A Single Amino Acid Exchange, Arg-45 to Ala, Generates an Epidermal Growth Factor (EGF) Mutant with High Affinity for the Chicken EGF Receptor*

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The finding that human epidermal growth factor (hEGF) and human transforming growth factor (hTGF) α bind with similar affinity to the human EGF receptor but differ in their affinity for the chicken EGF receptor was used as a model system to study ligand-receptor interaction of EGF receptor agonists. We previously constructed domain-exchange mutants of hEGF and hTGFα and found that the region COOH-terminal of the sixth cysteine residue in hTGFα is important for high affinity binding to the chicken EGF receptor (Krummer, R. H., Lenferink, A. E. G., Lammers van Buuren-Koornneef, L. van der Meer, A., van de Poll, M. L. M., and van Zoelen, E. J. J. (1994) J. Biol. Chem. 269, 8708—8711). To analyze this domain in more detail, we now constructed four additional chimeras in which either the region between the sixth cysteine residue and the highly conserved Leu-47 was exchanged or the region COOH-terminal of Leu-47. A mutant in which the latter region in hEGF was replaced by hTGFα (designated E6TE) showed increased binding affinity, whereas replacement of the former region in hEGF by hTGFα was sufficient to generate a mutant (designated E6ET) with a similar high affinity for the chicken EGF receptor as wild type hTGFα. Furthermore, a deletion mutant of hEGF lacking three COOH-terminal amino acids, EGF50, showed intermediate binding affinity for the chicken EGF receptor similar to E6ET, but upon additional deletions (EGF49 and EGF48), this initial gain in affinity was lost. A systematic analysis of the region between the sixth cysteine residue and Leu-47 showed that the low affinity of hEGF for the chicken EGF receptor is mainly due to the presence of Arg-46. Replacement of the positively charged Arg-45 by Ala, the corresponding amino acid in hTGFα, was sufficient to generate a mutant growth factor with high affinity for the chicken EGF receptor. This indicates that in hEGF Arg-45 may play an important role in receptor binding. A model is proposed in which positively charged amino acids close to or within the receptor recognition site of hEGF prohibit high affinity binding to the chicken EGF receptor due to electrostatic repulsion of positively charged amino acids in the putative ligand binding domain of the chicken EGF receptor.

Human epidermal growth factor (hEGF) and human transforming growth factor (hTGF) α belong to the same family of growth factors. They both bind with high affinity to the human EGF receptor, but hEGF has a 10—50-fold lower affinity for the chicken EGF receptor than hTGFα (1). All members of the EGF family are characterized by the presence of six identically spaced cysteine residues, which form three intramolecular disulfide bridges. Together with some highly conserved glycine residues they are essential for the correct three-dimensional structure of the growth factor and for high affinity binding to the EGF receptor, which suggests that they form part of the binding domain (5—9). The crystal structure of hEGF or hTGFα is not available, and most of the information on the structure of these growth factors has come from detailed 1H NMR studies. Based on the observation that amino acids surrounding the second cysteine residue are in close contact with amino acids near the sixth cysteine residue, it has been postulated that Tyr-13/Leu-15/His-16 together with Arg-42/Gln-48/Leu-47 form the binding site in hEGF (10—12). The exact region involved in binding to the receptor is still not known, however, and this has hampered the design of receptor antagonists.

To gain more insight into the way hEGF and hTGFα bind to their receptor, we recently used the difference in binding affinity of these growth factors for the chicken EGF receptor as a model system. A total of 10 hEGFα/hTGFα chimeras were constructed in which regions bordered by the highly conserved cysteine residues were exchanged, and their relative binding affinity for the chicken EGF receptor was assessed (13). Introduction of the region COOH-terminal of the sixth cysteine residue of hTGFα into hEGF appeared to be sufficient to confer high affinity binding characteristics to hEGF, and, in line with this, an exchange of the same region in hTGFα with the corresponding hEGF sequence caused hTGFα to lose its high affinity for the chicken EGF receptor. These data indicate that the COOH-terminal region in EGF receptor agonists plays an important role in receptor binding. In a recent 1H NMR study (14), it has been shown that this region of hTGFα is flexible in the unbound molecule but that its mobility is strongly reduced upon receptor binding, which emphasizes again the role of the COOH-terminal domain in receptor-ligand interaction.

