The Relationship between Human Epidermal Growth-like Factor Receptor Expression and Cellular Transformation in NIH3T3 Cells*

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A collection of cell lines expressing each human epidermal growth factor receptor (HER) family member alone or in all pairwise combinations in a clone of NIH3T3 cells (3T3-7d) devoid of detectable epidermal growth factor receptor family members has been generated. Transformation, as measured by growth in soft agar, occurred only in the presence of appropriate ligand and only in cells expressing two different HER family members. Transformation of oncogenic neu (Tneu), conferred ligand-independent transformation only in cells which co-expressed HER1, HER3, or HER4, but not when expressed alone or with HER2. Cell lines were also tested for their ability to form tumors in animals. None of the cell lines expressing single HER family members was able to form tumors in animals with the exception of HER1, which was weakly tumorigenic. Although unable to form tumors when expressed alone, HER2 was tumorigenic when expressed with HER1 or HER3, but not HER4. Of all complexes analyzed, cells expressing HER1 + HER2 were the most aggressive. The relationship between HER1 activation, intracellular calcium fluxes, and phospholipase Cγ1 activation is well established. We found that activation of HER1 was required for the induction of a calcium flux and the phosphorylation of phospholipase Cγ1. These activities were independent of, and unaffected by, the co-expression of any other family member. Further, heregulin stimulation of all cell lines including those containing HER1 did not demonstrate any effect on intracellular calcium levels or phospholipase Cγ1 phosphorylation. This demonstrates that heregulin induced cellular transformation by activating HER3- and HER4-containing complexes does not require the activation of either phospholipase Cγ1 or the mobilization of intracellular calcium.

The involvement of tyrosine kinase growth factor receptors and their ligands in the regulation of cell growth and differentiation is well documented. The EGF1 family of type 1 tyrosine kinase receptors has been implicated in the progression of human carcinomas (1–5). This family consists of four members, EGF/HER1, c-neu/ErbB-2/HER2, ErbB-3/HER3, and ErbB-4/HER4 (hereinafter called HER for human EGF-like receptor) (6–9). Most noteworthy is HER2, whose overexpression resulting from increased transcriptional regulation or gene amplification, has been observed in a range of cancers including 30% of breast and ovarian cancers and correlates with a poor prognosis and decreased patient survival (4, 10–12). Overexpression of these receptors by gene transfection confers a transforming phenotype (5, 13–17). Unlike the other family members that require ligand activation, overexpression of HER2 alone is sufficient for promoting transformation (13, 15). This activity is analogous to the oncogenic form of HER2, Tneu, which was derived from chemically induced rat neuroblastoma containing a single point mutation in the transmembrane domain (18).

Activation of the HERs can occur by different EGF-like ligands that can be divided into three groups. Those that can only bind to HER1 include EGF, amphiregulin, transforming growth factor-α, and heparin-binding EGF (19, 20). Heregulin (HRG/neuregulin or NDF (neu differentiation factor), as well as other related molecules such as glial growth factor and ARIA represent the second group which bind to HER3 and HER4 (9, 21–26). The last group, represented by betacellulin, initially identified with specificity for HER1 (27) was later shown to activate both HER1 and HER4 (28).

Recent studies have established that this family of receptors can associate with each other to form an array of heterodimer complexes (17, 29–33). Therefore, the activation of one receptor could modulate the activities of other HER family members, when co-expressed in the same cell, and expand the repertoire of downstream signaling events. This is clearly the case for HER2, which in the absence of any HER2-specific ligand, can be transmodulated within a heterodimer complex by associating with other HER family members able to respond to their respective ligand(s). With the ability of these receptors to heterodimerize, it is essential that the study of any one receptor or heterodimer be done in a cell system devoid of endogenous HER expression. This became especially apparent in HER4-transfected NIH3T3 cells, that were able to form colonies in agar in response to both HRG and EGF (17). The responsiveness to EGF correlated with the formation of a heterodimer complex between HER4 and the endogenous mouse EGF receptor (17).

To further examine the biochemical and biological properties of individual HER complexes, we have generated a collection of cell lines expressing all HERs alone or in all pairwise combinations in a clone of NIH3T3 cells (3T3-7d) (34) devoid of detectable EGFR family members. We were able to demonstrate that transformation, as defined as growth in soft agar, was dependent upon the expression of multiple HER family members in the same cell, and was independent of intracellular calcium mobilization or PLCγ1 phosphorylation.

