Distinctive Effects of the Carboxyl-Terminal Sequence of the Insulin-Like Growth Factor I Receptor on Its Signaling Functions

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We have shown previously that the extracellular sequences of the human insulin receptor (IR) and the insulin-like growth factor I receptor (IGFR) have an inhibitory effect on protein tyrosine kinase (PTK) activity and on the biological functions of their respective Gag-receptor fusion proteins. To study the role of IGFR carboxyl sequence in modulation of the Gag-IGFR PTK and biological activities, five mutants, CM1, CM2, CM3, CM4, and CM5, containing carboxyl deletions of 17, 27, 47, 67, and 88 amino acids (aa), respectively, were constructed from the parental virus UIGFR encoding the Gag-IGFR. Deletion of up to 27 aa had little effect on the cell-transforming and PTK activities of UIGFR. Deletions of 47 aa in CM3 abolished PTK and transforming activities. Surprisingly, a further deletion of 20 aa in CM4 beyond that in CM3 reactivated the kinase and transforming activities. CM5, containing a deletion of 20 aa beyond that in CM4, had only marginal transforming and PTK activities. We conclude that deletion of the carboxyl region of the Gag-IGFR inactivates, instead of activating as in the case with Gag-IR, its transforming activity and the amino acid sequence 1250 to 1310 is essential for PTK and transforming activities. Analysis of the ability of the full-length IGFR and its mutant receptors described above to associate with phosphatidylinositol 3 kinase indicated that the association required PTK activity and tyrosine phosphorylation of the receptors and correlated well with their transforming activities. The carboxyl 88 aa are not essential for the association.

Protein tyrosine kinase (PTK) receptors share significant amino acid sequence homology in their PTK domains, yet they have distinctive pathways for signal transduction (3, 11). The specificity of signal transduction by PTK receptors may involve sequences in the juxtamembrane region (1, 13, 24), kinase insert region (9), and/or carboxyl-terminal sequences following the PTK domain. Carboxyl-terminal sequences appear to be the most divergent regions among PTK receptors. Deletion of C-terminal sequences has been found to be a common mechanism for activation of proto-oncogenes like *erbB*, *fms*, and *kit* (2, 6, 7). The C-terminal deletions frequently include important autophosphorylation sites.

The role of the C-terminal tail of the human insulin receptor (IR) in signal transduction has been investigated in both rat-1 and Chinese hamster cells (CHO) by deletion of its C-terminal 43 amino acids (aa) (18–20, 27) or by specific mutation of the two tyrosine residues (positions 1316 and 1322) to phenylalanines (26). These studies suggest that the IR C-terminal 43 aa play a positive role in the receptor's metabolic function but have a negative role in its mitogenic activity and have no effect on the receptor's PTK activity.

The IR, the insulin-like growth factor I receptor (IGFR), and the IR-related receptor (IRR) (25) are three closely related PTK receptors. We have previously shown that the extracellular sequences of human IR and IGFR have negative regulatory effects on their tyrosine kinase activities and biological functions (15, 16, 22, 29). Both truncated PTK receptors have constitutively activated PTK and oncogenicity. However, no information regarding the role of the IGFR C terminus in modulation of its PTK activity and signal transduction is available. Even though the PTK domains of IR, IGFR, and

IRR have over 80% identity in their amino acid sequences, their carboxyl sequences are only 19 to 45% homologous (25). This finding implies that their C-terminal sequences might have different roles in modulation of their PTK activity and signal transduction.

In this study we investigated the role of the IGFR C-terminal sequence in modulating biological functions and biochemical properties of the IGFR PTK.