In the present study, we investigated in more detail which amino acids in the putative ligand binding domain of the chicken EGF receptor...

1 The abbreviations used are: hEGF, human epidermal growth factor; hTGFα, mouse EGF; hTGF, human transforming growth factor; RP-HPLC, reverse-phase high pressure liquid chromatography; DMEM, Dulbecco's modified Eagle's medium; NCS, newborn calf serum; BSS, Na-bis(2-hydroxyethyl)glycine hydrochloride; 2-aminoethanesulfonic acid.
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Experimental Procedures

DNA Constructs—The gene encoding the mature 50-amino acid sequence of human TGFα was made as previously described (13). The synthetic gene for human EGF was obtained from British Biotechnol-ology (Oxford, United Kingdom). The genes were linked at the 5'-end to the sequence coding for the recognition sequence of the proteolytic enzyme factor X (Ile-Glu-Gly-Arg) (15). In experiments (13), all constructs had been cloned into the plHoma153 expression vector. Because in our hands a higher level of expression of biologically active protein was obtained using the pEZZ/FX/ growth factor/EGF and pHoma153/FX/EGF pEZZ18 constructs were cloned into the corresponding sites of pEZZ18. In this way, the constructs encoding hEGF and hTGFα were placed in frame 3' of the sequence coding for the two synthetic IgG binding domains (so-called Z domains) of pEZZ18. All further constructs were cloned into pEZZ18 using the SacI site at the 3'-end and the EcoRI site at the 5'-end. To generate constructs of TETE and TETET (for definition, see Fig. 1 and "Results"), the gene coding for hTGFα was cleaved at the sixth cysteine codon by DraI and at the 3'-end by SacI. The gap was filled in using synthetic double-stranded oligonucleotides. For the construction of E6ETE, hEGF was digested at the fourth cysteine codon by SphI, and TETET was cleaved at the sixth cysteine codon by DraI. The fragments were ligated to a double-stranded oligonucleotide separating the region between the SphI site and the DraI site. To generate E6ETET, the SphI site and the SacI site of the hEGF construct were used, and the gap was filled in using two double-stranded oligonucleotides. DNA constructs that code for hEGF mutants truncated at the COOH-terminal end, EGF50, EGF49, and EGF48, were made by polymerase chain reaction using pEZZ/FX/EGF as a template. Three different antisense primers sequences NH2-termind and KGK sequences ([Hbtrrininal COOH-terminal domain were investigated on Western blot as shown in Fig. 1). In addition, hKGF deletion mutants FGPF60, FGPF50, FGPF40, and FGPF30 were constructed to evaluate the role of the region between the sixth cysteine residue and the COOH-terminal domain of the sixth cysteine residue, has a low affinity. In the present study, the importance of specific amino acids within the linear COOH-terminal domain was investigated in more detail. First, EGF/TGFα chimeras were constructed in which either the region between the sixth cysteine residue and the highly conserved Leu-47 (Leu-48 in hTGFα) or the region (Leu-48 in hTGFα) of the tracer into cellular DNA was determined 24 h after growth factor addition. For this, cells were washed twice with phosphate-buffered saline and incubated with methanol at room temperature. After 15 min, the methanol was aspirated, and the cells were lysed in 1 ml of 0.2 N NaOH for 30 min at 37° C as described (27). Radioactivity was determined by liquid scintillation counting. Experiments were performed in duplicate.

Results

Mutant Growth Factors—In previous work (13), the COOH-terminal region in hTGFα was identified as an important domain for high-affinity binding of hTGFα to the chicken EGF receptor. Introduction of this region into hEGF generated a chimeric growth factor (designated rETE) with high affinity for the chicken EGF receptor, whereas rTEE, a chimera with TGFα sequences NH2-terminal and EGF sequences COOH-terminal of the sixth cysteine residue, has a low affinity. In the present study, the importance of specific amino acids within the linear COOH-terminal domain was investigated in more detail. First, EGF/TGFα chimeras were constructed in which either the region between the sixth cysteine residue and the highly conserved Leu-47 (Leu-48 in hTGFα) or the region (Leu-48 in hTGFα) of the tracer into cellular DNA was determined 24 h after growth factor addition. For this, cells were washed twice with phosphate-buffered saline and incubated with methanol at room temperature. After 15 min, the methanol was aspirated, and the cells were lysed in 1 ml of 0.2 N NaOH for 30 min at 37° C as described (27). Radioactivity was determined by liquid scintillation counting. Experiments were performed in duplicate.

