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Characteristics of EGFR Family-Mediated HRG Signals in Human Ovarian Cancer

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Abstract The ability of the epidermal growth factor receptor (EGFR) family members, EGFR, HER2, HER3, and HER4, to form homo- and heterodimers after interaction with different ligands expands the signal diversity of these proteins. We investigated their mechanism of activation by exogenous EGF and heregulin (HRG) in human ovarian carcinoma cell lines which express different amounts and combinations of the four receptors. Consistently the predominant interaction after EGF treatment was between EGFR and HER2, whereas activation of HER3 and HER4 depended on the relative abundance of the four receptors in the cells. Remarkably HER3 activation by HRG could occurs independent of HER2, and in one cell line almost no HER4 activation by HRG was detected despite high levels expression. Both EGF and HRG induced activation of mitogen-activated protein kinase (MAPK), but the time course of MAPK activation differed depending on the hetero-dimers induced. EGF and HRG mediated cell growth through the EGFR/HER2 heterodimer and HER4, respectively, but not through HER3 when it was the only HRG receptor expressed and phosphorylated in the cells. These findings reveal a distinct pattern of HRG induced EGFR family interaction in ovarian cancer that is distinct from that described in human breast cancer. Moreover EGF and HRG can exert distinct biological functions depending on the receptor complexes induced in a given ovarian cancer cell line. J. Cell. Biochem. 73:522–532, 1999.

Key words: EGFR family; ovarian cancer; HRG; EGF; signal transduction

The human EGF receptor (EGFR) family consists of four membrane-bound proteins with intrinsic tyrosine kinase activity: EGFR, HER2, HER3, and HER4 [Plowman et al., 1993a; Kraus et al., 1989a; Yamamoto et al., 1986a; Ullrich et al., 1984a]. These four receptors are expressed in normal epithelial, mesenchymal, and neuronal tissues, but are also frequently amplified and/or overexpressed in some human cancers [Rajkumar et al., 1994; Hynes et al., 1994; Gullick, 1991; Earp et al., 1995], suggesting a role for the EGFR family in tumorigenesis and cancer progression. Several growth factors are known to bind and activate members of the EGFR family: EGF, TGFα, amphiregulin, heparin-binding EGF-like growth factor, epiregulin can all activate EGFR [Toyoda et al., 1998a; Toyoda et al., 1998a; Groenen et al., 1994a], while heregulin (HRG) or the rat homologous neu-differentiation factor (NDF) [Ben-Baruch et al., 1994] can bind and activate both HER3 and HER4 [Tzahar et al., 1994; Carraway, III et al., 1994; Plowman et al., 1993]. Recently, betacellulin, a protein with overall homology to the different forms of the EGF family, has been demonstrated to bind and activate HER2/HER3 complexes and HER4 [Alimandi et al., 1997; Riese, II et al., 1996]. Binding and activation of HER4 was reported even for epiregulin [Riese, II et al., 1998]. None of these growth factors binds directly to HER2.

The cross-talk between members of the EGFR family was initially analyzed in cells in which the receptors were ectopically expressed alone or in combination. Besides the predominant transactivation between EGFR and HER2 [Wada et al., 1990; Qian et al., 1992], EGF can also induce dimers formation between EGFR and HER3 [Soltoff et al., 1994; Kim et al., 1994] or HER4 [Riese, II et al., 1998; Cohen et al., 1996]. Heterodimerization is also induced by HRG. By using HRG as ligand, HER3 can recruit HER2 to form a high-affinity receptor, in which HER3 is unidirectionally tyrosine phosphorylated by the HER2 kinase [Wallasch et

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al., 1995; Sliwkowski et al., 1994; Alimandi et al., 1995]. Bidirectional transphosphorylation between HER4 and HER2 has been described in transfected Chinese hamster ovary (CHO) cells [Plowman et al., 1993b] and in transformed rat thyroid epithelial cell [Mincione et al., 1998]. These observations suggest that the homo- and heterodimerization between the members of the EGFR family as well as the ability of their ligands to bind and activate more than one receptor underlie the complex signaling pathway of these membrane-bound proteins. The inter-receptor interactions allow the EGFR to recruit the PI3 kinase through heterodimerization of EGFR with HER3, the only member of the family able to bind the p85 subunit of the PI3 kinase [Soltoff et al., 1994; Kim et al., 1994].

In analyses of the HRG signaling pathway in breast cancer cells, HER3 activation was found to be strictly HER2-dependent in breast cancer epithelium [Graus-Porta et al., 1997; King et al., 1988; Karunagaran et al., 1996]. HER2 retention in the endoplasmic reticulum by coexpression of single chain (ScFv) anti-HER2 antibody in mammary cancer cell lines was shown to reduce the binding affinity of HRG to its receptors. In addition, HRG-induced phosphorylation of HER4 appears to be cell-specific [Egan et al., 1993; Chen et al., 1996; Beerli et al., 1995].

