5 × 10⁶ COS-1 cells by electroporation (450 V/250 μF) using a Bio-Rad Gene-Pulser. Between 48 and 72 h after transfection, the cells were harvested for immunoblot analysis or processed for immunofluorescence. For stable DNA transfection, purified plasmid DNA was introduced by using the transfection reagent FuGENE 6 (Roche) as recommended by the manufacturer.

Immunofluorescence—NIH3T3 cells grown on glass coverslips were transfected with the appropriate plasmids, and 48 h later the cells were fixed and processed as previously described (28). For surface immunostaining, the cells were incubated with antibodies without permeabilizing or, alternatively, cells were incubated with the antibodies at 4 °C in the presence of 0.05% sodium azide prior to fixation. After washing in PBS, the stained cells were mounted in 90% glycerol containing 1% PBS, the stained cells were mounted in 90% glycerol containing 1% PBS. Subsequently, the incorporation mixture was replaced with DMEM plus 10% FCS. The test plasmid DNAs were introduced by using the transfection reagent FuGENE 6 (Roche) and, after 24 h, 8 × 10⁶ cells were plated into 10-cm diameter dishes. In the delayed focus formation assay, the cells were cultured in DMEM, 10% FCS plus 1 mg/ml neomycin until a cytotropic effect could be visualized (normally 1–2 days). Within 7 or 8 days the G418-resistant colonies grew to near confluence, the medium was changed to DMEM plus 3% FCS, and cells were cultured another 6 days. Cell foci were scored microscopically. For the agar colony assay, transfected cells were maintained in G418 as above, after which duplicate samples of 5 × 10⁶ cells were suspended in 1.5 ml of DMEM with 10% FCS containing 0.3% agar (Difco). The plates were incubated at 37 °C for a minimum of 10 days, and the cell foci were counted.

RESULTS

Expression of Dominant Negative FGFR Receptor in DMI Cells—FGF3 presumably transforms NIH3T3 cells by establishing an autocrine loop mediated by endogenous FGFRs. DMI-1 cells were isolated as a transformed colony following transfection of a cDNA encoding secreted FGFR3. The 92-kDa secreted FGFR3 protein (p92.5) is predicted to contain a transmembrane domain uniquely at the cell surface from which it can be quantitatively displaced by heparin as demonstrated by FGFR3 cell surface iodination, and the labeled cells were recovered as monolayer by incubating in PBS, 0.5% Triton X-100 leaving the ECM on the culture dish (Fig. 1). Using receptor binding competition assays, FGF3 was shown to bind with high affinity to the IIIb isoforms of FGFR1 and FGFR2 but with 10× lower affinity to the IIC isoform of FGFR2.
affinity to the IIIC variant of FGFR2. No binding affinity could be demonstrated for FGFR1(IIIC), the isoforms of FGFR3 or for FGFR4. NIH3T3 cells predominantly express the IIIC isoforms of both FGFR1 and FGFR2 and low levels of FGFR4. A reverse transcriptase-PCR analysis showed that the DMI-1 cells had retained the same FGFR expression pattern as the parental NIH3T3 cells (25).

Reversion of the Transformed Phenotype of DMI Cells—To show that the transformed phenotype of DMI-1 cells was due to FGF3 expression, a DN-FGFR was used to block FGF3 signaling. DMI cells were stably transfected with DN-FGFR cDNAs inserted into a MuLV-based retrovirus vector containing a puromycin-resistant gene to enable selection of transformants (Fig. 2A). DMI-1 cells expressing high levels of DN-FGFR receptor 2 isoform IIb and IIc were identified by Northern blot analysis (Fig. 2B). Overexpression of the DN-FGFR results in an FGF-mediated sequestration of the wild type receptor by the DN-FGFR to form an inactive complex. Although the DN-FGFR2(IIlc) isoform resulted in a partial reversion of the transformed DMI phenotype (Fig. 3E), overexpression of the DN-FGFR2 isoform IIb resulted in the morphological reversion of most colonies to an untransformed phenotype (Fig. 3D). The greater effect of the DN-FGFR2(IIib) isoform is consistent with its 10-fold higher affinity for FGF3. To exclude the possibility that revertants were due to the selection procedure, recombinant retroviruses were used to infect rather than transfect DMI cells to express mutant FGF receptors. This resulted in most cells showing a non-transformed phenotype. These results confirmed the presumption that DMI-1 cells were transformed by FGF3.