Analysis of Fusion Proteins by SDS-Polyacrylamide Gel Electrophoresis and Western Blotting—Aliquots of 10 μg of purified periplasm were analyzed by gel electrophoresis on a 12.5% SDS-polyacrylamide gel in the absence or presence of 2% β-mercaptoethanol as a reducing agent. Proteins were transferred to nitrocellulose, and the Western blots were probed with a rat-anti-goat antibody linked to horseradish peroxidase. Enzyme activity was detected by incubation with tetramethylbenzidine/sodium dihydrothiobisulfocyanate/H2O2 in phosphocitrate buffer (pH 5).
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EGF  ECGYRIDLWKWyELR
TGFα  ECGHADLWL
E6T  ECGHADLWL
T6E  ECGYRIDLWKWyELR
E6ET  ECGHADLWL
E6TE  ECGYRIDLWKWyELR
T6ET  ECGYRIDLWKWyELR
Q43E  ECGHADLWL
Y44H  ECGHADLWL
R45A  ECGYRIDLWKWyELR
EGF48  ECGYRIDLWK
EGF49  ECGYRIDLWK
EGF50  ECGYRIDLWK

Figure 1. Schematic representation of mutant growth factors. Amino acids COOH-terminal of the sixth cysteine residue are indicated: circles, hTGFα-derived amino acids; squares, hEGF-derived amino acids; diamonds, the conserved sixth cysteine residue and Leu-47 (Leu-48 in hTGFα). The sequence N-terminal of the sixth cysteine residue is either hEGF (B) or hTGFα (T).

Figure 2. Identification of ZZFX/growth factor fusion proteins by SDS-polyacrylamide gel electrophoresis and Western blotting. Aliquots of 10 μl of unprepared periplasm were run on a 12.5% SDS-polyacrylamide gel under non-reducing (A) or reducing (B) conditions. Proteins were transferred to nitrocellulose, and the Western blots were probed with rat-anti-goat antibody linked to horseradish peroxidase: wild type hEGF, lanes 1 and 6; Q43E, lanes 2 and 7; Y44H, lanes 3 and 8; R45A, lanes 4 and 9; control periplasm (ppZZ18 without insert), lane 5.

on Western blot but disappeared under reducing conditions, indicating the presence of a small amount of (inactive) multimeric forms of the growth factor fusion proteins. One unidentified band of ~24 kDa, also present in control periplasm (ppZZ18 without insert), is thought to be due to nonspecific binding of IgG-peroxidase. Wild type and mutant growth factors were purified as described under "Experimental Procedures," and the presence of fusion protein or growth factor activity after each purification step was monitored by Western blotting, protein A enzyme-linked immunosorbent assay, and/or 125I-mEGF binding competition assay. The growth factors were finally purified by RP-HPLC using a linear gradient of CH3CN in 0.1% trifluoroacetate (Fig. 3A). Fractions were analyzed for biological activity in a binding competition assay with 125I-mEGF (Fig. 3B). In general, one major (peak 1) and two minor (peak IIa/b) biologically active products eluted between 25 and 35% CH3CN. When peak I and peak IIa/b were assayed separately (as was done for EGF50 and R45A), no differences were detected in their relative affinity for the chicken EGF receptor. With all other growth factors, peaks I and IIa/b were pooled. The identity of peaks I and IIa/b was not established, but it is assumed that the major peak represents "unmodified" growth factor, whereas the two minor peaks may represent chemically modified products or NH2-terminally truncated forms (2). Truncations at the COOH-terminal end are less likely since none of the biologically active peaks identified in EGF50 coincided with those of EGF49 or EGF48 on the RP-HPLC column. Degradation of EGF upon storage has been reported to involve oxidation of Met-21, deamination of Asn-29 and/or succinimide formation of Asp-11 (2, 20). Oxidation of Met-21 and NH2-terminal deletions up to three amino acids does not significantly alter the biological activity of EGF (2).