Unlike the mammary cell system, the activation of the EGFR family in ovarian cancer cells is still poorly understood. Conflicting data have been reported about HRG-induced signaling in the ovarian carcinoma cell line OVCAR3, in which HER3 and HER4 phosphorylation induced by HRG appears to be HER2-independent [Beerli et al., 1995], suggesting another level of complexity in the cross-talk of the EGFR family in different histological types and different environments.

We analyzed the mechanism of activation of the EGFR family in human ovarian carcinoma cell lines endogenously expressing various amounts and combination of these receptors. We also examined the ability of the different combinations of receptors to generate signals leading to activation of the mitogen-activated protein kinase (MAPK) and to cell proliferation. Our findings suggest that HRG-induced signaling is mediated through a different pattern of interaction in ovarian cancer than that described in the human breast carcinoma. In particular, HER2/HER3 complexes, which are reportedly the predominant and stronger interaction in both human breast cancer cells and transfected cells, are not detected in ovarian tumor cells.

MATERIALS AND METHODS

Tumor Cell Lines and Antibodies

SKOV3, IGROV1, and SKOV8 cells were maintained in RPMI 1640 supplemented with 10% FCS; PA1, OVCAR3, 2780, and 2774 cells were maintained in minimum essential medium (MEM) supplemented with 10% FCS and 1% nonessential amino acid; OAW42 cells were maintained in MEM supplemented with 10% FCS; CAOV3 cells were maintained in Dulbecco's modified Eagle medium (DMEM)/high glucose supplemented with 10% FCS; CAOV4 cells were maintained in L15 (Leibovitz medium) supplemented with 20% FCS. IGROV1 cells were obtained from Dr. Bénard (Institut. Roussy, Villejuif, France) and OAW42 cells were from DKFZ (Germany); all other cell lines were from the American Type Culture Collection (ATCC; Rockville, MD).

The following polyclonal and monoclonal antibodies (Mab) were used: mouse MAb 108.1 [Honegger et al., 1989], raised against the extracellular domain of the EGFR; rabbit polyclonal RK2 [Kris et al., 1985] raised against residues 984–996 of the human EGFR; rabbit polyclonal anti-HER2 against residues 1006–1027 of the human HER2; rabbit polyclonal anti-HER3 Ab, against a peptide corresponding to the C-terminal 17 amino acid residues of human HER3, mouse anti-HER4 Ab (Santa Cruz Biotechnology, Santa Cruz, CA) against the residues 1291–1308 of the human HER4 and mouse MAb 5E2 (41) against phosphotyrosine.

Heregulin Preparation

Human recombinant GST-αHRG [Wallasch et al., 1995] was produced in *E. coli* and purified with glutathione beads according to the manufacturer purification protocol (Pharmacia, Gaithersburg, MD). The purified recombinant protein was dialyzed overnight against a 1× PBS, 10% glycerol solution.

Receptor Phosphorylation Analysis

Human ovarian cancer cell lines were stimulated with 20 nM EGF and 6 μg/ml α-HRG for 10 min and, as a control, with GST alone for 10

min at 37°C. Cells were then washed with cold 1× PBS and lysed with lysis buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 5 mM EDTA, 0.5% Triton, 10% glycerol, 1 mM phenylmethylsulphonyl-fluoride, 2 mM Na-orthovanadate, 10 mM leupeptin, 100 mM Na-Fluoride, 10 mM Na-pyrophosphate). The crude lysate was then centrifuged at 12,000 rpm for 5 min at 4°C and 3 mg of pre-cleared lysate was immunoprecipitated with the appropriate antiserum and 15 µl of protein A-Sepharose for 3 h at 4°C. Immunoprecipitates were washed three times with 1 ml of washing buffer (20 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.1% Triton, 10% glycerol and the same protease and phosphatase inhibitors as in the lysis buffer) and eluted with 15 µl of Laemmli buffer. Samples were then boiled for 5 min at 95°C.

Western Blot Analysis

Cell lines were grown to confluence and lysed with lysis buffer (see above). Equal amount of proteins was separated in 7.5% SDS-polyacrylamide gels and electrophoretically transferred onto nitrocellulose filters. For immunoblot analysis, each filter was preincubated with 0.2% gelatine in 1× NET buffer (150 mM NaCl, 0.5 mM EDTA, pH 8.0, 50 mM Tris-HCl pH 7.5, 0.05% Triton) and incubated with primary antibodies for 2 h or overnight at 4°C. Membranes were then incubated with goat anti-mouse or goat anti-rabbit antibodies coupled to peroxidase for 1 h at room temperature, washed and the signal was revealed by chemiluminescence (ECL kit, Amersham, Arlington Heights, IL) according to the manufacturer's instructions.

