Tyrosine Phosphorylation of Paxillin and Focal Adhesion Kinase during Insulin-like Growth Factor-I-stimulated Lamellipodial Advance*  

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In the current studies, we examined whether focal adhesion kinase (FAK) and paxillin play a role in insulin-like growth factor-I (IGF-I)-stimulated morphological changes in neuronal cells. In SH-SY5Y human neuroblastoma cells, 10 nM IGF-I enhanced the extension of lamellipodia within 30 min. Scanning electron microscopy and staining with rhodamine-phalloidin showed that these lamellipodia displayed ruffles, filopodia, and a distinct meshwork of actin filaments. Immunofluorescence staining identified focal concentrations of FAK, paxillin, and phosphotyrosine within the lamellipodia. Immunoprecipitation experiments revealed that FAK and paxillin are tyrosine-phosphorylated during IGF-I-stimulated lamellipodial extension. Maximal phosphorylation of FAK and paxillin was observed 15–30 min after the addition of 10 nM IGF-I, whereas maximal IGF-I receptor phosphorylation occurred within 5 min. FAK, paxillin, and IGF-I receptor tyrosine phosphorylation had similar concentration-response curves and were inhibited by the receptor blocking antibody aIR-3. These results indicate that FAK and paxillin are tyrosine-phosphorylated during IGF-I-stimulated lamellipodial advance and suggest that the tyrosine phosphorylation of these two proteins helps mediate IGF-I-stimulated cell and growth cone motility. These responses contrast directly with recent reports showing insulin-stimulated dephosphorylation of FAK and paxillin.

Insulin-like growth factor-I (IGF-I) is a key growth factor in fetal development (1, 2), and in vitro, IGF-I is a potent mitogen and promoter of cell motility (1, 3, 4). These effects of IGF-I are mediated by the type I IGF receptor (IGF-IR), a member of the receptor tyrosine kinase family (1). The earliest detectable morphological change induced by IGF-I is the redistribution of the actin cytoskeleton associated with the formation of membrane ruffles (5). Ruffles are rapidly moving membrane protrusions that often extend several micrometers perpendicular to the leading edges of the cell lamella (6, 7). IGF-I-stimulated membrane ruffling involves activation of phosphatidylinositol-3-kinase (8). Ruffling is followed by protrusion of membranes from the ventral surface of the lamella (9).

When the protruding lamellar membranes adhere to specific extracellular matrix (ECM) molecules, adhesion foci form, and the lamellae are stabilized (10, 11). One important family of adhesion receptors in this regard is the integrins, a group of heterodimeric transmembrane proteins that lack intrinsic enzymatic activity (12). Integrin-mediated adhesion to the ECM results in downstream tyrosine phosphorylation of focal adhesion proteins including paxillin and focal adhesion kinase (FAK) (13). These tyrosine phosphorylations help direct integrin-cytoskeletal interaction and assembly of focal adhesion complexes (14). Thus, tyrosine phosphorylation of FAK and paxillin is thought to assist in ECM-stimulated cytoskeletal remodeling and stabilization of adhesions (13, 14). Formation of adhesions on the lamella is necessary not only for lamellar stability but also for continued lamellar advance (10, 11). Actively advancing regions of lamella are known as lamellipodia and mediate cell migration and growth cone translocation (15–17).

In our laboratory, we have been studying the role of FAK and paxillin in the regulation of neuronal morphology. We showed recently that FAK and paxillin are tyrosine-phosphorylated during integrin-mediated cell spreading and neurite formation in human neuroblastoma SH-SY5Y cells (18). Because IGF-I also promotes neurite outgrowth in SH-SY5Y cells (19, 20), we were interested in determining the function of FAK and paxillin in the response of SH-SY5Y cells to IGF-I. We began our investigations by characterizing the morphological effects of IGF-I on SH-SY5Y cells in detail. We found that the initial effect of IGF-I in these cells is to promote the extension of lamellipodia. FAK and paxillin were concentrated in the lamellipodia within focal adhesion-like streaks. We also found that IGF-I stimulates the tyrosine phosphorylation of FAK and paxillin as the lamellipodia advance over the substrate. These findings are discussed in terms of a general model for growth factor-stimulated cell and growth cone motility.