125I-mEGF Binding Competition Assays on HER-14 and CER-108—To determine the binding affinity of the mutant growth factors for the human EGF receptor, the total binding activity present under peak I and peak IIa/b was measured in a 125I-mEGF binding competition assay on HER-14 cells. Wild type mEGF (Bioproducts for Science Inc., Indianapolis, IN) was used as a standard, and the binding activity of the mutant growth factors was expressed as ng mEGF equivalents. The ratio between binding activity and the amount of protein rep-
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Binding affinities of hEGF mutants for the human EGF receptor

The ratio (R) between binding activity present under peaks I and IIab in the RP-HPLC chromatogram (ng mEGF equivalents) and the amount of protein represented by the peak area (mm²) was calculated for each mutant as well as for wild type mEGF (Huproducts for Science Inc., Indianapolis, IN). The binding affinities of the mutant growth factors are expressed as percentage of wild type mEGF: R(mutant)/R(wild type) × 100%.

<table>
<thead>
<tr>
<th>hEGF species</th>
<th>Binding affinity % of wild type</th>
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<tbody>
<tr>
<td>Wild type mEGF</td>
<td>100</td>
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<tr>
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<td>124</td>
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<td>106</td>
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<tr>
<td>EGF56 (peak IIab)</td>
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<tr>
<td>R45A (peak I)</td>
<td>106</td>
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<tr>
<td>R45A (peak IIab)</td>
<td>124</td>
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Table I

represented by the peak area (absorption at 229 nm) was then calculated and compared with the ratio obtained with wild type mEGF (Table I). For most of the mutant growth factors the binding affinity was calculated to be close to the binding affinity of mEGF. Only the affinity of EGF48 was approximately 2-fold lower. No such data are available for the exchange mutants E67E, E67E, T67E, and T67E.

To determine the differential binding characteristics of the mutant growth factors for the human and the chicken EGF receptors, all recombinant proteins were eventually calibrated to give the same 50% competition of 125I-mEGF binding to HER-14 cells as wild type mEGF. An example of this is shown in Fig. 4 for the EGF point mutants Q43E, Y44H, and R45A.

E67E, E67E, T67E, and T67E—After calibration on HER-14 cells, the relative affinity of the mutant growth factors for the chicken EGF receptor was, subsequently, compared with the affinity of wild type hTGFα and hEGF. Replacement of the region between the sixth cysteine residue and Leu-47 in hEGF for the corresponding region in hTGFα (E67E), was sufficient to increase the affinity of hEGF for the chicken EGF receptor to the level of hTGFα (Fig. 5A). Interestingly, also the region COOH-terminal of Leu-47 influenced the affinity of hEGF for the chicken EGF receptor. Replacement of the relatively long COOH-terminal tail in hEGF (KWWELR) for the much shorter corresponding region in hTGFα (LA) resulting in E67E, caused a substantial increase in binding affinity for CER-109 cells. The increase in affinity was even larger when this region was replaced in T6E making T6ET; in contrast, T6E binds with a similar low affinity to the chicken EGF receptor as wild type hEGF (13).

EGF50, EGF49, and EGF48—One striking difference between hEGF and E67E is the relatively long COOH-terminal tail (KWWELR) in hEGF as compared to a short tail (LA) in E67E and hTGFα. To investigate whether this difference in tail length determines the difference in affinity between hEGF and E67E, COOH-terminally truncated forms of hEGF were prepared. Deletion of three COOH-terminal amino acids ELR of hEGF (EGF50) caused a 4-fold increase in relative affinity for the chicken EGF receptor similar as seen with E67E. Upon additional deletion (EGF49 and EGF48), however, this initial gain in affinity was lost (Fig. 5B).

Q43E, Y44H, and R45A—Of all modifications of hEGF tested thus far in this study, replacement of the region between the sixth cysteine residue and Leu-47 with the corresponding hTGFα sequence (E67E) gave the largest increase in binding affinity. This region in hEGF differs from hTGFα only by three amino acids. To determine the individual role of these residues in high affinity binding to the chicken EGF receptor, each amino acid in this region in hEGF was successively exchanged with the corresponding amino acid in hTGFα. In this way, the point mutants Q43E, Y44H, and R45A were generated. Mutation of glutamine on position 43 to glutamate or mutation of tyrosine on position 44 to histidine hardly affected the relative affinity of hEGF for the chicken EGF receptor. However, exchanging arginine on position 45 in hEGF with alanine in hTGFα generated a hEGF mutant with a similar relative affinity for the chicken EGF receptor as hTGFα (Fig. 5C). Also, the absolute affinity of R45A for the chicken EGF receptor will be close to hTGFα since its affinity for the human EGF receptor was estimated to be similar to wild type mEGF (Table I).