MAP Kinase Assay

Cells lysates were prepared as described above and 30 µg of total protein was subjected to electrophoresis in a 12% SDS-polyacrylamide gel polymerized in the presence of 0.5 mg/ml of myelin basic protein (MBP; Upstate Biotechnology, Inc., Lake Placid, NY). The gel was fixed in 20% isopropanol, 50 mM Tris-HCl, pH 8.0, for 1 h, followed by five washes in 50 mM Tris-HCl, pH 8.0, 5 mM β-mercaptoethanol. Proteins were denatured by incubating the gel for 1 h in 6 M guanidine-HCl (two changes), and renatured in 50 mM Tris-HCl, pH 8.0, 0.04% Tween 40, 5 mM µl β-mercaptoethanol for 16 h at 4°C. Gels were washed five times with 40 mM Hepes/NaOH, pH 8.0, 2 mM dithiothreitol, 10 mM $MgCl_2$ and incubated for 1 h at room temperature in 10 ml of kinase buffer (40 mM Hepes/NaOH pH 8.0, 50 µCi [γ-^{32}P]ATP, 40 mM ATP, 10 mM $MgCl_2$, 0.5 mM EGTA). Finally gels were washed in washing solution containing 5% (w/v) TCA, 1% Na-pyrophosphate (tetra-Na-salt) for almost 2 h, dried, and subjected to autoradiography.

[^{3}H] Thymidine Incorporation

Cells were grown in 96-well plates to 60% confluency, when medium was replaced with a serum free medium for 48 h. Cells were treated with different concentrations of EGF and α-HRG for 24 h labeled with 1 µCi/well of methyl-[^{3}H] thymidine (Amersham, 1 mCi/ml; 82 Ci/mmol) the last 6 h. Cell monolayers were washed twice with ice-cold 1 × PBS, precipitated with 10% TCA and solubilized in 100 µl of 0.2 N NaOH/1% SDS. Lysates were neutralized with 100 µl of 0.2 N HCl, and incorporated radioactivity was quantitated by scintillation counting.

RESULTS

Expression of EGFR, HER2, HER3, and HER4 Receptors in Human Ovarian Carcinoma Cell Lines

Figure 1 summarizes the relative expression of four EGFR family members by 10 human ovarian carcinoma cell lines determined by Western blot analysis on total lysates. Four of these cell lines representing different combinations of the receptors were analyzed for EGFR family signaling. EGFR expression (Fig. 2A) was maximal in OVCAR3 cells followed by SKOV3, OAW42 and IGROV1. HER2 receptor expression (Fig. 2B) was high only in SKOV3 cells. IGROV1 and OAW42 cells expressed intermediate HER2 protein levels, which were six- and nine-fold less than those in SKOV3, respectively, whereas OVCAR3 cells expressed low levels of HER2. OVCAR3 cells expressed the highest levels of HER3 (Fig. 2C) followed by IGROV1 and SKOV3; OAW42 cells expressed no detectable HER3 protein. HER4 levels (Fig. 2D) were highest in OAW42 cells, followed by OVCAR3 cells, in which levels were three-fold lower; HER4 protein was undetectable in SKOV3 and IGROV1 cells.

EGF- and HRG-Induced Tyrosine Phosphorylation of EGFR Family in Ovarian Cancer Cells

Inter-receptor interactions of the four members of the EGFR family were investigated by