EXPERIMENTAL PROCEDURES

Cell Culture—3T3-7d and HER-transfected 3T3-7d cell lines, previously characterized (34), were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum (Life Techno-
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gies, Inc.) and 500 μg/ml G418 at 37°C in 5% CO₂. All cell lines are massed except for the exception of HER2, HER2 + 4, and HER2 + 3-expressing lines where selected clones were isolated to equalize the level of HER2 expression. Transfection of Tneu (35) was done using Lipofectamine (Life Technologies, Inc.) as recommended by the manufacturer. Briefly, 1 × 10⁶ cells were plated into a 32-mm dish, incubated overnight, and then transfected with 1.0 μg of Tneu plasmid and Lipofectamine for 5 h. Cells were fed and then passed into selection media the following day.

Ligands—Heregulin β2 used in all assays was synthesized as described previously (36). Briefly, the EGF domain of heregulin β2 was fused to the Fc portion of a human IgG1 gene, and this construct was transfected and expressed in COS cells. The heregulin portion of the purified IgG1 fusion was cleaved away from the Fc domain with thrombin and purified. EGF was purchased from Life Technologies, Inc.

Soft Agar Assay—Cells were trypsinized and approximately 1 × 10⁴ cells were mixed with 0.4% agar, DMEM, 10% FBS (Life Technologies, Inc.), and plated on top of an existing basement layer of 0.6% agar. The basement agar layer consisted of DMEM, 10% FBS, plus 200 μg/ml EGF or HRG.

Tumor Growth in Nude Mice—1 × 10⁵ cells of 3T3-7d transfected cell lines expressing HER1, HER2, HER3, HER1 + 2, HER1 + 3, HER1 + 4, or HER2 + 3 cells were injected subcutaneously above the lateral aspect of the pelvis. Measurements were taken at week 4, and volumes were calculated.

Cell Extractions and Immunoprecipitation—Cells grown in 100 mm dishes were lysed by adding 1.0 ml of lysis buffer (1% Nonidet P-40, 150 mM NaCl, 50 mM Tris-HCl, pH 7.4, 0.5 mM EDTA, 5% glycerol, 1 mM sodium orthovanadate) to the plate. Each HER was immunoprecipitated from the extract using 2 μg of receptor-specific monoclonal antibody (HER1-AB1, Oncogene Science; HER2-AB2, Neomarkers Inc.; HER3-AB3, Neomarkers Inc.; HER4-10-4-7), with 40 μl of a 50% slurry of goat anti-mouse IgG-linked agarose (Sigma). The mixture was mixed for 60 min at 4°C. Samples were washed twice with lysis buffer and boiled in SDS sample buffer. Blotting for receptor expression was done using the following antibodies: HER1-06-129, Upstate Biotechnology; HER2-AB2, Oncogene Science; HER3-AB3, Neomarkers Inc.; HER4-SC-283, Santa Cruz Biotechnology. Anti-phosphotyrosine immunoblots were done with PY20 from Transduction Laboratories. Rabbit polyclonal antibodies to PLC-γ1 were developed against a synthetic peptide corresponding to residues 461–481, which were conjugated to ovalbumin. All immunoblotting experiments used 125I-labeled goat anti-mouse or rabbit second antibody for detection (Dupont NEN) at a 1:500 dilution. The exposure times and PhosphorImager profile for the data were noted on the top of each lane.

RESULTS

The 3T3-7d cell line which is devoid of detectable HER family expression were transfected with expression plasmids encoding each of the HER family members. Cell lines were isolated that expressed the receptors individually or all possible pairwise combinations. The level of receptor expression within each HER family subtype, as determined by immunoprecipitation and Western analysis using receptor specific antibodies, was similar in all cell lines (Fig. 1). The level of receptor activity in response to ligand, as measured by the change in receptor tyrosine phosphorylation was investigated. Cells were stimulated with EGF or HRG, and the HERs were immunoprecipitated with receptor-specific antibodies followed by immunoblott- ing with anti-phosphotyrosine antibody (Fig. 2). In the absence of added ligand, the steady state level of HER receptor phosphorylation was affected by the co-expression of other HER family members. Specifically, HER1 phosphorylation was elevated when expressed alone or only when co-expressed with HER3. However, when co-expressed with HER2 or HER4, the level of ligand independent phosphorylation decreased. HER2 phosphorylation was elevated in all cell lines except when co-expressed with HER3. HER3 phosphorylation was stimulated when co-expressed with HER1 and HER2 but not HER4. HER4 phosphorylation was high when expressed alone or with HER1, but low when co-expressed with HER2 or HER3. These data suggest that receptor complexes can form in the absence of ligand, and the kinase activity of these complexes is differentially regulated.