Biological functions of IGFR carboxyl mutants (CM). The IGFR C terminus contains 108 aa residues downstream of the PTK domain (28), 88 aa of which were sequentially deleted from the Gag-IGFR-encoding virus UIGFR that we constructed previously (15). CM1 contains a deletion of the C-terminal 17 aa. CM2 lacks an additional 10 aa (total of 27 aa) which includes a conserved YXXM motif (YAHM) involved in phosphatidylinositol 3 kinase (PI 3-kinase) binding (3). This 10 aa is highly conserved in the human IR C terminus but is absent in the IRR (25, 28). CM3 contains a further deletion of 20 aa (total of 47 aa). This 20-aa sequence (aa 1291 to 1310) is the most divergent region among the C termini of IGFR, IR, and IRR (25, 28). CM4 has a further 20-aa deletion (aa 1271 to 1290) beyond that in CM3, resulting in a total deletion of 67 aa. CM5 lacks the C-terminal 88 aa. The additional 21-aa deletion (aa 1250 to 1270) in CM5 has two consecutive tyrosine residues (1250 and 1251) which are absent in the human IR but are conserved in the IRR (25). This 21-aa sequence is remarkably rich in charged amino acids (Fig. 1).

The plasmids containing the proviral recombinants were transfected into primary chicken embryo fibroblasts (CEF) as described before (15). CM1, CM2, and CM4 induced morphological transformation of primary CEF about 2 weeks after DNA transfection. The transformed CEF became morphologically elongated (fusiform) and refractile (Fig. 2). CM5 caused a similar but less obvious morphological change around 3 to 4 weeks after transfection, whereas CM3 did not induce any

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A.

Stu5: 5' C (IGFR 3803) C AAG ATG AGG CCT TCC TTC 3'

CM1: 5' GC GGA TC CTA(IGFR 4147)/G(IGFR 4095)TT CAT GTG GGC GTA 3'

CM2: 5' GC GGA TC CTA(IGFR 4147)/G(IGFR 4065)AA GCT GGC GCG GAG 3'

CM3: 5' GC GGA TC CTA(IGFR 4147)/G(IGFR 4005)TG TCT GTC GGG CAG 3'

CM4: 5' GC GGA TC CTA(IGFR 4147)/C(IGFR 3945)AT GTT CTC TGG CTC 3'

CM5: 5' GC GGA TC CTA(IGFR 4147)/G(IGFR 3882)AA GGA GAC CTC CCG 3'

B.

L(1230) EIISSIKEEM EPGFREVSFY YSEENKLEP EELDLEPENM ESVPDPSPAS
SSSLPLPDH SGHKAENGPG PGVLVLRASF DERQPYAHMN GGRKNERALP LPQSSTC(1337)

Mutant	Mutation	aa deleted	NA deleted
UIGFR	gag-IGFR	0	0
CM1	-17aa	1321-1337	4096-4046
CM2	-27aa	1311-1337	4066-4046
CM3	-47aa	1291-1337	4006-4046
CM4	-67aa	1271-1337	3946-4046
CM5	-88aa	1250-1337	3883-4046

FIG. 1. Construction of recombinant plasmids. (A) The oligonucleotide primers used to generate DNA fragments containing C-terminal deletions (aa 1250 to 1337) of the human IGFR cDNA. Corresponding nucleotide positions in the original published nucleotide sequence of IGFR (28) are indicated; restriction sites for *StuI* and *BamHI* are underlined. Each primer contains an added termination codon. DNA fragments of CM1 to CM5 were individually made by employing PCR and the synthetic oligonucleotide primers Stu5 (sense primer) and one of the CM primers (antisense primers). The PCR reaction was done as described previously (15). The PCR-derived DNA fragments were each digested with *StuI* and *BamHI* and inserted individually into a previously described plasmid pBUIGFR-II (16), replacing the native IGFR sequence between *StuI* and *BamHI* (from residues 3805 to 4155 in IGFR cDNA). The final plasmid constructs were named pCM1, pCM2, pCM3, pCM4, and pCM5 after each CM primer. The DNA sequence of the PCR fragment containing the mutation in each CM was confirmed by dideoxy sequencing. (B) Summary of deletions in the Gag-IGFR CM. The carboxyl amino acid sequence (aa 1230 to 1337) is shown in the single-letter code. Number and positions of amino acids and nucleotides (NA) deleted are indicated for each mutant. The underlined amino acids denote the upstream boundary of the deletion in each mutant.

