Identification of Glypican as a Dual Modulator of the Biological Activity of Fibroblast Growth Factors*

(Received for publication, September 6, 1996, and in revised form, February 14, 1997)

Dafna Bonneh-Barkay‡, Meir Shlissel§, Bluma Berman‡, Ester Shaoul‡, Arie Admon‡, Israel Vlodavsky§, David J. Carey®, Vinod K. Asundi‡, Ronit Reich-Slotky‡, and Dina Ron‡

From the ‡Department of Biology, Technion-Israel Institute of Technology, Haifa 32000, Israel, the §Department of Oncology, Hadassah-Hebrew University Hospital, Jerusalem 91120, Israel, and the ©Geisinger Clinic, Sigfried and Janet Weis Center for Research, Danville, Pennsylvania 17822

Heparan sulfate moieties of cell-surface proteoglycans modulate the biological responses to fibroblast growth factors (FGFs). We have reported previously that cell-associated heparan sulfates inhibit the binding of the keratinocyte growth factor (KGF), but enhance the binding of acidic FGF to the KGF receptor, both in keratinocytes, which naturally express this receptor, and in rat myoblasts, which ectopically express it (Reich-Slotky, R., Bonneh-Barkay, D., Shaoul, E., Berman, B., Svahn, C. M., and Ron, D. (1994) J. Biol. Chem. 269, 32279–32285). The proteoglycan bearing these modulatory heparan sulfates was purified to homogeneity from salt extracts of rat myoblasts by anion-exchange and FGF affinity chromatography and was identified as rat glypican. Affinity-purified glypican augmented the binding of acidic FGF and basic FGF to human FGF receptor-1 in a cell-free system. This effect was abolished following digestion of glypican by heparinase. Addition of purified soluble glypican effectively replaced heparin in supporting basic FGF-induced cellular proliferation of heparan sulfate-negative cells expressing recombinant FGF receptor-1. In keratinocytes, glypican strongly inhibited the mitogenic response to KGF while enhancing the response to acidic FGF. Taken together, these findings demonstrate that glypican plays an important role in regulating the biological activity of fibroblast growth factors and that, for different growth factors, glypican can either enhance or suppress cellular responsiveness.

Proteoglycans are proteins bearing glycosaminoglycan side chains that exist in the extracellular matrix and on the surface of many cell types. These molecules are thought to play an important role in cell growth, morphogenesis, and cancer (1, 2). The most abundant proteoglycans are those that bear glycosaminoglycan chains consisting of heparan sulfate (HS).1 Heparan sulfate proteoglycans (HSPGs) interact with a variety of heparin-binding proteins such as extracellular matrix components and growth factors (1–3). Studies in recent years have strongly indicated that HSPGs are important modulators of the activity of heparin-binding growth factors (3), an issue that has been particularly well studied for fibroblast growth factors (FGFs).

FGFs constitute a large family of polypeptides that are important in the control of cell growth and differentiation and play a key role in oncogenesis and developmental processes including limb formation, mesoderm induction, and neuronal development (4). FGFs elicit their biological activities by interaction with four distinct cell-surface tyrosine kinase receptors (FGFR1–FGFR4) that display overlapping affinities for the various FGFs (5). Several members of the receptor family also exist in alternatively spliced forms that display altered ligand binding properties (5). For example, the KGF receptor (KGFR) is a splice variant of FGFR2. Whereas FGFR2 interacts with αFGF and bFGF, but not with KGF, KGFR binds αFGF and KGF and exhibits a significantly reduced affinity for bFGF (6).

Heparan sulfates or heparin can modulate the activities of FGFs by several mechanisms. They can stabilize FGFs by protecting them from proteolysis and thermal denaturation (7, 8). They can also increase the affinity of FGFs for their signaling receptors (8–10) and facilitate receptor dimerization and subsequent signaling (10–13). On the other hand, these molecules can also inhibit the activities of FGFs (14). The mechanisms by which HS exert these multiple effects are not very well understood.