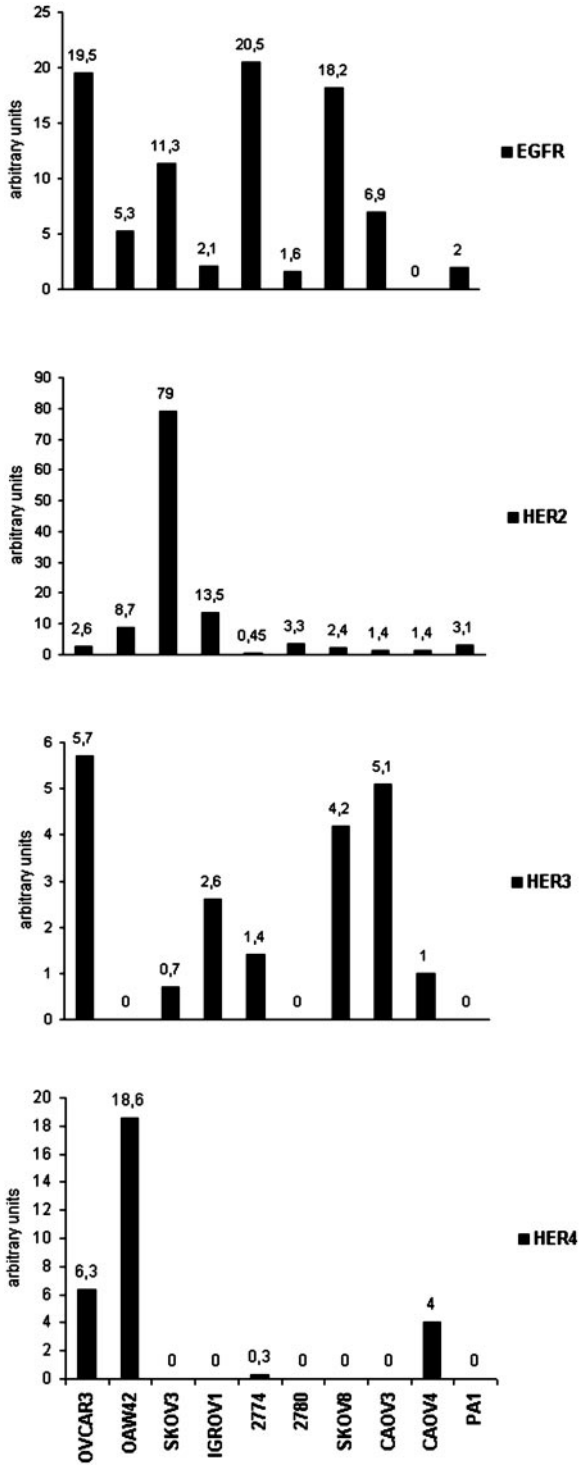

Fig. 1. Expression of EGFR family members in ovarian cancer cell line. Relative amounts of EGFR, HER2, HER3, HER4 protein were determined by Western blot analysis. Total lysate (50 µg) of each cell line was separated in a 7.5% SDS-polyacrylamide gel and immunoblotted with Abs against the receptors. Relative signal intensities was evaluated by densitometry and displayed as bar diagram.

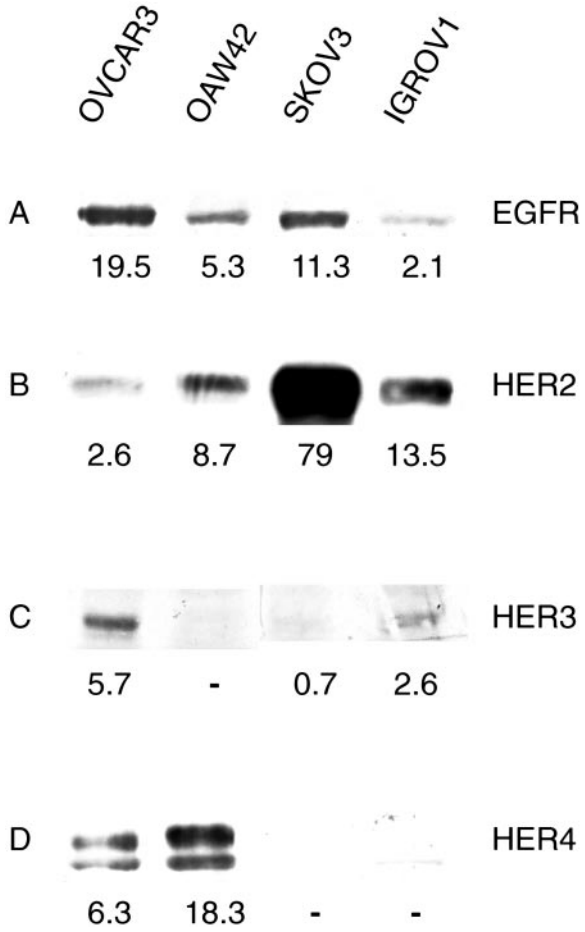

Fig. 2. Expression of EGFR (**A**), HER2 (**B**), HER3 (**C**), HER4 (**D**) protein in ovarian cancer cell lines. Total lysate (50 µg) of each cell line was separated in a 7.5% SDS-polyacrylamide gel and immunoblotted with Abs against the receptors. Numbers below each lane indicate the amount of protein as determined by densitometry.

analysis of EGF- and HRG-induced tyrosine phosphorylation in the selected ovarian cell lines. EGF stimulation of OVCAR3 cells (Fig. 3A) resulted in an increase in tyrosine phosphorylation relative to basal levels of the EGFR (lanes 1 and 2), HER2 (lanes 4 and 5), HER3 (lanes 7 and 8), and HER4 (lanes 10 and 11), indicating heterodimerization between the EGFR and the three other members of the receptor family or, alternatively, an initiation of the heterodimerization cascade beginning with EGFR activation. Stimulation of this cell line with HRG led to low level increase in HER3 (lanes 7 and 9) and a large increase in HER4 (lanes 10 and 12) tyrosine phosphorylation, but no increase in EGFR (lanes 1 and 3) or HER2 (lanes 4 and 6) phosphotyrosine content.