recognizable morphological change. The CM were further assessed for their ability to promote anchorage-independent growth of transfected CEF. When compared with parental UIGFR-transfected CEF, CM1- and CM4-transfected CEF had a similar colony formation potency, whereas CM2 seemed to be slightly weaker (fewer and smaller colonies) than UIGFR; CM3 failed to induce colony formation of transfected cells, but CM5 reproducibly caused the formation of clearly recognizable but tiny colonies after a longer latent period (around 4 weeks) (Fig. 2). The CM were injected into the wing webs of 1- to 3-day-old chickens to assay their tumorigenicity. None induced tumors up to 10 weeks after injection. Surprisingly, 60% of CM4-injected chickens (9 of 15) developed tiny tumor nodules (about 5 mm in diameter) which became apparent about 9 to 10 weeks after injection. The tumors grew very slowly. The tumor-bearing chickens were physically healthy and never succumbed to the tumors. The cell-transforming and

weak tumorigenic activity of CM4, with the 67-aa deletion, is surprising since CM3, with a 47-aa deletion, lost all activity. To exclude the possibility that unexpected mutation outside the polymerase chain reaction (PCR)-derived region in pCM4 was responsible for the observed biological activity, the CM4 fragment derived from PCR was confirmed by sequencing and subcloned into the pCM3 backbone, replacing the CM3 fragment. The newly derived pCM4 in a pCM3 background demonstrated same biological and PTK activities as the original pCM4 and has been used ever since. The possibility that a further mutation(s) of CM4 in vivo was responsible for the observed weak tumorigenicity seems unlikely, since viruses recovered from CM4-induced tumor tissues, CM4t, had a similar latency (9 weeks) and incidence (50%) for tumor development. An in vivo-derived tumorigenic mutant would have resulted in a markedly shorter latency (10 to 14 days) and 100% incidence of tumorigenicity as observed in other cases (12, 17). Besides, the CM4t-encoded Gag-IGFR protein had identical sodium dodecyl sulfate-polyacrylamide gel electrophoretic (SDS-PAGE) mobility to that of CM4 (data not shown). Therefore, the observed transforming activity of CM4 indeed reflects the nature of the construct and is not due to additional mutations.

Expression and kinase activity of CM proteins. All five CM-encoded fusion proteins (PCM1 to PCM5) were expressed to a similar level, as determined by metabolic labeling with [³⁵S]methionine (Fig. 3A; see Fig. 5C). Like the parental Gag-IGFR fusion protein P57-75^{UIGFR}, the five CM fusion proteins all have a glycosylated form (the slower-moving broad band) (15, 16). The pulse-chase labeling study showed that the CM3, CM4, and CM5 mutant proteins and the parental UIGFR proteins have a similar turnover half-life of about 2 h (data not shown). Although comparable amounts of fusion proteins were detected by metabolic labeling and immunoprecipitation (Fig. 3A; see Fig. 5C), the immunoblotting signals for PCM4 and PCM5 were drastically lower (Fig. 3B). Apparently, our antiserum α IB is not as efficient in recognizing PCM4 and PCM5 in Western immunoblotting as in immunoprecipitation (Fig. 3B). The in vitro autokinase activities of PCM1 and PCM2 proteins were comparable with that of the parental Gag-IGFR protein P57-75^{UIGFR}, whereas those of PCM3 and PCM5 were barely detectable (Fig. 3C). The lower autophosphorylation signal of PCM4 was apparently due to the fact that less PCM4 was brought down by the α IB antiserum (Fig. 3A). The specific activity of PCM4 is comparable to that of P57-75^{UIGFR}. The degree of tyrosine phosphorylation of CM proteins in infected cells was assessed by immunoblotting with the anti-phosphotyrosine antibody 4G10 (Upstate Biotechnology Inc.). Intracellular tyrosine phosphorylation of the mutant proteins was generally consistent with their in vitro autokinase activities, namely, PCM1, PCM2, and PCM4 were comparable with P57-75^{UIGFR}, whereas PCM3 had a negligible level of tyrosine phosphorylation. Again, the extent of phosphorylation of PCM4, when normalized to its protein amount in the immunoprecipitate, was comparable to that of P57-75^{UIGFR}. However, despite the lack of detectable in vitro autokinase activity, PCM5 had a significantly higher level of tyrosine phosphorylation in the living cells than did PCM3 (Fig. 3D). All five CM proteins were able to form disulfide-bond-linked dimers under nonreducing conditions, as did the parental P57-75^{UIGFR} in the presence of a sulfhydryl alkylating agent, iodoacetamide (10 mM), during the protein extraction (data not shown), indicating that the loss of PTK activity of PCM3 and PCM5 was not due to their failure to dimerize. These data indicate that PTK activity and tyrosine phosphorylation of the Gag-IGFR fusion proteins correlate well with