EXPERIMENTAL PROCEDURES

Materials—Laminin/polylysine-coated coverslips were from Collaborative-Becton Dickinson (Bedford, MA). Anti-paxillin monoclonal antibody (mAb) and anti-phosphotyrosine mAb PY20 were from Transduction Laboratories (Lexington, KY). Anti-IGF-I mAb ZA7 and rabbit anti-FAK serum were generously provided by Dr. J. T. Parsons (University of Virginia). Anti-phosphotyrosine mAb 4G10 and chicken anti-IGF-IR antibody were from Upstate Biochemicals, Inc. (Lake Placid, NY). Streptavidin-fluorescein and protein A/G-agarose were from Santa Cruz Biotechnology (Santa Cruz, CA). Horseradish peroxidase-conjugated goat anti-mouse and goat anti-anti antibodies were from Vector Laboratories (Burlingame, CA). Rhodamine-phalloidin was from Molecular Probes (Eugene, OR). Rabbit anti-IGF-IR serum IB was a

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1 The abbreviations used are: IGF-I, insulin-like growth factor-I; IGF-IR, type I IGF receptor; ECM, extracellular matrix; FAK, focal adhesion kinase; mAb, monoclonal antibody; DMEM, Dulbecco's modified Eagle's media.
kind gift from Dr. Lu-Hai Wang (Mount Sinai School of Medicine). Enhanced chemiluminescence reagents were from Amersham Corp. IGF-I was a gift from Cephalon Corp. (West Chester, PA). Dulbecco's modified essential media (DMEM) with high glucose, t-glutamine, and 110 mg/ml sodium pyruvate was from Life Technologies, Inc. and was pH buffered with 3.7% sodium bicarbonate.

Cells—SH-SY5Y human neuroblastoma cells (21) were kindly provided by Dr. Stephen Fisher (University of Michigan). The SH-SY5Y cells were grown in DMEM containing 10% calf serum and maintained at 37°C in a humidified atmosphere with 10% CO2. 18–24 h prior to use, media was replaced with DMEM (serum free).

Scanning Electron Microscopy—SH-SY5Y cells on glass coverslips were fixed for 45 min in cacodylate buffer (100 mM sodium cacodylate, pH 7.2, 120 mM sucrose, and 2 mM CaCl2) containing 0.5% glutaraldehyde. After extensive washing with cacodylate buffer, coverslips were incubated for 15 min in 1% OsO4 in cacodylate buffer. Coverslips were again washed with cacodylate buffer and then dehydrated by successive 5-min incubations in 10, 30, 50, 70, and 90% ethanol. The coverslips were incubated 3 times for 5 min each in 100% ethanol, followed by 5 min in hexamethyldisilazane. Dehydration was completed by drying coverslips in a dessicating chamber. Coverslips were sputter-coated with gold/palladium and imaged using a International Scientific Instruments DS-130 scanning electron microscope.

Immunocytochemistry—All solutions were made up in phosphate-buffered saline, pH 7.2. SH-SY5Y cells on laminin/poly(lysine)-coated coverslips were fixed in 4% paraformaldehyde, permeabilized in 0.15% Triton X-100 plus 1% bovine serum albumin, and incubated for 2 h with 25 μg/ml of one of the following: anti-paxillin mAb, anti-FAK mAb 2A7, anti-phosphotyrosine mAb PY20, or anti-IGF-IR antibody. The coverslips were then incubated for 45 min in 10 μg/ml biotinylated horse- antimouse or goat anti-chicken antibody, followed by 15 min in 5 μg/ml streptavidin-fluorescein or -rhodamine. Staining for actin filaments was accomplished by incubating fixed, permeabilized cells for 15 min with 2 units/ml rhodamine-phalloidin.

Immunoprecipitation and Anti-Phosphotyrosine Immunoblotting—Immunoprecipitation was performed as described previously (18, 22). Briefly, cell lysates were incubated overnight with one of the following: 10 μg/ml anti-FAK polyclonal antibody BC3, 4 μg/ml anti-paxillin mAb, or 1:500 polyclonal anti-IGF-IR antibody IB1. Next, the proteins were immunoprecipitated by mixing lysates for 2 h with protein A/G-agarose. The immunoprecipitated proteins were separated by SDS-polyacrylamide gel electrophoresis and electrophoretically transferred to nitrocellulose. Anti-phosphotyrosine immunoblotting was performed as described previously (18, 22) using 1 μg/ml mAb PY20 and 0.4 μg/ml mAb 4G10, followed by 0.2 μg/ml horseradish peroxidase-conjugated goat anti-mouse mAb. Tyrosine-phosphorylated proteins were visualized with enhanced chemiluminescence reagents.