Mitogenic Activity—To test whether the mutant growth factors are biologically active, HER-14 cells rather than CER-109 cells were used. We have noticed that wild type hEGF and hTGFα are equally mitogenic on CER-109 cells, whereas clear differences were observed using primary chicken adipocyte precursor cells. The presence of endogenous mEGF receptors (3,000–10,000 receptors/cell) might cause a potentiation of the mitogenic response to growth factors that have a low affinity for the chicken EGF receptor but a high affinity for the mEGF receptor. Therefore, mitogenic assays on CER-109 cells are of limited value.

Because the binding affinity of all recombinant proteins was calibrated based on their ability to give 50% competition of 125I-mEGF binding to HER-14 cells, any difference in relative mitogenic activity is most easily detected on the same cells. In Fig. 6 is shown that all growth factors were biologically active when tested for their ability to stimulate [3H]thymidine incorporation into serum-starved HER-14 cells. Most of the mutant growth factors were similarly active as wild type hEGF or hTGFα, but one mutant, EGF48, induced a slightly higher mitogenic response. The absolute affinity of EGF48, however, was calculated to be ~2-fold lower than the affinity of the wild type growth factors (Table I). We therefore expect that EGF48 will induce a similar mitogenic response as wild type hEGF when assayed on a protein basis. Loss of binding affinity without a concomitant decrease in mitogenic activity has been reported before by Walker et al. (5) for mEGF in which Leu-47

Fig. 4. Inhibition of binding of 125I-mEGF to HER-14. The binding activity of the mutant growth factors was calibrated to give the same competition of binding of 125I-mEGF to HER-14 as wild type mEGF. Representative curves of the hEGF point mutants Q43E, Y44H, and R45A are shown after the final calibration. Experiments were repeated at least three times.

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was mutated to Ala. In data to be published elsewhere, we will show that, even on a protein basis, some of the hEGF/hTGFα chimeras constructed previously by us (13) are truly superagonistic for HER-14 when compared with wild type hEGF or hTGFα.

**DISCUSSION**

Mammalian EGF and TGFα bind with similar high affinity to the human EGF receptor, but their affinity toward the chicken EGF receptor differs substantially (1). Human EGF has a 10-50-fold lower affinity for the chicken EGF receptor than human TGFα, and the affinity of mouse EGF is ~5-fold lower than of human EGF (1, 13). We have previously used the model to study ligand-receptor interactions (13). A total of 10 chimeras of hEGF and hTGFα were constructed, and it was found that chimeras with hTGFα sequences COOH-terminal of the sixth cysteine residue all had a similar high affinity for the avian EGF receptor as wild type hTGFα, whereas those having hEGF sequences in this region showed EGF-like binding characteristics. This indicates the importance of the COOH-terminal domain in discriminating between hEGF and hTGFα.

To identify amino acids involved in high affinity binding to the EGF receptor, a detailed analysis of the COOH-terminal domain of hEGF was made in the present study. Here, we show that the low affinity of human EGF for the avian EGF receptor is mainly due to the presence of arginine on position 45. Re-
placement of the positively charged Arg-45 for alanine, the corresponding amino acid in hTGFα, was sufficient to generate a hEGF mutant with high affinity for the chicken EGF receptor. Thus far, point mutation studies of the carboxyl-terminal region of hEGF and hTGFα have focused mainly on the highly conserved Asp-46 and Leu-47 (Asp-47 and Leu-48 in hTGFα). Leu-47 and (less stringently) Asp-46 have been shown to be crucial for receptor binding and activation (5, 7, 9, 12). By using a domain exchange strategy, however, a systematic survey of the importance of non-conserved amino acids can be made. The present finding that Arg-45 discriminates between hEGF and hTGFα with respect to their affinity for the chicken EGF receptor, implicates that this amino acid lies close to or forms part of the receptor recognition site.