With respect to ligand stimulation of cell lines expressing only one family member, HER1 was partially tyrosine phosphorylated in the absence of ligand and was further stimulated by addition of EGF. Similarly, HER2 was phosphorylated in the absence of ligand, however, as expected its level of phosphorylation did not change in response to either EGF or HRG. HER3, previously shown to be a defective tyrosine kinase (38), demonstrated a weak response to HRG. HER4 was partially activated in the absence of ligand, and could further be phosphorylated in response to HRG. The lower band in the HER4 immunoprecipitates from HER2 + 3 and HER2 + 4 cell lines is not HER4 (data not shown). In cells expressing multiple receptors, stimulation of either receptor partner by EGF or HRG resulted in the increased phosphorylation of both receptors indicative of a heterodimer complex. However, in the context of a HER1 + 3 heterodimer, it was not possible to see a HRG dependent increase in HER1 phosphorylation.

To address the biological activity of the 3T3-7d/HER transfectants, cells were seeded in soft agar to determine if colony formation occurred in the presence or absence of ligand stimulation (Fig. 3). All cell lines expressing only one HER family member were unable to promote colony growth even in the presence of ligand. In contrast, all cell lines expressing multiple HER family members were able to form colonies in agar in response to the appropriate ligand. Cell lines expressing HER1 + 3 and HER1 + 4 share the capacity to respond to both EGF and HRG unlike other cell lines that can respond to only one
ligand. However, only the HER1-expressing cell line formed colonies in response to both ligands, whereas the HER13 cells responded to HRG but not EGF. This was surprising given the fact that EGF is able to activate both HER1 and HER3 tyrosine phosphorylation in the same cells (Fig. 2). The oncogenic form of HER2 (Tneu) which contains an activating mutation in the transmembrane domain, is able to dimerize and transform cells in the absence of ligand (18). To further support our hypothesis that heterodimer formation was required for efficient growth in agar, Tneu was tested for its ability to induce ligand independent colonies in agar when expressed alone or in the presence of other HER family members. Cell lines were generated by transfecting a Tneu expression vector into the 3T3-7d parent or 3T3-7d HER1-, HER2-, HER3-, or HER4-expressing cell lines and the level of Tneu expression determined by immunoblot analysis (Fig. 4A). Since the antibody used to detect Tneu also cross reacts with HER2, we were unable to clearly demonstrate the presence of Tneu expression independent of HER2 expression within the HER2/Tneu-expressing cell line. Transformation by Tneu was only evident in cells that co-expressed HER1, HER3, or HER4 (Fig. 4B). Cells expressing Tneu alone or co-expressed with HER2 were unable to form colonies in agar. These data demonstrate that the oncogenic activity of Tneu requires the co-expression of other HER members, excluding HER2, and demonstrates for the first time that Tneu is able to functionally heterodimerize with other HER family members.

The requirement for heterodimer expression in cells to promote a transforming signal was further tested in vivo by determining the tumorigenic potential of 3T3-7d HER transfec-
tants in nude mice. In general, cells expressing HER homodimers were unable to induce tumor growth. HER1-expressing cells, however, were able to induce a low level of tumor formation (Fig. 5). Co-expression of HER2 with HER1 was highly tumorigenic, identifying that HER1 + HER2 is much better than either receptor alone. Similarly, co-expression of HER2 with HER3 conferred tumor growth where each receptor alone did not. Interestingly, although able to form colonies in agar, cells expressing HER1 + 3 and HER1 + 4 were unable to form tumors in animals. In fact, co-expression of HER3 and HER4 with HER1 reduced the tumorigenic potential of HER1 alone, suggesting that these receptors are regulating the activity of HER1 in vivo. Together with the soft agar results, our data demonstrate that expression of multiple HER family members are required to efficiently transform cells in vitro and in vivo.