Glycophospholipid Membrane Anchor Modification of FGF3—Although mitogenic stimulation of NIH3T3 cells by FGF3 is dependent on the presence of soluble heparin, heparin at the same dose inhibits the morphological transformation in DMI-1 cells (1, 25, 28). Furthermore, purified FGF3 from DMI cells induces mitogenesis of NIH3T3, but even at higher concentrations this does not lead to the transformation of NIH3T3 cells. Therefore, the localization of FGF3 at the cell surface through binding to HSPGs could be seen as the essential prerequisite for its transforming potential.

To test this hypothesis, a chimeric FGF3 protein was created and attached to the cell surface via a glycosphospholipid anchor, which renders the FGF3 cell surface localization independent of its binding to HSPGs. To generate a chimeric protein of FGF3 linked to a GPI, the signal sequence of decay accelerating factor (CD55; DAF), which directs attachment of a GPI anchor, was fused to the C terminal of FGF3 (Fig. 4A). The previously described plasmid pK3.2 (28) contains a FGF3 cDNA where the AUG initiation codon was optimized for efficient translation of an FGF3, which is exclusively directed into the secretory pathway. COS-1 cells transiently transfected with pK3.2 express several FGF3-related products that can be detected by immunoblotting with specific antisera raised against the C terminal peptide of mouse FGF3. Two major intracellular species of 31.5 and 30.5 kDa (gp31.5 and gp30.5) and two much less abundant non-glycosylated forms, 28.5 and 27.5 kDa, can be observed (Ref. 28 and Fig. 5A). As previously shown (28, 31), each pair differs with respect to the presence or absence of the signal peptide. The last 37 amino acids of DAF were fused in-frame to the C terminal of FGF3 to create a fusion protein. To confirm the presence of the GPI anchor, COS-1 cells were transfected with pK3.2-DAF and metabolically labeled with [3H]ethanolamine, a component of the GPI anchor. 3H-labeled FGF3-related proteins were immunoprecipitated with FGF3-specific antisera only from pK3.2-DAF-transfected cells encoding the fusion protein but not from pK3.2-transfected con-
control cells. Two products of 32.5 and 33.5 kDa, analogous to the major glycosylated FGF3 species of 30.5 and 31.5 kDa, could be identified by autoradiography (Fig. 4B).

FGF3-DAF Protein Remains Cell-associated—The function of GPI modification is to direct proteins efficiently to the cell surface where they stay integrated into the membrane by their phospholipid moiety. To test the ability of the GPI anchor to stably attach FGF3 at the cell surface, COS-1 cells transfected with pKC3.2 and pKC3.2-DAF cells were analyzed for cell-associated and secreted products in the presence or absence of heparin. The apparent molecular mass of the three major intracellular FGF3-DAF-related proteins were increased by 2 kDa consistent with the presence of the GPI moiety (Fig. 5A). These isoforms are analogous to the FGF3 products gp31.5 and gp30.5, which are both glycosylated but with and without the signal peptide respectively, and gp28.5, the non-glycosylated form with a retained signal peptide. The secreted 32.5-kDa form associates with the cell surface and ECM by binding to HSPGs, which can be reversed by incubation with soluble heparin. Hence, in the presence of heparin the concentration of FGF3 in the culture medium increases (Fig. 5B). In contrast, the exported chimeric FGF3-DAF could not be displaced from its binding to the cell surface by the addition of heparin. The results are consistent with the conclusion that the chimeric FGF3-DAF contains a functional GPI anchor, which leads to a stable integration of a substantial amount of FGF3 into the cell membrane.