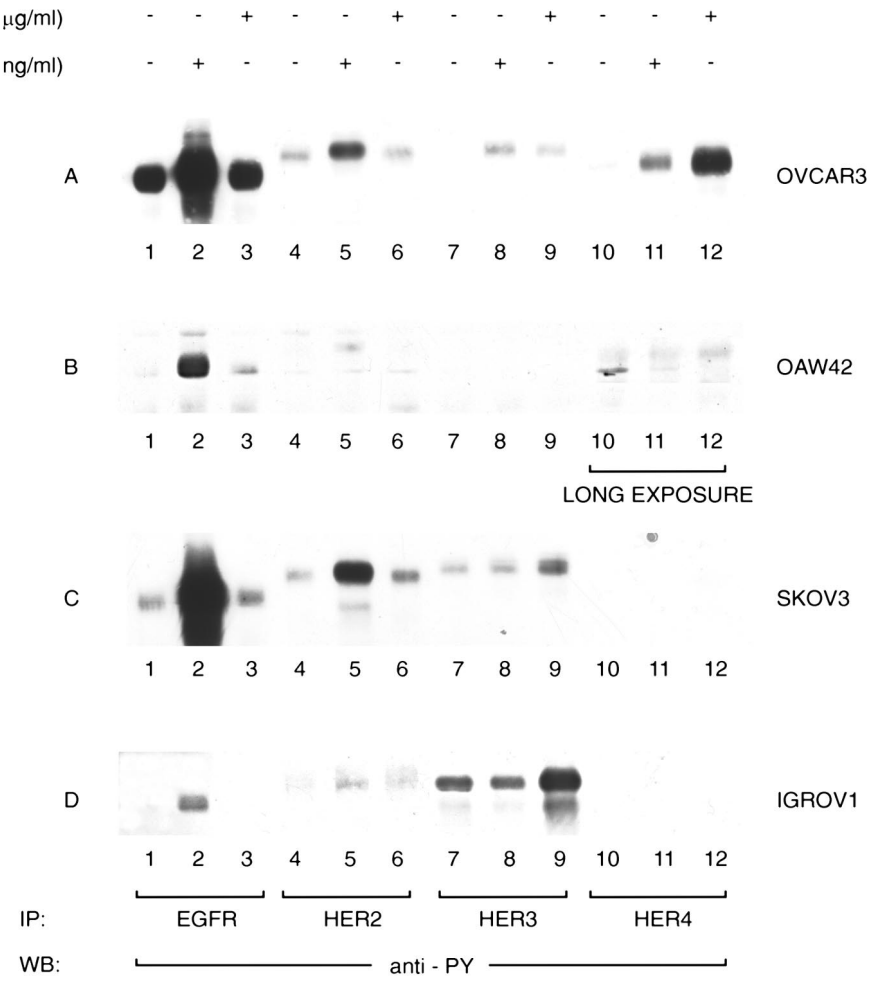

Fig. 3. Activation of EGFR family members as a function of combinatorial assortment of the receptors in OVCAR3 (**A**), OAW42 (**B**), SKOV3 (**C**), and IGROV1 (**D**) cells. Cells were lysed after treatment with EGF (20 ng/ml), αHRG (6 μg/ml), or GST alone as control. EGFR, HER2, HER3, and HER4 were immunoprecipitated with the appropriate antibodies. Bound proteins were separated in 7.5% SDS polyacrylamide gels, transferred to nitrocellulose, and reacted with anti-phosphotyrosine antibody. IP, Immunoprecipitation; WB, Western blot.

OVCAR3 cells appears to respond mainly through HER4 homodimer formation.

EGF treatment of OAW42 cells (Fig. 3B) which do not express detectable level of HER3, enhanced EGFR (lanes 1 and 2) and HER2 (lanes 4 and 5) tyrosine phosphorylation, but did not affect HER4 activation (lanes 10 and 11). This observation suggests a role of HER3 in EGF-stimulated tyrosine phosphorylation of HER4 as observed in OVCAR3 cells. HRG stimulation of OAW42 cells led to a small increase in HER4 tyrosine phosphorylation (lanes 10 and 12) detectable only after a long exposure, despite high expression levels (Fig. 2D, lane 2). A light increase in EGFR phosphorylation after HRG stimulation was detectable (lanes 1 and 3) but no HER2 activation was observed (lanes 4 and 6). These results suggest that cross-talk mediated by HER3 is critical for transmission of the HRG signal in ovarian carcinoma.

SKOV3 cells, which overexpress HER2 but no detectable HER4 (Fig. 3C), showed tyrosine phosphorylation of EGFR (lanes 1 and 2) and HER2 (lanes 4 and 5) over basal levels upon EGF stimulation. Unlike OVCAR3 cells, no increase in tyrosine phosphorylation of HER3 was detectable in SKOV3 cells (lanes 7 and 8). HRG stimulation of these cells induced tyrosine phosphorylation of HER3 (lanes 7 and 9) but not of EGFR (lanes 1 and 3) or HER2 (lanes 4 and 6) suggesting that HER3 can undergo tyrosine phosphorylation following HRG stimulation without HER2 activation and in the ab-

sence of HER4. In IGROV1 cells, the other cell line lacking HER4 expression, (Fig. 3D), EGF stimulation increased the tyrosine phosphorylation of EGFR (lanes 1 and 2) and barely of HER2 (lanes 4 and 5). HER3 phosphorylation was not affected (lanes 7 and 8). HRG stimulation of these cells induced tyrosine phosphorylation over basal level only for HER3 (lanes 7 and 9) and not for EGFR (lanes 1 and 3) or HER2 (lanes 4 and 6), an observation which further supported the possibility that HER3 activation can occurs via an alternative mechanism which is independent of HER2 or HER4.