FIG. 2. CEF transformation by CM. (A) Morphology of normal and transformed CEF on monolayer culture. The preparation and maintenance of CEF were done according to published procedures (10, 15). Avian sarcoma virus UR2-associated helper virus UR2AV and its molecular clone have been described previously (21). CEF were transfected with pCM plasmids containing CM DNAs together with *SacI*-digested pUR2AV. The transfected CEF were transferred at 3- to 4-day intervals and overlaid with soft agar medium after each transfer to enhance the transformed cells. Recovery of viruses from transfected CEF and tumors was done as described previously (16). Viruses recovered from CM-transfected cells were named CM1, CM2, CM3, CM4, and CM5, correspondingly. The pictures were taken 2 weeks after transfection. (B) Colony formation by transfected CEF. Equal numbers of normal or uniformly infected CEF were plated in soft agar medium for assaying the anchorage-independent growth. Pictures were taken 3 weeks after plating.

their transforming activities. The C-terminal 47 aa of human IGFR, unlike the corresponding 43 aa of human IR, is of primary importance for the PTK activity of the Gag-IGFR.

The above results demonstrate that the C-terminal 27 aa (residues 1311 to 1337) are dispensable for the autokinase and cell-transforming activities of the Gag-IGFR fusion protein. However, further deletion of 20 aa (residues 1291 to 1310) completely abolished its PTK activity and the biological function. This suggests that aa 1291 to 1310 are important for the PTK activity of IGFR and likely for its function as well. However, introduction of this deletion into native IGFR would

be necessary to substantiate this implication. Surprisingly, further deletion of 20 aa beyond that in CM3 restores the PTK and transforming activity of the Gag-IGFR. An additional 20-aa deletion, as in CM5, compromised the reactivated fusion receptor. CM5 protein retains a low but significant level of intracellular PTK activity although it lacks detectable in vitro autokinase activity. This is probably due to its enzymatic lability during in vitro manipulation. It is likely that the 20-aa sequence from 1291 to 1310 is essential for maintaining a correct conformation of the active IGFR and thus may be a sequence element involved in a positive modulation, whereas

FIG. 3. Expression, autokinase activity, and intracellular tyrosine phosphorylation of the CM proteins. Transfected CEF were either unlabeled or labeled with [35 S]methionine for 4 h. The radiolabeled and unlabeled CEF were lysed in Nonidet P-40 buffer, and equal amounts of cell lysates were immunoprecipitated with α IB (15). The [35 S]methionine-labeled immunoprecipitates were analyzed by SDS-PAGE (A). The immunoprecipitates from unlabeled CEF were washed and divided equally into three aliquots. One aliquot was used for in vitro kinase assay (C), and the other two aliquots were analyzed for protein amount of the mutant IGFRs by immunoblotting with α IB antiserum (B) and for tyrosine phosphorylation of the mutant IGFRs by immunoblotting with anti-p-tyr antibody 4G10 (D). These procedures were done as described previously (15). Lanes: C, CEF; UI, UIGFR; 1 to 5, CM1 through CM5, respectively.