The modulatory effects of the low affinity binding sites were studied mainly with HS extracted from cells or with heparin, which shares structural similarity with HS and thus can mimic the action of cell- or matrix-associated HSPGs (15). Because the level of expression of HSPGs and the ability of cells to synthesize HS side chains of a defined structure are developmentally regulated (16–18), it is critical to identify the core proteins bearing such modulatory side chains, to elucidate whether the cores influence the structure and function of the glycosaminoglycans attached to them, and to determine the structural requirements for growth factor interaction with the HS moiety. Until now, most of the attempts were concentrated on the identification of native HSPGs that modulate interaction of bFGF with FGFR1. Perlecan, the large basal lamina proteoglycan, was identified as a major candidate for the bFGF low affinity accessory receptor (19). In addition, syndecans and glypican can either inhibit or stimulate bFGF/FGFR1 interactions and signaling depending on the cell type in which they are expressed or their level of expression (14, 20, 21). Little is known about the identity of HSPGs that bind and modulate the activities of other members of the FGF family.

In a previous study, we reported that cell-associated HSPGs

---

1 The abbreviations used are: HS, heparan sulfate; HSPG, heparan sulfate proteoglycan; FGF, fibroblast growth factor; FGF2, fibroblast growth factor receptor; αFGF, acidic fibroblast growth factor; βFGF, basic fibroblast growth factor; KGF, keratinocyte growth factor; KGFR, keratinocyte growth factor receptor; FCS, fetal calf serum; PAGE, polyacrylamide gel electrophoresis; GAG, glycosaminoglycan; HPLC, high pressure liquid chromatography.

This paper is available online at http://www-jbc.stanford.edu/jbc/12415
exert a differential effect on the binding of KGF and aFGF to KGFR (22), which binds both growth factors equally well (23). Thus, treatment of cells with a metabolic inhibitor of sulfation or with HS-degrading enzymes reduced the binding of aFGF to KGFR, but enhanced the binding of KGF. Addition of heparin reversed the effect (22). This differential effect was observed both in keratinocytes, which naturally express KGFR, and in the rat myoblast cell line L6E9, which ectopically expresses this receptor (22). This study was carried out to identify the proteoglycan that may be responsible for this effect. Here, we report the purification of such a proteoglycan from rat myoblasts and its identification as glypican (24). We show that glypicans exert either a stimulatory or an inhibitory activity that is dependent on the type of growth factor.

EXPERIMENTAL PROCEDURES

Materials—Recombinant aFGF, bFGF, and KGF were produced in bacteria as described (25, 26). Bovine brain aFGF was purchased from R&D Systems. Carrier-free Na125I and Na2124I were purchased from DuPont NEN. Fetal and newborn calf sera and media were purchased from Cell Technologies, Inc. Heparin and bovine testicular alkaline phosphatase activities were purchased from Sigma. Chondroitinase ABC was from Seikagaku, and heparinases I and III were from Ibex Technologies. Fibronectin was purchased from Upstate Biotechnology, Inc.

Tissue Culture—The rat myoblast cell line L6E9 and L6E9 cells transfected with KGFR (27) were grown in Dulbecco’s modified Eagle’s medium containing 10% FCS. Balb/MK cells were grown in low calcium medium containing 5 ng/ml epidermal growth factor and 10% dialyzed FCS as described previously (25). The lymphocyte cell line BaF3 transfected with mouse FGFR1 (designated F32 (29)) was grown in RPMI 1640 medium supplemented with 10% FCS and 10% conditioned medium from WEHI-3B cells (30).

Construction and Production of Soluble Human FGFR1—A soluble extracellular domain of the short isoform of human FGFR1 (hR1 (31)) was cloned into the ApTag vector to produce an in-frame fusion of hR1 with secreted placentical alkaline phosphatase (32). This plasmid was cotransfected with the selectable NeoR marker into NIH/3T3 cells. Conditioned medium from G418-resistant colonies was screened for alkaline phosphatase activity (32). The clone that produced the highest level of activity was expanded and used to purify the hR1/alkaline phosphatase fusion protein using bFGF affinity chromatography.

Radioiodination of HSPGs and FGFs—Purified glypican and FGFs were radioiodinated using chloramine T (33) as described previously (27). Radiolabeled glypicans were separated from free iodine by chromatography on DEAR-Sephadex, and radiolabeled FGFs were purified by heparin-Sepharose affinity chromatography. Specific activities of iodinated HSPGs and FGFs were calculated from the radioactivity incorporated and the protein concentration determined by either standard chemistry on an Applied Biosystems sequencer (Model 476A) or by SDS-PAGE and autoradiography.