RESULTS

IGF-I-stimulated Lamellipodial Extension in SH-SY5Y Human Neuroblastoma Cells—Several laboratories including ours have used SH-SY5Y human neuroblastoma cells to characterize the effects of IGFs on neuronal cells (19, 20, 24). These cells grow in serum-free media, allowing analysis of the effects of IGFs in the absence of other growth factors. Furthermore, the SH-SY5Y cells express a relatively high level of the IGF-IR (22, 24, 25). In SH-SY5Y cells, long-term incubation (i.e. 1–3 days) with IGF-I increases neurite length (19, 20), although the mechanism of this effect has not been identified. For this reason, we first sought to perform a detailed characterization of the effect of IGF-I in SH-SY5Y cells.

In preliminary experiments, we found that IGF-I induces the extension of lamellipodia (flattened veil-like membranes). Increased lamellipodial extension was readily observed within 30 min after IGF-I addition (Fig. 1B, arrowheads). The lamellipodia may either be extended as fan-shaped protrusions at the back of the cell as well as from long neurites or from growth cones. Untreated cells appeared to have fewer and smaller lamellipodia than IGF-I-treated cells (Fig. 1A). Membrane ruffles were visible on the lamellipodia as darkened areas. Examination of lamellipodial dynamics by time lapse imaging revealed that lamellipodia are very dynamic, rapidly changing in size and shape, and continually extend and retract on a time scale of several minutes (data not shown).

FIG. 1. IGF-I stimulates the extension of lamellipodia in SH-SY5Y human neuroblastoma cells. Serum-starved SH-SY5Y cells were treated for 30 min with DMEM containing no addition (A) or 10 nM (B) IGF-I. Cells were fixed and imaged by phase contrast microscopy. Arrowheads denote extended lamellipodia. Bar, 25 μm. Results are representative of 10 independent experiments.

Characteristics of Lamellipodia on SH-SY5Y Cells—Because we were interested in the details of lamellipodial morphology, we examined the cells using scanning electron microscopy. Shown in Fig. 2 are representative SH-SY5Y cells treated for 30 min with DMEM alone (A) or DMEM containing 10 nM IGF-I (B–D). Scanning electron microscopy highlighted the dramatic increase in lamellipodial extension in the IGF-I-treated cells. These lamellipodia were roughly 0.1-μm thick, although these images do not allow for exact quantitation. Filopodia can be seen protruding from the edge of the lamellipodia in both the untreated and IGF-I-treated cells. Fig. 2, C and D, show that the lamellipodia are often rich in “ruffles” (6, 7), jagged membranes that protrude up to several micrometers perpendicular to the lamellipodial surface. The ruffles occurred at the leading edges of the lamellipodia and were not found within the cell bodies. These micrographs also suggest that the bodies and neurites of the SH-SY5Y cells do not adhere strongly to the substrate, but that cell-substrate attachment is mediated mostly by the lamellipodia.

Changes in the organization of the actin cytoskeleton are critical for lamellipodial extension (26, 27). We, therefore, analyzed the distribution of actin filaments in the SH-SY5Y cells by staining them with rhodamine-phalloidin. As seen in Fig. 3, this staining highlights filopodia and stress fibers in both control (A) and IGF-I-treated (B) SH-SY5Y cells. Additionally, the IGF-I-treated cells possess a unique brush-like meshwork of actin filaments at the leading edges of lamellipodia.

IGF-I-stimulated Tyrosine Phosphorylation of FAK and Paxillin—We found previously that tyrosine phosphorylation of the focal adhesion proteins FAK and paxillin occurs in SH-SY5Y cells during integrin-mediated spreading and neurite formation (18). Given that lamellipodial advance is dependent on the organization of the integrin cytoskeleton, we were interested in the details of lamellipodial morphology, we examined the cells using scanning electron microscopy. Shown in Fig. 2 are representative SH-SY5Y cells treated for 30 min with DMEM alone (A) or DMEM containing 10 nM IGF-I (B–D). Scanning electron microscopy highlighted the dramatic increase in lamellipodial extension in the IGF-I-treated cells. These lamellipodia were roughly 0.1-μm thick, although these images do not allow for exact quantitation. Filopodia can be seen protruding from the edge of the lamellipodia in both the untreated and IGF-I-treated cells. Fig. 2, C and D, show that the lamellipodia are often rich in “ruffles” (6, 7), jagged membranes that protrude up to several micrometers perpendicular to the lamellipodial surface. The ruffles occurred at the leading edges of the lamellipodia and were not found within the cell bodies. These micrographs also suggest that the bodies and neurites of the SH-SY5Y cells do not adhere strongly to the substrate, but that cell-substrate attachment is mediated mostly by the lamellipodia.
FIG. 2. Characterization of SH-SY5Y cell morphology by scanning electron microscopy. Serum-starved SH-SY5Y cells were treated for 30 min with DMEM containing no addition (A) or 10 nM (B–D) IGF-I. Cells were imaged by scanning electron microscopy. Bars, 10 μm.