Ithasbeenproposedthatthemobilizationofcalciumisakey component in the regulation of cell proliferation (39). Depletion of calcium leads to a reversible growth arrest in normal cells, whereas, transformed cells are still able to grow (39). Stimulation of HER1 by EGF results in the activation of PLCg1 (40, 55), which stimulates the release of two second messengers, inositol 1,4,5-trisphosphate and diacylglycerol, which can in turn induce the release of calcium and activate protein kinase C, respectively (41, 42). To determine if activation of other HER
family members also contribute to the mobilization of intracellular calcium, HER transfected 3T3-7d cell lines were stimulated with HRG and the level of intracellular calcium determined using the fluorescent calcium chelator Indo-1. In cell lines expressing HER1 alone or when co-expressed with HER2, HER3, or HER4, EGF induced an increase in intracellular calcium levels (Fig. 6A). In contrast, HRG did not have any effect on intracellular calcium in all cell lines tested including cells expressing HER2 + 3 and HER2 + 4 (Fig. 6, A and B). Stimulation of the endogenous mouse PDGF receptor with PDGF was used as a positive control to demonstrate that these cells are able to mobilize intracellular calcium flux in an unrelated receptor pathway (Fig. 6B). Removal of extracellular calcium by the addition of 5 mM EGTA to the extracellular media decreased the late phase of the calcium response, indicating that in addition to an intracellular component, extracellular calcium also contributes to the overall response (Fig. 6C).

Having shown that HRG is unable to induce a detectable calcium response, we set out to determine whether HRG was also unable to induce PLCγ1 phosphorylation. Stimulation of the 3T3-7d transfectants expressing HER1, HER1 + 2, HER1 + 3 or HER1 + 4 with EGF or HRG resulted in an EGF, but not HRG, dependent increase in PLCγ1 tyrosine phosphorylation (Fig. 7). The level of PLCγ1 phosphorylation did not significantly change by the co-expression of other HER family members with HER1, suggesting HER1 by itself is responsible for this action. Cells expressing HER2 + 3 and HER2 + 4 were also unable to induce PLCγ1 phosphorylation in response to HRG (data not shown). Together, these data demonstrate that the activation of HER complexes by HRG and subsequent induction of cellular transformation does not require the immobilization of intracellular calcium or the activation of targets related to the generation of second messengers such as PLCγ1.

**DISCUSSION**

In analyzing the expression profile of HER family members in both normal and tumor tissues, it is often the rule and not the exception that multiple members are expressed within a given cell type. Our data suggest that expression of multiple HERs within a cell is required for efficient transformation of cells in response to ligand. None of the receptors when expressed alone could efficiently induce growth in soft agar. The lack of transformation by homodimer expression was not due to a lack of receptor kinase activity, for all homodimer complexes, especially HER1, demonstrated an increase in receptor tyrosine phosphorylation in response to ligand. This is supported by the fact that expression of Tneu, a potent transforming oncogene, induced colony formation in cells that co-expressed either HER1, HER3, or HER4 but not when expressed alone or co-expressed with HER2. The complementation of Tneu with HER1, HER3, and HER4 also suggests that Tneu can heterodimerize with other family members, although the biochemical identification of this heterodimer was not investigated. Lastly, cells expressing HER1 + 2 were able to form aggressive tumors in animals in a short time frame in contrast to cells expressing HER1 or HER2 alone. This was also reflected in the cells expressing HER2 + 3 in comparison to cells expressing HER2 and HER3 alone. Interestingly, cells expressing HER1 + 3 and HER1 + 4 were not tumorigenic. In fact, co-expression of HER3 or HER4 with HER1 decreased the low level of tumor formation seen in HER1-expressing cells alone. This suggests that HER3 and HER4 are regulating the activity of HER1 in vivo, and clearly distinguish themselves from the activity seen with HER2 co-expression. Combining the soft agar results with the in vivo data identifies that the expression of multiple HERs is required for efficient transformation of cells. Further, the HER1 + 2 and HER2 + 3-expressing cells appear to be more biologically active. This correlates with studies that identified the HER1 + 2 complex as being tumorigenic (29, 43) and parallels the finding that HER1 + 2 and HER3 + 2 complexes are preferentially formed in response to ligand (44).