Subcellular Localization of FGF3-DAF Protein—To investigate further the influence of the GPI tag on the subcellular distribution of FGF3, COS-1 cells were transfected with pKC3.2 and pKC3.2-DAF, grown in the presence or absence of heparin, and examined by immunofluorescence. The staining patterns of the intracellular FGF3 and FGF3-DAF proteins displayed a typical juxtanuclear distribution characteristic for proteins that are located in the Golgi complex, confirming that both wild type FGF3 and mutant proteins accumulate in the Golgi stacks. The GPI tag appears not to change the primary intracellular distribution of FGF3 (Fig. 6A and B). The cell surface staining analysis of fixed and non-permeabilized COS-1 cells expressing FGF3 and FGF3-DAF demonstrated a typical juxtanuclear distribution characteristic for proteins that are located in the Golgi complex, confirming that both wild type FGF3 and mutant proteins accumulate in the Golgi stacks. The GPI tag appears not to change the primary intracellular distribution of FGF3 (Fig. 6A and B). However, in the presence of heparin the concentration of FGF3 in the culture medium increases (Fig. 5B). In contrast, the exported chimeric FGF3-DAF could not be displaced from its binding to the cell surface by the addition of heparin. The results are consistent with the conclusion that the chimeric FGF3-DAF contains a functional GPI anchor, which leads to a stable integration of a substantial amount of FGF3 into the cell membrane.

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FGF3 Transforming Ability

Transformation of NIH3T3 Cells by FGF3-DAF—DMI-1 cells are transformed by secreted FGF3 through an autocrine signaling loop (Fig. 3). However, in addition to the interaction of FGF3 with the FGF receptors, the association of FGF3 with HSPGs of the cell surface is also necessary to maintain the transformed phenotype (1). To see whether the GPI anchor may overcome the need for binding to HSPGs at the cell surface, FGF3-DAF fusion protein (pBabeneo-3.2-DAF) was isolated. Positive clones were identified by Northern blot analysis. Representative examples are shown.

Experimental Procedures—Transformation of NIH3T3 Cells by FGF3-DAF—Cos-1 cells transfected with pKC3.2, pKC3.2-DAF, or the control vector pKC4. A, extracts of COS-1 cells transfected with pKC3.2, pKC3.2-DAF, or the empty vector pKC4 were separated by SDS-PAGE, and the FGF3-related proteins were detected by immunoblotting with a rabbit polyclonal antiserum against FGF3. B, COS-1 cells transfected with pKC3.2, pKC3.2-DAF, or the empty vector pKC4 were harvested after 48 h, and the culture fluid was recovered. The cells were washed with PBS and removed from the culture dish with 0.5% Triton X-100 in PBS. The material remaining on the dish was operationally defined as ECM and recovered in dissociation buffer as described under "Experimental Procedures." Samples of ECM and culture medium were fractionated by SDS-PAGE on a 12.5% gel and immunoblotted with the antiserum against FGF3. The + and – indicate whether the cells were grown in the presence or absence of 10 μg/ml heparin. The immunocomplexes were visualized by ECL using a specific anti-rabbit secondary antibody (for details see "Experimental Procedures"). The protein sizes were calculated relative to prestained protein standards.

Fig. 3. The effect of GPI-anchored FGF3 on its secretion. Immunoblot analysis of cell extracts, ECM, and culture medium from COS-1 cells transfected with pKC3.2, pKC3.2-DAF, or the control vector pKC4. A, extracts of COS-1 cells transfected with pKC3.2, pKC3.2-DAF, or the empty vector pKC4 were separated by SDS-PAGE, and the FGF3-related proteins were detected by immunoblotting with a rabbit polyclonal antiserum against FGF3.
fected NIH3T3 demonstrate a nearly 100% transformed phenotype (Fig. 8).

**Growth in Soft Agar**—As a more stringent assay of transformation, pools of transfected cells were analyzed for their ability to grow as anchorage independent colonies in soft agar in the presence or absence of 10 ng/ml heparin. The results are expressed as the number of G418-resistant colonies in relation to those determined by the delayed focus assay. The results clearly show that FGF3-DAF- and FGF4-transformed cells were not affected by heparin at this concentration (Fig. 9). These results strongly suggest that anchoring FGF3 on the plasma membrane significantly influences the transforming potential of FGF3 and emphasize the importance of cell surface localization of the growth factor as a prerequisite for the morphological transformation of NIH3T3 cells by FGF3.