Lax et al. (21) analyzed the differential binding characteristics of the human and chicken EGF receptor using a domain-exchange strategy, and they demonstrated that domain III of the EGF receptor extracellular domain is most important for ligand recognition. Within this region the sequence between amino acids 351 and 364 was found to be the epitope recognized by ligand-competitive monoclonal antibodies (22). The participation of this epitope in the formation of a ligand binding site, however, was recently questioned since exchange of this region in the human EGF receptor for the corresponding sequence in the chicken EGF receptor did not alter the affinity of mouse EGF or human TGFα for the receptor (23). On the other hand, one might expect that the epitope recognized by an antibody that competes with the natural ligand for binding to the receptor lies close to the ligand binding site of the receptor. Immediately COOH-terminal of the epitope two lysine residues are found in the chicken EGF receptor that are not conserved in the human EGF receptor (24). For one of these lysine residues, a conservative replacement (Arg) is found in the murine EGF receptor; this amino acid is, therefore, less likely to discriminate between the chicken and the mammalian EGF receptor. The second lysine residue, however, is only found in the chicken EGF receptor, whereas in the human EGF receptor this positively charged amino acid is replaced by a negatively charged glutamate. We hypothesize that the lysine residue on position 367 in the chicken EGF receptor lies close to or forms part of the ligand binding domain and that the positively charged Arg-45 in the putative receptor recognition site of hEGF prohibits the interaction of hEGF with the chicken EGF receptor due to electrostatic repulsion.

Other positively charged amino acids in hEGF that might interfere with ligand-receptor interaction, are Lys-48 and Arg-53 in the carboxyl-terminal tail of hEGF. Replacement of this region in hEGF (KWWEIR) for the corresponding uncharged sequence in hTGFα (LA), making EβET, caused an increase in binding affinity. A similar improvement of binding affinity was found upon truncation of the carboxyl-terminal tripleptide ELR (EGF50) removing Arg-53 as the COOH-terminal amino acid. Additional deletion of Trp-50, however, resulted in a decrease in affinity for the chicken EGF receptor.

Besides a difference in charge distribution, there is also a difference between hEGF and hTGFα in conformation of the COOH-terminal tail. In hEGF, this region adopts an α-helix conformation involving Leu-47–Glu-51, whereas in hTGFα the COOH-terminal dipeptide is flexible in solution and lacks a defined structure (10, 14, 25). The α-helix in hEGF has an amphipathic character with Lys-48 and Glu-51 on the hydrophobic site and Leu-47/Trp-50 and Trp-49/Leu-52 on the hydrophilic site. In addition, Trp-50 interacts with other hydrophobic amino acids in the protein such as Val-34 and Tyr-37 (10). One might speculate that in EGF50 the carboxyl-terminal tail can still adopt an α-helix conformation, which is stabilized by hydrogen bond formation between Leu-47 and Trp-50 as well as by Vander-Waals interactions between Trp-50 and other hydrophobic side chains. In contrast to EGF50, no α-helix structure will be formed in EGF49 or EGF48. The relatively high binding affinity of EGF50 for the chicken EGF receptor suggests that α-helix formation of the carboxyl-terminal tail of hEGF will prevent the positively charged Lys-48 from interfering with ligand-receptor interaction, whereas it does interfere in the case of EGF49 and EGF48.

In conclusion, we propose a model in which positively charged amino acids close to or within the putative receptor recognition site of hEGF (Arg-45 and to a lesser extent Lys-48 and Arg-53) prohibit high affinity binding to the chicken EGF receptor due to electrostatic repulsion of positive charges in or near the putative binding domain of this receptor. Comparing the carboxyl-terminal sequences of EGF receptor agonists of different origin, the proposed model would predict that EGF derived from human, mouse, or rat will have a low affinity for the avian EGF receptor, whereas human and rat TGFα but also EGF from guinea pig will have a high affinity for the avian EGF receptor.

In addition to amino acids in the COOH-terminal domain, residues in other domains are thought to form part of the binding domain in EGF and TGFα. 1H NMR studies have shown, for instance, that amino acids near the sixth cysteine residue are in close contact with residues surrounding the second cysteine residue (10), and in a recent study Richter et al. (26) hypothesized that amino acids in the β-loop β-sheets determine the difference in binding affinity between human EGF and mouse EGF for the chicken EGF receptor. Perhaps EGF receptor agonists contain two distinct binding domains that each can bind one receptor monomer similar as seen for the interaction of human growth hormone with its receptor (28). Data in favor of this model have been discussed previously by Gullick (29). Additional studies will be necessary, however, to increase our understanding of the way EGF and TGFα interact with their receptor and to make the design of receptor antagonists feasible.

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