The lack of transformation by expression of a single HER was surprising given the historical accumulation of data in which the overexpression of one HER member in NIH3T3 cells conferred growth in soft agar in response to ligand; and overexpressed or activated HER2 was shown to be transforming in the absence of ligand (5, 13–17). An explanation for the differences between our results and the previous studies is that the earlier studies made use of NIH3T3 cells which express low levels of EGFR and c-neu. This low level of endogenous receptor expression may be able to synergize with an overexpressed HER family member. This hypothesis is supported by the observation that HER4 transfected NIH3T3 cells were able to form colonies in agar in response to both EGF and HRG whereas the parent cell line could not (17). Further, this should send a strong message of caution upon investigation of the role of HERs in tumor cell lines since multiple HER expression is generally the rule (45).

Within the set of HER transfectants, cells expressing HER1 + 3 and HER1 + 4 have the capacity to be activated by both HRG and EGF. However, only the HER1 + 4-expressing cell line formed colonies in response to both ligands, whereas the cells expressing HER1 + 3 responded only to HRG and not EGF. The lack of soft agar growth of the HER1 + 3-expressing cells was not due to a lack of ligand responsiveness. EGF induces the phosphorylation of both HER1 and HER3 (Fig. 2) as well as the downstream targets SHC and the p85 regulatory subunit of phosphatidylinositol-3 kinase. A possible explanation is that EGF stimulation of a HER1 + 3 complex may be inducing a differentiating signal, as was demonstrated by nerve growth factor stimulation of 3T3 cells expressing TrkA (46). The level of ligand independent HER tyrosine phosphorylation varied depending upon which other receptor was co-expressed in the same cell. These data suggest that receptors can associate with one another and regulate their kinase activity in the absence of ligand. Perhaps these complexes function in vivo by demonstrating an increased sensitivity to low concentrations of ligand. Stimulation of the transfectants with ligand induced the phosphorylation of homodimers as well as both receptors within a heterodimer complex. The level of ligand-
FIG. 6. Mobilization of intracellular calcium in 3T3 transfectants by EGF, but not HRG. Changes in intracellular calcium were monitored as outlined under "Experimental Procedures." A, stimulation of mobilization of intracellular calcium in HER 1, HER 1 + 2, HER 1 + 3, or HER 1 + 4 transfectants. EGF (400 ng/ml) or HRG (400 ng/ml) were added at the times indicated by the arrows. B, stimulation of mobilization of calcium by PDGF but not EGF or HRG in HER2 + 3 and HER2 + 4 transfectants. Cells were stimulated with either single ligands alone (HER2 + 4) or with EGF (400 ng/ml) or HRG (400 ng/ml) followed by PDGF (80 ng/ml). The ligands were added at the times indicated by the arrows. C, effect of depletion of extracellular calcium on EGF-stimulated mobilization of calcium in HER1 + 2 and HER1 + 3 transfectants. Cells were resuspended in the analysis buffer containing calcium (labeled -EGTA). Where appropriate, 5 mM EGTA was added (at the first arrow; labeled +EGTA) and the cells were then stimulated with 400 ng/ml EGF (second arrow).
induced receptor phosphorylation was dependent upon the co-expressed receptor. For example, the level of EGF-induced HER1 phosphorylation was higher when expressed as a homodimer, with HER3 or HER4 as compared to when co-expressed with HER2. This suggests that HER1 is regulated differently in a HER1 + 2 complex than in other heterodimer complexes. We did not see a significant induction of HER1 phosphorylation by HRG in cells expressing HER1 + 3. This is in contrast to studies examining HER expression in hematopoietic cells (33). The lack of detectable response could be due to an already elevated level of HER1 phosphorylation which would make small changes difficult to detect. This might have been expected since previous studies have demonstrated that HER3 is a partially defective kinase (Fig. 2) (38). The implication that HER3 is an impaired kinase within the HER1 + 3 cells is in contrast to what was found when analyzing the HER2 + 3 heterodimer. HRG stimulation of HER2 + 3-expressing cells resulted in an increase in both HER2 and HER3 phosphorylation. We and others have seen similar results in other cell lines expressing HER2 and HER3. This implies that HER3 may not be a defective kinase, but rather limited in its specificity for appropriate substrate sites or regulated by its dimerizing partner (i.e. HER2). Our data demonstrate that HER3 kinase activity is weak when expressed as a homodimer or co-expressed with HER1 or HER4, but is more active when co-expressed with HER2. This is supported by the observation that stimulation with heregulin can elevate the level of HER2 phosphorylation in cells expressing HER2 and HER3, but not when HER3 contains an ATP-binding mutation (47).