FIG. 3. Organization of actin filaments in SH-SY5Y cells. Serum-starved SH-SY5Y cells were treated for 30 min with DMEM containing no addition (A) or 10 nM (B) IGF-I. Actin filaments were localized by staining cells with rhodamine-phalloidin. Bar, 10 μm. Results are representative of four independent experiments.

ment had no effect on the ability of FAK and paxillin to be immunoprecipitated (data not shown). Maximal tyrosine phosphorylation of the IGF-IR β subunit was observed within 5 min (Fig. 4A). In contrast, maximal phosphorylation of FAK and paxillin was evident 15–30 min after addition of IGF-I. Thus, there is a lag of at least 10 min between maximal IGF-IR and FAK/paxillin tyrosine phosphorylation. Although there was a difference between the temporal dependence of these phosphorylations, they had similar concentration dependences, reaching maximum phosphorylation at 1–10 nM IGF-I (Fig. 4B).

To verify that the effects of IGF-I were mediated by the IGF-IR, rather than by IGF-binding proteins (29) or hybrid insulin/IGF receptors (30), we assessed the effect of the IGF-IR receptor blocking antibody aIR-3 (31). In this experiment, paxillin and FAK phosphorylations were assessed as in Fig. 4, A and B, but IGF-IR phosphorylation was examined in whole-cell lysates because the presence of aIR-3 precluded IGF-IR immunoprecipitation. We found that aIR-3 inhibited the IGF-I-stimulated tyrosine phosphorylation of FAK and paxillin (Fig. 4C). In the whole-cell lysates, the IGF-IR β subunit is clearly visible as an approximately 97 kDa band, and as expected, aIR-3 blocked IGF-I stimulation of IGF-IR tyrosine phosphorylation. The addition of aIR-3 also inhibited IGF-I-stimulated lamellipodial extension (data not shown).

Localization of FAK, Paxillin, and Phosphotyrosine in the Lamellipodia of IGF-I-treated SH-SY5Y Cells—To help determine the role of FAK and paxillin tyrosine phosphorylation in IGF-I-stimulated lamellipodial advance, we examined the distribution of these two proteins in IGF-I-treated SH-SY5Y cells. In these experiments, we stained for the proteins using mAbs, followed by a two-step fluorescence amplification procedure. FAK (Fig. 5A) and paxillin (Fig. 5B) were found in the lamellipodia of IGF-I-treated cells. Specifically, these proteins were concentrated within distinct radial streaks, typically 1–3 μm in length. Similar to FAK and paxillin, phosphotyrosine (Fig. 5C) was concentrated in the lamellipodia within short radial streaks. Finally, in contrast to FAK, paxillin, and phosphotyrosine, only diffuse staining for the IGF-IR was observed in IGF-I-treated SH-SY5Y cells (data not shown).

DISCUSSION

In the current studies, we investigated the role of FAK and paxillin in the morphological changes induced in SH-SY5Y cells by IGF-I. We show for the first time that IGF-I promotes the extension of lamellipodia. These lamellipodia were highly motile, flattened protrusions extending from the cell body, neurites, and growth cones. Within the lamellipodia, there was a distinct brush-like meshwork of actin filaments that was concentrated at the leading edges. Along with actin stress fibers, this meshwork of actin filaments appeared to provide structure to the lamellipodia. The lamellipodia also displayed ruffles and membrane ruffling. Similarly, IGF-I has been shown to promote membrane ruffling in KB human epidermoid carcinoma cells (5, 8). Finally, the lamellipodia appeared to be the points of strongest cell-substrate adhesion. These characteristics of lamellipodia in SH-SY5Y cells are essentially identical to those found in migrating fibroblasts (6, 10, 16). Furthermore, these results indicate that lamellipodia confer IGF-I-stimulated cell and growth cone motility in SH-SY5Y cells.
Results are representative of four independent experiments.