**Painting of NIH3T3 Surfaces with FGF3-DAF Protein**—Biologically active mouse FGF3 can be purified from DMI cells by retention on heparin-Sepharose columns. In the presence of heparin, FGF3 purified from DMI cells is mitogenic for NIH3T3 cells, but it is not able to induce morphological transformation (25). DAF-linked proteins can be easily isolated from cell membranes, reinserted into heterologous cell membranes (painting), and still retain their specific biological function (33). To determine whether FGF3-DAF purified from membranes of transfected COS-1 cells is able to transform NIH3T3 cells, COS-1 cell extracts expressing pKC3.2 and pKC3.2-DAF were isolated and enriched by heparin-Sepharose column chromatography (Fig. 10). The FGF3-related proteins were identified by immunoblotting. Incubation of NIH3T3 cells with purified FGF3 had no

![FGF3 Transforming Ability](image_url)
noticeable effect (Fig. 10B); however, when cells were incubated with FGF3-DAF protein the flat morphology of non-transformed NIH3T3 cells changed to cells with a highly refractile appearance typical of NIH3T3 cells transformed by FGF4 or other oncogenes. The cells tend to grow into stellar formations as seen with DMI cells. This transformed phenotype appeared independently of cell density, as demonstrated by incubating different cell densities with the FGF3-DAF fusion protein.

DISCUSSION

Because DMI cells were selected for their ability to grow in defined medium and anchorage-independent in soft agar after FGF3 transfection, it was possible that the transformed phenotype might be partly due to additional mutations occurring during the selection process. However, DMI cells show the same phenotype seen with NIH3T3 cells transfected and transformed with a FGF5-FGF3 chimera in which the N terminal containing the signal sequence of FGF3 was exchanged for the corresponding part of FGF5 to improve the secretion efficiency. In particular, the transformed phenotype of FGF5-FGF3-transformed NIH3T3 cells is reverted to a normal morphology by adding heparin similar to DMI cells. In contrast, the transformed phenotype of Ki-ras-transformed and FGF4-transformed NIH3T3 cells are not affected by heparin (28).

To confirm the FGF3 signaling pathway, which is necessary for the morphological transformation of NIH3T3 cells by FGF3, we overexpressed DN-FGFRs in DMI cells and showed a reversal of the transformed phenotype. This strongly suggests that an intact FGF receptor signaling pathway is essential for maintaining the transformed morphology in DMI cells. NIH3T3 cells express the IIIc isoforms of the FGF receptor 1 and 2 and very weakly FGFR4. DMI cells have the same FGFR expression pattern as the parental NIH3T3 cells. Using receptor binding competition assay, we previously demonstrated that mouse FGF3 binds with high affinity to the IIIb isoforms of FGFR1 and FGFR2 and with a 10-fold lower affinity to the IIIc variant of FGFR2. No competition for binding was observed for FGFR1 (IIIc), FGFR3, or FGFR4 (25). The greater effect of the DN-FGFR2(IIIb) to reverse the FGF3-transformed phenotype reflects the affinity of the ligand for the DN receptor mutants. The poor transforming ability of FGF3 on NIH3T3 cells can also be explained by the availability of only the lower affinity receptor, FGFR2(IIIb).

FIG. 9. Soft agar cloning efficiency of NIH3T3 cells transformed by FGF3. The diagram illustrates the data of Table II. NIH3T3 cells were transfected with murine leukemia virus-based vector expressing FGF3 (3.2), the GPI-anchored FGF3 fusion protein (3.2DAF), and FGF4 and tested for their ability to form colonies in soft agar in the presence or absence of 10 µg of heparin per ml (see “Experimental Procedures”). As an additional comparison, stable FGF3-transformed DMI-1 cells were also plated. The arithmetical means of four different experiments are shown.

| Cell line | with 10 µg/ml Heparin | without Heparin |
|-----------|----------------------|----------------|
| NIH3T3: 3.2 | 3.6 | 417 | 58.4 |
| NIH3T3:3.2DAF | 70 | 614 | 68.5 |
| NIH3T3:FGF4 | 38 | 861 | 73 |
| DMI-1 | 1.5 | 451 | 48.1 |