the upstream 20-aa sequence (residues 1271 to 1290) may be involved in a negative modulation. Removal of this latter 20 aa thus restored the Gag-IGFR PTK and transforming activities lost in CM3. Alternatively, deletion of the 20 aa (residues 1271 to 1290) in CM4 may simply result in a compensatory effect on the misfolded CM3 protein such that an active conformation of the molecule is restored. The 21-aa residues adjacent to the putative PTK domain (residues 1250 to 1270, additional deletion in CM5) seems to be also important for the PTK and cell-transforming activities of the Gag-IGFR. The 21 aa further deleted in CM5 are remarkably rich in acidic residues and contain two consecutive tyrosine residues (tyrosine 1250 and 1251). The twin tyrosine residues are absent in the corresponding region of IR (28). Interestingly, the sequence including the twin tyrosine residues is identical to that of the IRR (25). Whether these twin tyrosine residues play any role in regulating IGFR PTK remains to be elucidated. In general, in vitro and intracellular PTK activities correlate well with the cell-transforming activity of the mutants.

The CM3 mutant lacking the C-terminal 47 aa is structurally similar to an IR mutant with a C-terminal 43-aa deletion (18–20, 27). The effects of the deletion are however quite different. CM3 lost PTK activity and transforming ability, whereas the IR mutant has a normal PTK and stronger mitogenic activity. Our parallel study employing a similar fusion protein, Gag-IR, indicated that deletion of up to 70 aa of the carboxyl terminus of the Gag-IR protein did not affect its PTK activity; in fact, the deletion enhanced its transforming

FIG. 4. Tyrosine phosphorylation of cellular substrates by CM. Equal numbers of normal or infected CEF were treated with 200 μ M of sodium orthovanadate for 4 h to preserve tyrosine phosphorylation prior to protein extraction. The protein extracts were separated by SDS-PAGE and transferred to nitrocellulose membranes. Tyrosine-phosphorylated proteins were detected by immunoblotting with a mixture of the monoclonal anti-p-tyr antibodies PY20 (ICN) and PT22 (prepared in our laboratory) (5). Positions of molecular size markers are indicated in kilodaltons. Lanes: C, CEF; N, NM1; UI, UIGFR; 1 to 5, CM1 to CM5, respectively. NM1 is a strong-transforming Gag-IGFR mutant of UIGFR described previously (16).

activity (4). The 20-aa sequence (residues 1291 to 1310) deleted in CM3 is the most diverged sequence in the carboxyl tails of the IR and IGFR. This difference in primary structures of the C termini of these two PTK receptors may be in part responsible for their different biological functions.

A recent study using a chimera consisting of the epidermal growth factor receptor ligand binding domain and the platelet-derived growth factor receptor (PDGFR) cytoplasmic domain showed a surprisingly similar observation (23). A mutant containing a 74-aa deletion in the PDGFR C terminus of the chimera had a low level of tyrosine phosphorylation and lost completely the oncogenic potential, whereas an additional 6-aa deletion restored the receptor phosphorylation and transforming activities to the wild-type level. Further deletion of 35 aa (115-aa deletion) yielded a receptor with little PTK or biological activity. The biological and biochemical activities of those three chimeric mutants are very similar to those of the mutants CM3, CM4, and CM5. Although the sequences of the IGFR and PDGFR carboxyl tails are very different, these regions seem to have similar modulatory effects on the biological and biochemical properties of these receptors.

Phosphorylation of cellular proteins by CM. To evaluate tyrosine phosphorylation of cellular proteins by the CM PTKs, total cell lysates of transfected CEF were analyzed by immunoblotting with the anti-p-tyr antibodies PT22 and PY20. Cells cultured in parallel were labeled with [35 S]methionine to monitor the mutant IGFR proteins. The result of metabolic labeling was similar to that shown in Fig. 3A and 5C, indicating that a comparable amount of mutant IGFR protein was expressed in the infected CEF. The overall tyrosine phosphorylation of cellular proteins was markedly increased in cells transfected by CM1, CM2, and CM4 (Fig. 4). As expected, cellular proteins from the CM3-infected CEF did not have a significant increase in tyrosine phosphorylation. However, the CM5-infected CEF appeared to have slightly elevated tyrosine phosphorylation of cellular proteins. Low levels of autophosphorylation (Fig. 3D) and phosphorylation of cellular proteins (Fig. 4) by PCM5 correlate with its weak transforming activity. Overall, the extent of tyrosine phosphorylation of cellular proteins correlates with the CEF-transforming activities of the CM mutants.