In addition to promoting lamellipodial advance, we found that IGF-I stimulated the tyrosine phosphorylation of FAK and paxillin. This observation that IGF-I-stimulated FAK and paxillin tyrosine phosphorylation contrasts with a recent report showing IGF-I-induced dephosphorylation of these proteins in CHO cells (32). However, the reported IGF-I-dependent dephosphorylation occurred in CHO cells transfected with the insulin receptor and does not occur via the IGF-IR. Also, insulin-stimulated FAK and paxillin dephosphorylation occurs in insulin receptor-transfected Swiss 3T3 fibroblasts (33) and CHO cells (34, 35). This is surprising, given the many similarities between IGF-IR and insulin receptor structure and signal transduction (1). We suspect that the difference in the effects of insulin and IGF-I lies in how these growth factors affect cell motility and the cytoskeleton. Specifically, IGF-I-activated FAK and paxillin phosphorylation occurs in parallel with an enhancement of cell motility, whereas insulin-dependent dephosphorylation of FAK and paxillin likely correspond with a decrease in cell motility (14).

Inhibition of FAK and paxillin tyrosine phosphorylation by α1r-3 indicates that IGF-I acted through the IGF-IR. However, our results suggest that IGF-I-stimulated FAK and paxillin tyrosine phosphorylation occur not as a direct signaling event from the IGF-IR but rather subsequent to lamellipodial advance: (a) there was a 10-min lag between maximal FAK/paxillin and IGF-IR tyrosine phosphorylation, which indicates that these phosphorylations occur far downstream of the IGF-IR; and (b) the localization of FAK, paxillin, and phosphotyrosine in the lamellipodia imply that these proteins become phosphorylated as the lamellipodia are extended. Specifically, FAK, paxillin, and phosphotyrosine were concentrated in the lamellipodia within streaks reminiscent of focal adhesions (36, 37). Such focal concentrations of paxillin, FAK, and phosphotyrosine are also found in migrating fibroblasts (13, 38), in the growth cone lamellipodia of mouse retinal neurons (39) and in the lamellipodia of cdc42-injected fibroblasts (40). This pattern of focal staining for paxillin, FAK, and phosphotyrosine in the SH-SY5Y cells suggests that FAK and paxillin are tyrosine phosphorylated as the extending lamellipodia form new adhesions.

Collectively, these results suggest a straightforward sequence of events to explain how IGF-I stimulates FAK and paxillin tyrosine phosphorylation: (a) IGF-I promotes the extension of lamellar membranes; (b) the advancing lamellipodia bind to the ECM; and (c) lamellipodial adhesion leads to FAK and paxillin tyrosine phosphorylation. Because tyrosine phosphorylation of FAK and paxillin phosphorylation are associated with assembly of adhesion foci (14), their phosphorylations may help stabilize the advancing lamellipodia (10, 11). Such a role for FAK and paxillin in lamellipodial advance could explain why FAK expression correlates with cell motility (41–43). Lamellipodial extension is also critical for growth cone migration (16), but at this time, the function of paxillin and FAK in this regard remains to be addressed.

There are many similarities between these effects of IGF-I and those of other motility-enhancing factors. For example, platelet-derived growth factor acts through a receptor that, like the IGF-IR, is a tyrosine kinase (44). Similar to IGF-I, platelet-derived growth factor stimulates the tyrosine phosphorylation of FAK and paxillin (45). Also, both IGF-I and platelet-derived growth factor activate phosphatidylinositol-3-kinase, which helps direct membrane ruffling (8, 46). Therefore, we suspect that, like IGF-I, platelet-derived growth factor induces FAK and paxillin phosphorylation as lamellipodia advance and adhere to the surrounding ECM. For this reason, current investigations in our laboratory are focusing on the connection between phosphatidylinositol-3-kinase and FAK and paxillin tyrosine phosphorylation in IGF-I-stimulated cell motility.

This relationship between lamellipodial advance and FAK and paxillin tyrosine phosphorylation may be a key aspect of tumor cell invasion. Lamellipodial-like protrusions, known as “invadopodia,” mediate cellular invasion by tumor cells (47). These invadopodia contain focal concentrations of paxillin and other tyrosine-phosphorylated proteins at sites of adhesion (48). Furthermore, high levels of FAK expression are found in invasive human tumors (49). Finally, many tumors produce IGF-II (50), which could act through the IGF-IR (1) to promote lamellipodial advance and associated FAK and paxillin tyrosine phosphorylation. In fact, the SH-SY5Y neuroblastoma cells are an excellent model system to investigate this possibility because they secrete IGF-II, which could function by an autocrine mechanism (24) to promote cell motility and tissue invasion.