MAPK Activation Induced by EGF and HRG

A major intracellular signaling pathway activated in response to growth factors involves MAPK (28–30). Analysis of MAPK activation in response to EGF and HRG in the four ovarian carcinoma cell lines (Fig. 4) indicated that both EGF and HRG activated MAPK in the four cell lines (Fig. 4), but with different kinetics. Indeed, maximal induction (eight-fold) was observed in OVCAR3 cells upon EGF stimulation (Fig. 4A), which matched the concerted activation of all EGFR family members by EGF as described above (Fig. 3). The time course of MAPK induction in response to EGF treatment indicated that EGF stimulation in OVCAR3 (Fig. 4A), OAW42 (Fig. 4C), and SKOV3 (Fig. 4E) led to sustained MAPK signal, whereas in IGROV1 cells, in which EGF induced only a barely detectable tyrosine phosphorylation of HER2, MAPK activation was transient since the signal strongly decreased during the observation time (Fig. 4G). These observations suggest a role for EGFR/HER2 cross-talk in determining the duration of MAPK activation in response to EGF. HRG treatment also induced MAPK activation in all cell lines examined. However, induction was low and transient in OAW42 cells (Fig. 4D), implying that HER3, which is not expressed in this cell line, plays an important role in transmitting the HRG signal that activates the MAPK signal. In OVCAR3 cells (Fig. 4B) MAPK activation was more sustained than in SKOV3 (Fig. 4F) or IGROV1 cells (Fig. 4H), which indicates that both HER4 and HER3 activation is required for sustained MAPK activation, whereas transient activation of MAPK may only require HER3.

Induction of DNA Synthesis in Response to EGF and HRG Treatments

Analysis of the mitogenic response induced by EGF revealed a similar extent of DNA synthesis in OVCAR3, OAW42, and SKOV3 (Fig. 5A,B, and C, respectively), in which the maximal mitogenic response was reached using EGF at concentrations between 1 and 10 ng/ml, whereas no stimulation was found in IGROV1 cells (Fig. 5D). The mitogenic effect of EGF is consistent with the activation pattern of the receptors and the MAPK pathway, suggesting that formation of an EGFR-HER2 heterodimer potentiates the biological effect of EGF.

HRG at the concentrations between 1 and 10 μg/ml induced DNA synthesis in OVCAR3 (Fig. 5E) and in OAW42 cells (Fig. 5F); HRG at 100 μg/ml was toxic for all cell lines. No effect of HRG was detected in SKOV3 (Fig. 5G) or IGROV1 (Fig. 5H), both of which lack detectable HER4 receptors. These results suggest that HER4 is involved in transmitting a proliferative effect.

DISCUSSION

Our analysis of the EGF- and HRG-induced signaling in human ovarian carcinoma cell lines indicates major differences from the cross-talk of EGFR family members observed in breast carcinomas and in cells transfected with these receptors.

EGF-Induced Transphosphorylation of All Four EGFR Family Members When Coexpressed in the Same Cell Line

When EGFR and HER2 are coexpressed in a cell line, EGF treatment leads to the activation of both receptors. As reported in different systems, EGF binds with a higher affinity to EGFR/HER2 complexes than to EGFR homodimers [Goldman et al., 1990; Wada et al., 1990]. Our results also indicate that EGFR/HER2 is the favored receptor interaction in ovarian cancer cells in response to EGF, except in IGROV1 cells, which express both receptors, but show barely detectable HER2 activation. Thus, other variables such as alteration in the HER2 extracellular domain or defects in receptor translocation from the cytoplasm to the membrane may play a role in heterodimer formation. While the prognostic value of HER2 in breast carcinomas is well-accepted [Slamon et al., 1987], it is less clear in ovarian carcinomas [Rubin et al., 1993;