Enzymatic and Chemical Deglycosylations—Enzymatic deglycosylation was carried out in Dulbecco’s phosphate-buffered saline containing 125I-HSPG and 0.5 unit/ml heparinases I and III or chondroitinase ABC. Incubation was carried out for 2 h at 37 °C. Deaminative scission of HS with HNO2 was performed as described (38). Anhydrous trifluoromethanesulfonic acid was used to strip the peripheral sugars (39).

Isolation of GAG Side Chains—The core protein of the HSPG was digested with a mixture of endo-H and peptidease K (0.5 mg/ml) in 0.4 M NaCl and 0.05 M Tris (pH 7.5) for 1 h. Digestion was complete, a parallel incubation was carried out in the presence of radioiodinated HSPG, and digestion was monitored by SDS-PAGE and autoradiography. The GAG side chains were then separated from the protein and degradation products by DEAE-Sepharose chromatography.

Protein Sequencing—Affinity-purified HSPG was digested with modified trypsin (Promega). The peptides were loaded onto DEAE-Sepharose in the presence of 0.2 N NaCl to absorb GAG-containing peptides. Non-absorbed peptides were resolved by reverse-phase HPLC on a 1-mm diameter Vydac C18 column. The peptides were sequenced using standard chemistry on an Applied Biosystems sequencer (Model 476A). Mass spectrometry was done using a matrix-assisted laser desorption/ionization-time-of-flight mass spectrometer (Fisons Instrument). Isolated peptides were identified by measuring the radioactivity in a liquid scintillation counter, by SDS-PAGE and autoradiography, or by safranin O staining (36). Samples containing HSPGs were identified following nitrous acid deamination and solid-phase assay on cationic nylon (Zeta-probe) as described (37). Fractions containing HSPGs were pooled, diluted, and the remaining salt concentration to 0.2 M NaCl, and applied to a 4-mL aFGF affinity column. The column was extensively washed with 0.2 M NaCl, and elution was performed by a stepwise increase in the NaCl concentration (see below).

Enzymic and Chemical Deglycosylation—Enzymic deglycosylation was carried out in Dulbecco’s phosphate-buffered saline containing 125I-HSPG and 0.5 unit/ml heparinases I and III or chondroitinase ABC. Incubation was carried out for 2 h at 37 °C. Deaminative scission of HS with HNO2 was performed as described (38). Anhydrous trifluoromethanesulfonic acid was used to strip the peripheral sugars (39).

RESULTS

Mode of Association of the Modulatory HSPG with L6E9 Cells—HSPGs exist either as integral or peripheral membrane proteins or as part of the extracellular matrix (1, 42). To determine the mode of association of the HSPG with parental L6E9 cells, we treated L6E9 cells expressing KGFR (designated L6/KGFR cells) with increasing concentrations of salt, a condition that is known to remove peripheral membrane proteins. L6/KGFR cells were then assayed for binding of radioiodinated aFGF and KGF. We reasoned that if the HSPG is salt-extractable, its removal should differentially affect the binding of aFGF and KGF to KGFR. As shown in Fig. 1, extraction of L6/KGFR cells with increasing salt concentrations resulted in a progressive reduction of the binding of aFGF to both low (Fig. 1A) and high (Fig. 1B) affinity receptors. In samples that were extracted at 1.5 M NaCl, the binding of aFGF to low and high affinity receptors was reduced by 60 and 80%, respectively. By contrast, salt extraction enhanced the binding of KGF to KGFR by up to 1.6-fold (Fig. 1B), whereas little or no effect was
observed with respect to binding to low affinity receptors (Fig. 1A). These results are in accordance with our previous observations obtained following treatment of L6/KGFR cells with heparan sulfate-degrading enzymes or with a metabolic inhibitor of sulfation (22) and further suggest that the differential effects on the binding of aFGF and KGF to KGFR is mediated by a peripheral membrane HSPG. The lack of reduction of the binding of KGF to low affinity receptors in the salt-extracted cells is probably due to the presence of non-heparan sulfate-binding sites, which account for >90% of the low affinity sites for KGF in L6E9 cells (22).