FIG. 5. Association of PI 3-kinase activity with mutant and native IGFR proteins. CEF were maintained in F10 medium supplemented with (A) or without (B) serum for 15 h. The CEF were lysed, and an equal amount of cell lysates was immunoprecipitated with α IB. The immunoprecipitates were washed extensively and used for the PI 3-kinase assay (16). The phosphoinositol phosphate (PIP) products were analyzed on thin-layer chromatography plates (A and B) (16). The level of fusion protein expression was monitored by [35 S]methionine labeling as described in the legend to Fig. 3 (C). PIP and protein bands were visualized by autoradiography, with exposure times within the linear range of the signals. Lanes: C, CEF; UI, UIGFR; 1 to 5, CM1 to CM5, respectively. To study PI 3-kinase association with full-length IGFR proteins, the full-length IGFR-transformed CEF were incubated overnight in serum-free F10 medium and stimulated with (+) or without (-) human IGF-I (15). One half of the cell lysates was immunoprecipitated with α IB, the other half was immunoprecipitated with PY20. Each immunoprecipitate was further divided into three aliquots. One aliquot was used for PI 3-kinase assay (D), the second aliquot was analyzed for IGFR tyrosine phosphorylation by immunoblotting with monoclonal anti-p-tyr antibody PY20 (E), and the third aliquot was analyzed for IGFR protein amount by immunoblotting with α IB (F). The positions of the 180-kDa IGFR precursor and the 95-kDa IGFR β subunit are indicated.

Association of PI 3-kinase with IGFR proteins. Association of PTK proteins with PI 3-kinase has been implicated in cellular transformation by PTK oncogenes (3). The association of the PI 3-kinase activity with that of N-terminally truncated IGFR mutants described previously correlated with their transforming ability (16). We therefore studied the PI 3-kinase association with CM proteins. When normalized to the mutant IGFR protein amounts in the immunoprecipitates (Fig. 5C), it appeared that PCM1, PCM2, and PCM4 had comparable levels of associated PI 3-kinase activity as that of P57-75^{UIGFR}, whereas PCM3 had negligible associated PI 3-kinase activity (Fig. 5). PCM5 had a significant level of associated PI 3-kinase activity in the regular but not in the serum-free culture medium (Fig. 5A and B). The amount of PI 3-kinase activity associated with UIGFR, PCM1, PCM2, and PCM4 was also decreased under serum-free conditions but not as drastically as for PCM5 (Fig. 5B).

The PTK-positive CM1, CM2, and CM4 proteins were able to associate with PI 3-kinase, whereas the PTK-negative or weak CM3 and CM5 proteins either failed to associate or associated only poorly with the PI 3-kinase. Thus, the association of PI 3-kinase with Gag-IGFR proteins appears to require PTK activity and/or tyrosine phosphorylation of the fusion proteins. This relationship was investigated by using native IGFR, since its PTK activity and tyrosine phosphorylation can be regulated. The PI 3-kinase activity associated with IGFR increased more than 20-fold after stimulation of IGFR-overexpressing CEF with recombinant human insulin-like growth factor 1 (IGF-1) (Fig. 5D). Most of the anti-p-tyr antibody-precipitated PI 3-kinase was also brought down by the anti-IGFR antiserum (Fig. 5D), indicating that the observed PI 3-kinase activity was mostly associated with tyrosine-