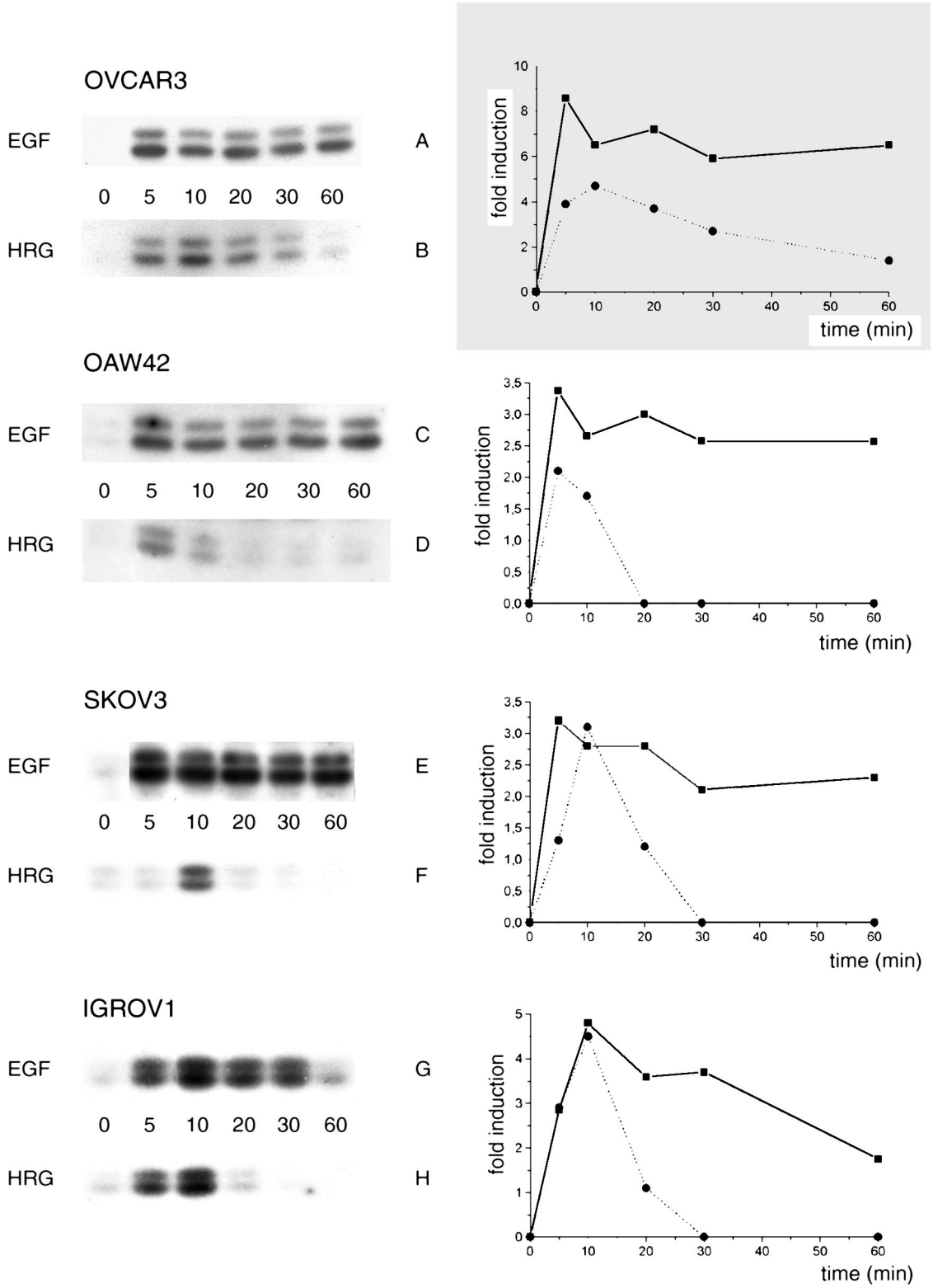

Fig. 4. Time course of MAPK activation in response to EGF and αHRG treatment in OVCAR3 (**A,B**), OAW42 (**C,D**), SKOV3 (**E,F**), and IGROV1 (**G,H**) cells. Cells were treated with 20 ng/ml of EGF and 6 µg/ml of αHRG for the indicated times. Total lysate (30 µg) was subjected to electrophoresis in a 12% SDS polycrilamide gel containing 0.5 mg/ml of MBP as substrate (left panel). Stimulation of MAPK activity (right panel) was calculated as fold-induction of the untreated cells (0 min). ■, EGF; ●, HRG. The experiment was performed two times with similar results.