In summary, we have shown that IGF-I stimulates lamellipodial advance in SH-SY5Y human neuroblastoma cells. In parallel, IGF-I promotes the tyrosine phosphorylation of FAK and paxillin. The tyrosine phosphorylation of FAK and paxillin appears to occur as the lamellipodia advance and form new adhesions. This relationship between growth factor-stimulated lamellipodial advance and FAK and paxillin tyrosine phosphorylation may be a key aspect of cell and growth cone motility as well as tumor cell invasion.

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REFERENCES
1. Hembel, R. E. (1990) Eur. J. Biochem. 190, 445–462
2. Daughaday, W. H. (1992) The Insulin Like Growth Factors, pp. 5–11, Oxford University Press, Oxford
3. Stracke, M. L., Engel, J. D., Wilson, L. W., Rechler, M. M., Liotta, L. A., and Schiffmann, E. (1989) J. Biol. Chem. 264, 21544–21549
4. Doerr, M. E., and Jones, J. J. (1996) J. Biol. Chem. 271, 2443–2447
5. Kadowaki, T., Koyasu, S., Nishida, E., Sakai, H., Takaku, F., Yahara, I., and Kasuga, M. (1996) J. Biol. Chem. 261, 16141–16147
6. Abercrombie, M., Heaysman, J. E. M., and Peagram, S. M. (1970) Exp. Cell Res. 60, 437–444
7. Ridley, A. J. (1994) BioEssays 16, 321–327
8. Kotani, K., Yonezawa, K., Hara, K., Ueda, H., Kitamura, Y., Sakai, H., Asano, A., Chavanieu, A., Calas, B., Grigorescu, F., Nishiyama, M., Waterfield, M. D., and Kasuga, M. (1994) EMBO J. 13, 2313–2321
9. Cheng, T. P. O. (1992) Exp. Cell Res. 203, 25–31
10. Abercrombie, M., Heaysman, J. E. M., and Peagram, S. M. (1971) Exp. Cell Res. 67, 359–367
11. Kolega, J., Shure, M. S., Chen, W.-T., and Young, N. D. (1982) J. Cell Sci. 54, 23–34
IGF-I-stimulated FAK and Paxillin Phosphorylation

12. Hynes, R. O. (1992) Cell 69, 11–25
13. Burridge, K., Turner, C. E., and Romer, L. H. (1992) J. Cell Biol. 119, 893–903
14. Miyamoto, S., Teramoto, H., Coso, O. A., Gutkind, J. S., Burbelo, P. D., Akiyama, S. K., and Yamada, K. M. (1995) J. Cell Biol. 131, 791–805
15. Kleitman, N., and Johnson, M. I. (1996) Cell Motil. Cytoskel. 34, 288–300
16. Abercrombie, M., Heaysman, J. E. M., and Pegrum, S. M. (1970) Exp. Cell Res. 59, 393–398
17. Goldberg, D. J., and Burmeister, D. W. (1986) J. Cell Biol. 103, 1921–1931
18. Leventhal, P. S., and Feldman, E. L. (1986) J. Biol. Chem. 261, 5957–5960
19. Ishii, D. N., and Recio-Pinto, E. (1987) Insulin, Insulin-like Growth Factors, and Their Receptors in the Central Nervous System, pp. 315–348, Plenum Publishing Corp., New York
20. Lavenius, E., Parrow, V., Nånberg, E., and Påhlman, S. (1994) Growth Factors 10, 29–39
21. Izzard, C. S., and Lochner, L. R. (1980) J. Cell Sci. 42, 81–116
22. Matsumoto, K., Matsumoto, K., Nakamura, T., and Kramer, R. H. (1994) J. Biol. Chem. 269, 31807–31813
23. Akasaka, T., Van Leeuwen, R. L., Yoshinaga, I. G., Mihm, M. C., and Byers, H. E. (1995) J. Invest. Dermatol. 105, 104–108
24. Claesson-Welsh, L. (1994) J. Biol. Chem. 269, 32923–32926
25. Chen, W.-T. (1989) J. Exp. Zool. 251, 167–185
26. Mueller, S. C., Yeh, Y., and Chen, W.-T. (1992) J. Cell Biol. 119, 1309–1325
27. Owens, L. V., Xu, L., Craven, R. J., Dent, G. A., Weiner, T. M., Kornberg, L., Liu, E. T., and Cance, W. G. (1995) Cancer Res. 55, 2725–2735
28. Daughaday, W. H. (1990) Endocrinology 127, 1–4

5218