An issue that is relevant to ligand stimulation and receptor activity is whether these cells produce any endogenous ligand that could impact these data. Clearly, addition of exogenous ligand is required to evoke a biological response. Further, conditioned media from these transfectants were unable to induce receptor phosphorylation (data not shown). HER1, HER2, and to a moderate extent HER4 are each phosphorylated in the absence of exogenous ligand (Fig. 2). This phosphorylation could either be the result of interaction with endogenous ligand or ligand-independent receptor dimer activity, as we have proposed. However, upon co-expression of additional HER family members we find that phosphorylation in the absence of exogenous ligand is often decreased. Further, these receptors are still responsive to the addition of exogenous ligand. If phosphorylation of single receptor transfectants were the result of endogenous ligand stimulation, one would expect multiple transfectants to be similarly, if not more, activated than their single transfectant counterparts.

The role of calcium in cell growth as well as the reduced requirement for calcium in transformed cells is well established (39). EGF activation of HER1 has been shown to stimulate PLCγ1 phosphorylation and intracellular calcium mobilization (40, 48, 55). Our data identified that EGF, but not HRG, stimulation of HER transfectant cell lines resulted in the mobilization of calcium from both intercellular and extracellular stores. This activity was dependent upon the expression of HER1, and was not markedly affected by the co-expression of any other HER family member within the same cell. Similarly, EGF stimulated the phosphorylation of PLCγ1, whereas HRG did not. This demonstrated that HRG-mediated transformation through HER3- and HER4-containing HER complexes does not require a detectable mobilization of calcium or the activation of PLCγ1. This is in support of other studies regarding a HER1/HER2 chimera (49) and the fibroblast growth factor receptor in which mitogenicity did not correlate with PLCγ1 activation (50, 56).

Several groups have identified that PLCγ1 is activated by, and is in a complex with, HER2 in cells overexpressing HER2 (51), oncogenic neu (52), or HER1/HER2 chimeric receptors that can be induced with EGF (52, 53). These results suggest that HER2 homodimers, formed by receptor overexpression, could result in the transphosphorylation of HER2 on the tyrosine residues required for the binding of PLCγ1. However, in our studies analyzing HRG-responsive, HER2-containing heterodimer complexes (HER2 + 3, HER2 + 4), HRG did not stimulate PLCγ1 phosphorylation or calcium mobilization, even though HER2 tyrosine phosphorylation was increased (Fig. 2). This suggests that neither HER3 nor HER4 is able to transphosphorylate HER2 on the same site(s) as found in the homodimer, and therefore a high affinity docking site for PLCγ1 may not exist. A similar scenario can be seen in analyzing the HER1 + 4 heterodimer complex. The activation of HER1 by EGF results in the autophosphorylation of tyrosine 992 which is the binding site for PLCγ1 (54). HRG activation of cells expressing HER1 + 4 results in the increased phosphorylation of both HER1 and HER4 (Fig. 2). However, HRG stimulation does not alter PLCγ1 phosphorylation or calcium mobilization, which suggests that HER4 cannot transphosphorylate HER1 at tyrosine 992 to create the appropriate PLCγ1 binding site. Taken together, HER1 seems to be the critical component for stimulating PLCγ1 and calcium-related pathways. This occurs only when cells are stimulated with EGF and not HRG, and is unaffected by the co-expression by other HER family members. This suggests that the HER1 homodimer is responsible for activating this pathway. Further, these data suggest that the pattern of receptor tyrosine phosphorylation within a HER dimer will depend upon the specificity of each HER kinase domain. This difference in substrate recognition expands the diversity of signals generated within different HER heterodimer complexes.

In summary, the raison d’être for the HER family seems to be that of complementation and diversity. The formation of heterodimer complexes, as opposed to homodimer complexes, is likely to be the predominant means to transmit a diversity of signals. Within the context of tumor cell lines that generally express three or more HER family members, the relative ratio of receptor expression will likely govern the biological outcome of receptor activation. A better understanding of which complexes are preferentially formed within a given cellular context and their relationship to ligand accessibility bring us one step closer in creating reagents that could potentially intervene in the progression of cellular transformation.

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