**Purification of the Peripheral HSPG**—The putative HSPG was purified from salt extracts of parental L6E9 cells that were metabolically labeled with [35S]sulfate. Purification was carried out by anion-exchange chromatography on DEAE-Sephacel followed by affinity chromatography on an aFGF column. The elution profile from DEAE-Sephacel is shown in Fig. 2A. Over 99% of the material was bound to the column as judged by the negligible amount of radioactivity in the flow-through fraction, and the material eluted at salt concentrations of up to 0.4 M NaCl. A major peak of [35S]eluted at >0.6 M NaCl. This peak contained predominantly heparan sulfates as judged by its sensitivity to deaminative cleavage with nitrous acid (Fig. 2A). The eluted material migrated on SDS-PAGE as a broad band of ~200 kDa (Fig. 2A, inset). The fractions containing heparan sulfates were pooled and subjected to aFGF affinity chromatography. About 80% of the pooled material bound to the column and could be eluted by 0.75–1 M NaCl (Fig. 2B). SDS-PAGE and silver staining of the eluted material revealed the presence of a broad high molecular mass band similar to that observed with the [35S]-labeled DEAE fractions (Fig. 2C). The material purified by aFGF affinity chromatography also bound to bFGF and KGF columns (data not shown). This ability to interact with all three growth factors is in agreement with the previously described ligand binding characteristics of L6E9 HSPG (22).

**Chemical Characterization of Purified Proteoglycan**—Enzymatic and chemical deglycosylations were carried out to characterize the GAG side chain of the purified material and to determine the molecular mass of the core protein. To increase the sensitivity of detection, the core protein was radiolabeled. Fig. 3A shows the results of enzymatic deglycosylation carried out with heparinase I/III or chondroitin ABC treatment. Heparinase, which specifically cleaves heparan sulfates, shifted the molecular mass of the broad band to a single band of ~64 kDa (Fig. 3A, lane 3). No shift in molecular mass was detected following treatment with chondroitinase ABC of either the intact HSPG or the preparation that had been treated with heparan sulfate-degrading enzymes (Fig. 3A, lanes 2 and 4).

Fig. 3B shows the results of chemical deglycosylation. Deaminitive cleavage with nitrous acid resulted in a shift in the molecular mass of the HSPG that was similar to that observed following treatment with heparinase (Fig. 3B, lane 2). Treatment with trifluoromethanesulfonic acid further shifted the molecular mass of the HSPG to 54 kDa (Fig. 3B, lane 3). We conclude that the purified HSPG does not carry chondroitin sulfate and that heparan sulfates account for about two-thirds of the mass of the protein. The results obtained with trifluoromethanesulfonic acid indicate that other types of glycosylations account for ~10 kDa of the molecular mass.

**Identification of the Purified HSPG as Glypican**—The purified HSPG was digested with modified trypsin; GAG-containing peptides were removed by absorption to DEAE-Sephacel; and GAG-free peptides were resolved by reverse-phase HPLC. Analysis of two peptides gave the sequences LSDVPQAEISGEHLR and QAEALRPFGDAPR. A search in the GenBank™ using the Blast program revealed that the above sequences are identical to residues 46–60 and 195–207 of rat glypican, respectively (24). It is noteworthy that even though the peptide encompassing residues 46–60 contains the consensus sequence...
for GAG attachment (24), this peptide is apparently not glycosylated. Mass spectrometry of several additional peptides confirmed that the purified HSPG is glypicanc (data not shown). Furthermore, the purified HSPG was immunoreactive with antibodies directed against bacterially expressed rat glypican, whereas no recognition was observed using antibodies against syndecan-1 and fibroglycan (Fig. 4).

**Glypican Modulates Biological Activities of FGFs**—It is well established that heparin potentiates the binding of FGFs to FGFR1 and is required for receptor-mediated signaling (8–13). We therefore compared the ability of affinity-purified glypican and heparin to promote FGF receptor binding and mitogenic activity. Binding of bFGF and aFGF to FGFR1 was assayed in a cell-free system utilizing a soluble extracellular domain of human FGFR1 fused to alkaline phosphatase. As shown in Fig. 5, glypican augmented the binding of bFGF and aFGF to FGFR1 at concentrations as low as 10 and 25 ng/ml, respectively. Quantitation of the results from several experiments showed that both glypican and heparin gave a similar 4–6-fold augmentation of bFGF binding, whereas heparin was somewhat more effective than glypican in stimulating the binding of aFGF. High concentrations of glypican (but not heparin) inhibited the binding of aFGF, but did not decrease the binding of bFGF. Similar results were obtained using different preparations of affinity-purified glypican as well as in binding assays performed with Chinese hamster ovary mutant cells that are defective in heparan sulfate synthesis and that ectopically express FGFR1 (data not shown) (43).