TABLE 1. Summary of biological and biochemical properties of Gag-IGFR CM^a

Mutant virus	Mutation or no. of aa deleted	Transformation	Tumorigenicity ^b	Kinase activity	PI 3-kinase binding
UIGFR	Gag-IGFR	++++	0/15 ^c	++++	++++
CM1	17	++++	0/13	++++	+++
CM2	27	+++	0/8	++++	+++
CM3	47	—	0/5	—	—
CM4	67	++++	9/15	++++	+++
CM5	88	+/-	0/11	+/-	+/-

^a CEF transformation, PTK activity, and PI 3-kinase association are described in the text and summarized here. The relative amount of virus was assessed by slot blot analysis of the viral RNAs as described previously (15). Equivalent amounts of viruses were injected into the wing webs of 1- to 3-day-old chickens. Five to six chickens were injected per virus each time. The latency for tumor appearance in CM4-injected chickens was 9 to 10 weeks. Total observation period was 13 weeks. Activity of each mutant is indicated by + or —, +/-, marginal activity.

^b Tumorigenicity is expressed as number of tumor-bearing chickens per total number of chickens injected. The results for the tumorigenicity assay represent the sum of two to four independent injections for CM1, CM2, CM4, and CM5.

^c See reference 15.

phosphorylated IGFR proteins. Indeed, the association of PI 3-kinase with IGFR correlated well with tyrosine phosphorylation of the receptor (Fig. 5E). Most of the receptor proteins became tyrosine phosphorylated after IGF-1 stimulation (Fig. 5F). We conclude that the association of PI 3-kinase with IGFR and its mutants requires PTK activity and correlates well with tyrosine phosphorylation of the native or mutant IGFR proteins. The association of PI 3-kinase with CM mutant proteins correlated well in general with their abilities to induce cell transformation (Table 1).

PTK activity and tyrosine phosphorylation of several PTK receptors have been shown to be necessary for PI 3-kinase binding (8; also see reference 3). We demonstrate in this study that activated PTK activity of IGFR is essential for its association with PI 3-kinase which appears to bind only the tyrosine-phosphorylated IGFR. PI 3-kinase does not associate with CM3, which lacks 47 aa and PTK activity, but it binds to CM4, which lacks 67 aa and has normal PTK activity. This result indicates that the lack of kinase activity rather than the binding sequence is responsible for the failure of CM3 to associate with the PI 3-kinase. In general, the associated PI 3-kinase level is higher in the presence of serum. This is particularly obvious with CM5. The underlying mechanism for the serum effect is not clear, but it could be due to phosphorylation of PCM5 and activation of its PTK by endogenous IGFR in the presence of serum. The sequence deleted in CM2 contains an amino acid sequence, YAHM (aa 1316 to 1319), which corresponds to the suggested PI 3-kinase binding motif YXXM (3). Our results show that this sequence motif and the carboxyl 88 aa are not essential for the association of Gag-IGFR with PI 3-kinase. This result is consistent with results of previous studies on the carboxyl terminus of IR which contains a similar YXXM motif that is not essential for IR association with PI 3-kinase (20). The sequence motif important for PI 3-kinase binding in IR was mapped to the juxtamembrane region which was shown to be also essential for interaction of IR with IRS-1, a major cellular substrate for IR PTK (1, 13). It may be possible that, like IR, IGFR interacts with PI 3-kinase through its juxtamembrane region which contains two well-conserved tyrosine residues.

When the results from our previous studies on the extracellular sequence and this study on the C-terminal sequence of

the human IGFR are considered together, it appears that the extracellular sequence has mainly an inhibitory effect, while the C-terminal sequence has generally a positive effect, on PTK activity and transforming function of the IGFR. The extracellular sequences of IR and IGFR seem to have a similar inhibitory role, but their carboxyl termini have different effects on their PTK activities and biological functions. A previous study on IR and IGFR chimeras has shown that the specificity of their biological functions is determined by their intracellular domains (14). The differential effects of C-terminal sequences of IR and IGFR on their functions elucidated in this and other studies (18–20, 25) may bear some relevance to the distinct biological functions of these two receptors.

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