* a cells were plated in soft agar as described in the experimental procedures. Colonies were counted at the end of 3 weeks.
* b arithmetical mean of four parallel experiments
* c standard deviation

FIG. 10. Painting of NIH3T3 cells with GPI-anchored FGF3. A, high level expression of the FGF3-DAF chimera. COS-1 cells were transfected with 10 µg of pPKC3.2 or pPKC3.2-DAF-cDNA and cultured for 48 h. FGF3- and FGF3-DAF-related proteins were affinity purified on a heparin-Sepharose column (see “Experimental Procedures”). Samples of cell extracts and purified fractions were separated on a 12.5% SDS-PAGE, and FGF3 proteins were detected by immunoblotting with a rabbit polyclonal antiserum against FGF3 and the ECL technique. B–E, morphologies of NIH3T3 cells after incorporation of FGF3-DAF protein into the cell surface. Cells grown on a dish were washed three times with PBS before painting. NIH3T3 cells were left untreated (B), were incubated with ~10 µg of FGF3 protein (C), or were incubated with 2 µg of FGF3-DAF protein (D and E) in serum-free medium plus 0.01% Triton X-100 for 2 h at 37°C. Panel D shows 80% confluent cells, whereas in panel E the cells are confluent. Representative fields are shown.
A possible explanation for the effect of heparin on DMI cells could be that FGF3 binds with low affinity to the FGFR expressed on NIH3T3 cells, thus allowing heparin to more effectively compete it from the cell surface. Hence, the amount of FGF3 could easily fall below a threshold required for activation of the FGFR signal pathway. Alternatively, the cell surface HSPGs may facilitate a different configuration of FGF3 with the FGFR receptor. However, the fact that FGF3 attaches via a GPI anchor promotes transformation of NIH3T3 cells in the presence of an excess of heparin argues against such a mechanism.

The remarkable finding in our study is that the attachment of the GPI anchor confers to FGF3 a strong transforming activity very similar to those of FGF4. This result suggests that the localization of FGF3 at the cell surface is in fact essential for its transforming activity. FGF3 purified from DMI cells exhibits a mitogenic activity on NIH3T3 cells, which depends on the presence of heparin, but the soluble growth factor is not able to induce a transformed phenotype. The same is true for FGF3 purified from transfected COS-1 cells. However, GPI-FGF3 purified from transfected COS-1 cell membranes is transforming, suggesting that only the clustered ligand is able to produce a transforming effect on NIH3T3 cells. Therefore, the heparin sensitivity of FGF3 transformation may not only be due to a change in the local concentration and quantity to keep the ligand concentration above a certain threshold, but may be due to directing the ligand in a specialized area of the plasma membrane.

GPI anchored proteins are often concentrated in specialized membrane domains called rafts that are rich in cholesterol, glycosphingolipids, and lipid-anchored membrane proteins. Some of these membrane domains interact with caveolae that appear to be specialized membrane domains with specific functions (34, 35). Some tyrosine kinase receptors appear to be present in caveolae and juxtaposed to a pre-assembled mitogen-activated protein (MAP) kinase module (34, 36). Similar the presence of FGF receptors in caveolar-like structures has been reported (37). Glycans are a family of six exclusively heparan sulfate-substituted proteoglycans, that are linked to membrane lipids via a GPI anchor and may locate FGFs into such specialized microdomains. At least glypicans-1 and glypican-4 are expressed on most adult tissues and are also expressed by NIH3T3 cells and DMI cells (38). An excess of heparin would then compete with glycan for FGF3 binding, and displace the growth factor as a soluble heparin-FGF3 complex into the supernatant reducing dramatically the concentration of FGF3 in caveolar-like structures. A recent report demonstrated a very interesting connection between tumor progression and glypican-1. This study showed that glypican-1 expression is significantly increased in pancreatic tumors and that antisense glypican-1 inhibited the mitogenic response of cultured pancreatic tumor cells to FGF2 and heparin-binding EGF-like growth factor (39, 40). Using GPI-tagged FGFs will be a useful instrument to determine the importance of caveolae in FGF signaling and may be helpful in generating growth factors with distinct biological activities.

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