GAGs derived from glypican following digestion of the core protein were stimulatory for FGF binding, whereas digestion with heparinase abolished activity (see Fig. 5). These findings establish that the heparan sulfate moiety of glypican rather than the core protein is responsible for the modulation of growth factor-receptor interaction. Since heparinase I cleaves heparan sulfates in sulfate-rich regions (44), it is likely that such regions are responsible for the observed effects of glypican.

We next examined whether glypican-derived GAGs can promote the mitogenic response of cells to FGFs. Since Chinese hamster ovary mutant cells transfected with FGFR1 do not display a response to FGFs even in the presence of heparin (10), we have utilized the BaF3 lymphocytic cell line, which is both heparan sulfate- and FGFR-negative and engineered to express FGFR1 (designated F32 cells (29)). These cells are dependent on interleukin-3 for growth and can be relieved from interleukin-3 dependence in the presence of bFGF and heparin. Heparan sulfate derived from glypican acts like heparin to strongly stimulate the mitogenic response of F32 cells to bFGF, whereas it has no effect when added without the growth factor (Fig. 6).

The ability of glypican to modulate the interaction of aFGF and KGF with KGFR was examined utilizing Balb/MK cells, which express native KGFR. In this cell line, heparin inhibits the biological activity of KGF and potentiates that of aFGF (25). As shown in Fig. 7, both glypican-derived heparan sulfate and heparin augmented the mitogenic activity of aFGF over a similar concentration range (panel A). In contrast, glypican-derived HS, like heparin, inhibited the mitogenic response of Balb/MK cells to KGF (panel B), whereas it had no effect on
can (50); K-glypican (51); and dally members: human and rat glypican (24, 47); rat cerebro-
linkage (46). The glypican family includes several closely re-
to the plasma membrane by a glycosylphosphatidylinositol
families of cell-surface proteoglycans. While the syndecans con-
tivity of a given ligand. Because FGFs and their receptors
concentration, can either restrict or promote the biological ac-
tivity involving cell-associated HSPGs. In such a
HSPG. The dual activity of glypican points to a possible regu-

can accounts for 80% of the high salt-extracted proteoglycans.
The remaining 20% were inactive in modulating FGF receptor
binding and were not further characterized (data not shown).
We also observed that 40% of the heparan sulfate-binding
sites for FGFs were retained on cells following salt extraction
(see Fig. 1). Because treatment of L6E9 cells with chloride- or
heparan sulfate-degrading enzymes reduced the level of hepa-
sulfate-binding sites for FGFs by 95% (22) and since the
effect of these treatments on binding of aFGF and KGF to
KGFR was similar to that observed following salt extraction, it
may be the case that the remaining 40% represent the lipid-an-
chored form and as a peripheral membrane proteoglycan most
likely due to cleavage of the lipid anchor by a specific phospho-
lipase (61). The peripheral form of glypican is clearly present in
perlecan and syndecans (14, 19)) and that, like glypici-
dicated that proteoglycans may act as coreceptors for growth
factors. In the case of dally, the ligand was not identified. In the
case of glypican-3, insulin-like growth factor II was suggested
as a putative ligand (50). This work provides evidence that
glypican is involved in the regulation of cellular growth by
modulating the biological activities of members of the FGF
family. Such a role is supported by the reported similarity in
the cellular and tissue distribution of glypican, FGFs, and their
receptors (6, 27, 31, 51, 53–60).
Glypican exists on the surface of cells both as a lipid-an-
chored form and as a peripheral membrane proteoglycan most
likely due to cleavage of the lipid anchor by a specific phospho-
lipase (61). The peripheral form of glypican is clearly present in
L6E9 cells since it could be extracted at high salt concentra-
tions in the absence of detergents. In fact, calculation of the
relative amount of glypican in the extracts revealed that glypi-
can accounts for 80% of the high-salt-extracted proteoglycans.

Glypican-3 and Dally have been recently implicated in the
control of cellular growth. Mutations in Dally affect cell divi-
sion and produce morphological defects in certain tissues of the
fly, and mutations in glypican-3 are thought to cause the Simp-
son-Golabi-Behmel over-growth syndrome (50, 52). It was sug-
gested that proteoglycans may act as coreceptors for growth
factors. In the case of dally, the ligand was not identified. In the
case of glypican-3, insulin-like growth factor II was suggested
as a putative ligand (50). This work provides evidence that
glypican is involved in the regulation of cellular growth by
modulating the biological activities of members of the FGF
family. Such a role is supported by the reported similarity in
the cellular and tissue distribution of glypican, FGFs, and their
receptors (6, 27, 31, 51, 53–60).
Glypican exists on the surface of cells both as a lipid-an-
chored form and as a peripheral membrane proteoglycan most
likely due to cleavage of the lipid anchor by a specific phospho-
lipase (61). The peripheral form of glypican is clearly present in
L6E9 cells since it could be extracted at high salt concentra-
tions in the absence of detergents. In fact, calculation of the
relative amount of glypican in the extracts revealed that glypi-
can accounts for 80% of the high-salt-extracted proteoglycans.

Glypicans (16) and syndecans (45) represent the two major
families of cell-surface proteoglycans. While the syndecans con-
tain a membrane-spanning domain, the glypicans are anchored
to the plasma membrane by a glycosylphosphatidylinositol
linkage (46). The glypican family includes several closely re-
lated members: human and rat glypican (24, 47); rat cerebro-
glycan (48); rat OCI-5 (49) and its human homologue, glypi-
can-3 (50); K-glypican (51); and Drosophila Dally (52). Both

![FIG. 6. Effect of glypican on the mitogenic response of BaF3/FGFR1 cells to bFGF. Shown is the [3H]thymidine incorporation into BaF3 cells that were incubated with 5 ng/ml bFGF and the indicated concentrations of heparin or glypican-derived GAGs (Gly-HS). Each point is the mean value of triplicate samples. The results are representative of at least three different experiments.](Image)

![FIG. 7. Differential effect of glypican on the mitogenic response of keratinocytes to KGF and aFGF. Increasing concentrations of glypican-derived GAGs (Gly-HS) or heparin were added to serum-starved Balb/MK cells along with 5 ng/ml aFGF (A) or 2 ng/ml KGF (B) or without the addition of growth factors (C). [3H]Thymidine incorporation was determined as described under “Experimental Procedures.” 100% cpm were 2000 for aFGF (A) and 29,750 for KGF (B). Count/min in the absence of growth factors and in the presence of glypican-derived GAGs or heparin were ~200 (C). These results are representative of at least four different experiments.](Image)
regulatory role for glypican in the control of a variety of biological processes in which FGFs are implicated, including cellular growth and differentiation. Because glypican is a prototype of a family of HSPGs, it is likely that the other family members will play a similar role in modulating the biological activities of FGFs or other heparin-binding growth factors. 

Acknowledgments—We thank Drs. Dan Cassel and Gera Etan for critical review of this manuscript and Dr. Gera Neufeld for stimulating discussions.

REFERENCES

1. Hardingam, T. E., and Fong, A. J. (1992) FASEB J. 6, 861–870
2. Ruoslahti, E. (1989) J. Biol. Chem. 264, 13369–13372
3. Ruoslahti, E., and Yamaguchi, Y. (1991) Cell 64, 867–869
4. Basilico, C., and Moscatelli, D. (1992) Adv. Cancer Res. 59, 115–165
5. Johnson, D. E., and Williams, T. (1993) Adv. Cancer Res. 60, 1–41
6. Miki, T., Bottero, D. P., Fleming, T. P., Smith, C. L., Burgess, W. H., Chan, A. M.-L., and Aaronson, S. A. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 246–250
7. Saksela, O., and Rifkin, D. B. (1990) J. Cell Biol. 110, 767–775
8. Vlodavsky, I., Miao, H.-Q., Medalion, B., Danagher, P., and Ron, D. (1996) Cancer Metastasis Rev. 15, 177–186
9. Rognoni, M., Minzak, A., DeFeo, P., Bellotta, P., Basilio, C., Rifkin, D. B., and Moscatelli, D. (1994) J. Biol. Chem. 269, 3976–3984
10. Rapraeger, A. C., Guimond, S., Krufta, A., and Olinw, B. B. (1994) Methods Enzymol. 245, 219–245
11. Spivak-Kroizman, T., Lemmon, M. A., Dikic, I., Landbury, J. E., Pinchasi, D., Huang, J., Jaye, M., Crumley, G., Schlessinger, J., and Lax, I. (1994) Cell 79, 1015–1024
12. Pantoliano, M. W., Horlick, R. A., Van-Dyk, D. E., Tobery, T., Wetmore, D. M., Lear, J. D., Nahapetian, A. T., Bradley, J. D., and Sisk, W. P. (1994) Biochemistry 33, 10229–10234
13. Schlessinger, J., Lax, I., and Lax, L. (1995) Cell 83, 357–360
14. Mal, I., Klenius, K., Miettinen, H. M., and Jalkanen, M. (1993) J. Biol. Chem. 268, 24215–24222
15. Bottaro, D. P., Rubin, J. S., Ron, D., Finch, P., Florio, C., and Aaronson, S. A. (1990) J. Biol. Chem. 265, 12767–12770
16. Karthikeyan, I., Maurel, P., Gallagher, J. T., and Turnbull, J. E. (1994) J. Biol. Chem. 269, 18881–18885
17. Narambe, V., Ford, D., Wildschut, J., and Bartlett, P. F. (1993) Science 260, 103–106
18. Aviezer, D., Hecht, M., Safran, M., Eisinger, I., and Yaron, A. (1994) Cell 79, 1005–1018
19. Aviezer, D., Levy, E., Safran, M., Svanh, C., Buddecke, E., Schmidt, A., David, G., Vlodavsky, I., and Yaron, A. (1994) J. Biol. Chem. 269, 111–124
20. Steinfeld, R., Van Der Berghe, H., and David, G. (1990) J. Cell Biol. 133, 405–416
21. Reich-Slotky, R., Donahue, A., Safran, M., Gruberg, L., and Ron, D. (1994) J. Biol. Chem. 269, 32279–32285
22. Bottaro, D. P., Rubin, J. S., Ron, D., Finch, P., Florio, C., and Aaronson, S. A. (1990) J. Biol. Chem. 265, 12767–12770
23. Karthikeyan, L., Maurel, P., Gallagher, J. T., and Turnbull, J. E. (1994) J. Biol. Chem. 269, 18881–18885
24. Kupce, V., Ford, D., Wildschut, J., and Bartlett, P. F. (1993) Science 260, 103–106
25. Gitay-Goren, H., Sofer, S., Vlodavsky, I., and Neufeld, G. (1992) J. Biol. Chem. 267, 6993–6998
26. Ron, D., Reich, R., Chedid, M., Lengel, C., Cohen, O. E., Chan, A. M.-L., Neufeld, G., Miki, T., and Tronnick, S. R. (1995) J. Biol. Chem. 268, 5388–5394
27. Weissman, B., and Aaronson, S. A. (1983) Cell 32, 599–606
28. Orntz, D. M., Yaron, A., Flanagan, J. G., Svanh, C. M., Levi, E., and Leder, P. (1992) Mol. Biol. Cell. 12, 240–247
29. Treacy, D. J., Mored, P. A., Herberman, R. B., and Sakurai, M. (1991) Cancer Res. 51, 4355–4359
30. Eisenman, A., Ahn, J. A., Graziani, G., Tronnick, S. R., and Ron, D. (1991) Oncogene 6, 1195–1202
31. Flanagan, J. G., and Leder, P. (1990) Cell 63, 185–194
32. Hunter, W. M., and Greenwood, F. C. (1962) Nature 194, 495–496
33. Yuen, S. (1991) Methods Enzymol. 191, 91–95
34. Wilchek, M., and Miron, T. (1982) Biochem. Int. 4, 629–635
35. Lammi, M., and Tammi, M. (1988) Anal. Biochem. 168, 352–357
36. Rapraeger, A., and Yeaman, C. (1989) Anal. Biochem. 179, 361–365
37. Soneoka, C. J., and Farquhar, M. G. (1991) J. Cell Biol. 114, 1241–1244
38. Edge, A. B., and Sris, R. (1987) J. Biol. Chem. 262, 6893–6898
39. Furdest, R. W., Buttke, D. J., and Barrett, A. J. (1986) Biochem. Biophys. Acta 883, 173–177
40. Reichen-Slotky, R., Shaul, E., Berman, B., Graziani, G., and Ron, D. (1995) J. Biol. Chem. 270, 29813–29818
41. Vlodavsky, I., Bar-Shavit, R., Ishai-Michaeli, R., Bashkin, P., and Faks, Z. (1995) Trends Biochem. Sci. 16, 268–271
42. Yaron, A., Klagsbrun, M., Eelco, J. D., Leder, P., and Orntz, D. M. (1991) Cell 64, 841–848
43. Bondhull, J. E., and Gallagher, J. T. (1991) Biochem. J. 273, 553–559
44. Bernd, M., Kokenyesi, F., Miki, T., Kanke, M., Ine, T., Sring, G. L., and Ron, D. (1991) Proc. Natl. Acad. Sci. U. S. A. 89, 246–250
45. David, G., Lories, V., Decock, B., Andrenne, P., Cossin, J.-J., and Ven den Berghe, S. (1990) J. Cell Biol. 111, 3155–3176
46. Stipp, C. S., Litwack, E. D., and Lander, A. D. (1984) J. Cell Biol. 124, 149–160
47. Filmus, J., Church, G. J., and Buick, R. N. (1988) Mol. Cell. Biol. 8, 4243–4249
48. Plia, G., Rhiannon, M., Hughes, B., McKenzie, A., Baybayyn, P., Chen, E. Y., Huber, R., Neri, G., Cao, A., Forabosco, A., and Schlessinger, D. (1996) Nat.
51. Watanabe, K., Yamada, H., and Yamaguchi, Y. (1995) *J. Cell Biol.* **130**, 1207–1218

52. Nakato, H., Futch, T. A., and Selleck, S. B. (1995) *Development (Camb.)* **121**, 3687–3702

53. Carey, D. J., Stahl, R. C., Asundi, V. K., and Tucker, B. (1993) *Exp. Cell Res.* **206**, 10–18

54. Litwack, E. D., Stipp, C. S., Kumbasar, A., and Lander, A. D. (1994) *J. Neurosci.* **14**, 3718–3724

55. Campos, A., Nunez, R., Koenig, C. S., Carey, D. J., and Brandan, E. (1993) *Eur. J. Biochem.* **216**, 587–595

56. Mertens, G., Cassiman, J.-J., Van den Berghe, H., Vernooylen, J., and David, G. (1992) *J. Biol. Chem.* **267**, 20435–20443

57. Lories, V., Cassiman, J.-J., Van den Berghe, H., and David, G. (1992) *J. Biol. Chem.* **267**, 1116–1122

58. Yazaki, N., Hosoi, Y., Kawahata, K., Miyake, A., Minami, M., Satoh, M., Ohta, M., Kawasaki, T., and Itoh, N. (1994) *J. Neurosci. Res.* **37**, 445–452

59. Gomez-Pinilla, F., van der Wal, E. A., and Cotman, C. W. (1995) *Exp. Neurol.* **133**, 164–174

60. Finch, P. W., Cuhna, G. R., Rubin, J. S., Wang, J., and Ron, D. (1995) *Dev. Dyn.* **203**, 223–240

61. Carey, D. J., and Evans, D. M. (1989) *J. Cell Biol.* **108**, 1891–1897

62. Brunner, G., Gabrilove, J., Rifkin, D. B., and Wilson, L. (1991) *J. Cell Biol.* **14**, 1275–1283

63. Bashkin, P., Neufeld, G., Gitay-Goren, H., and Vlodavsky, I. (1992) *J. Cell. Physiol.* **151**, 126–137
Identification of Glypican as a Dual Modulator of the Biological Activity of Fibroblast Growth Factors
Dafna Bonneh-Barkay, Meir Shlissel, Bluma Berman, Ester Shaoul, Arie Admon, Israel Vlodavsky, David J. Carey, Vinod K. Asundi, Ronit Reich-Slotky and Dina Ron

J. Biol. Chem. 1997, 272:12415-12421.
doi: 10.1074/jbc.272.19.12415

Access the most updated version of this article at http://www.jbc.org/content/272/19/12415

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 63 references, 29 of which can be accessed free at http://www.jbc.org/content/272/19/12415.full.html#ref-list-1