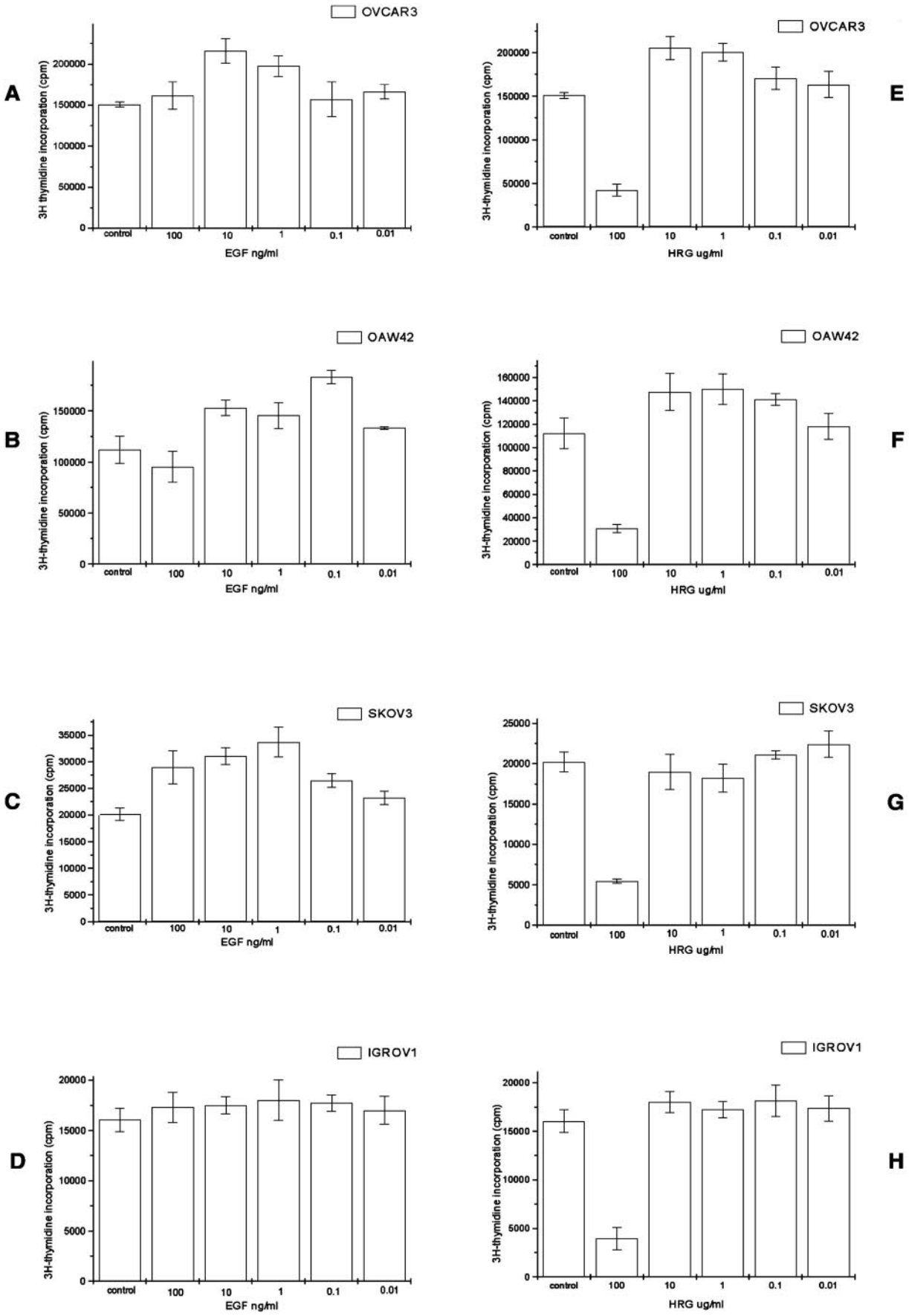

Fig. 5. Effect of EGFR family on the mitogenic responses of ovarian carcinoma cells to EGF and αHRG. OVCAR3 (**A,E**), OAW42 (**B,F**), SKOV3 (**C,G**), and IGROV1 (**D,H**) cells were grown to 60% confluence in 96-well plates. Medium was replaced with serum-free medium for 48 h, and cells were stimulated for 24 h with EGF and αHRG at the indicated concentrations. Cells were radiolabeled for the last 4 h with 1 μCi/well of [^{3}H] thymidine. Thymidine incorporation (cpm) is given as media plus SD of four determinations in two independent experiments with similar results.

Knyazev et al., 1992]. It seems possible that defects in cross-talk with EGFR might be the basis for the different behavior of the two histotypes.

HER3 activation by EGF has been observed in cotransfection experiments with EGFR and HER3 as well as in the epidermoid carcinoma cell line A431 [Soltoff et al., 1994], but not in T47D breast cancer cells, which coexpress EGFR and HER3 [Graus-Porta et al., 1995]. We found that EGF induces cross-talk between EGFR and HER3 in OVCAR3 cells but not in SKOV3 or IGROV1 cells. Similarly, HER4 is transphosphorylated by the activated EGFR in OVCAR3, but not in OAW42 cells despite a similar expression level of both receptors. The major criteria in which these cells differ is the level of HER2 expression: OVCAR3 cells express high levels of EGFR, HER3 and HER4 but very low levels of HER2, whereas in the other three cell lines in which EGFR showed no cross-talk with HER3 or HER4, expression of HER2 was sufficiently high to allow EGFR/HER2 heterodimer formation which is predominant.

HER3 Activation by HRG is Independent of HER2 and HER4

HRG-induced signaling of the EGFR family in ovarian cancer appears to be more complex. The HER3 receptor presents only an attenuated kinase activity due to the substitution of 3 amino acids in the catalytic domain that are normally shared between tyrosine and the serine/threonine protein kinases [Hanks et al., 1991]. HER3 "activation" has been reported to be mediated by heterodimerization with and transphosphorylation by other receptors of the EGFR family. This heterodimer formation was observed in breast carcinoma cell lines [Chen et al., 1996; Graus-Porta et al., 1997; Karunagaran et al., 1996] and in transfected cells [Kramarsky et al., 1996; Tzahar et al., 1996; Riese, II et al., 1995], in which the predominant form of the HRG-activated receptor was HER2/HER3. Because of the defective HER3 kinase, HER2 unidirectionally phosphorylated HER3 [Karunagaran et al., 1996; Wallasch et al., 1995; Alimandi et al., 1995]. Beerli et al. analyzed NDF-mediated signaling in one ovarian cancer cell, OVCAR3; surprisingly, the role HER2 plays in NDF-induced signaling appears to be of less importance to its role in breast cancer cell lines. In this study, we show that the HER3 activation-HER2 independent is not a characteristic of only the OVCAR3 cell line, but is related to an ovarian histotype, since three lines expressing both HER3 and HER2, HER3 was functionally activated in response to HRG without an increase in HER2 tyrosine phosphorylation.

Moreover, the absence of HER4 in two of these cell lines (SKOV3 and IGROV1) excludes the formation of HER3/HER4 heterodimers. The mechanism by which HER3 undergoes tyrosine phosphorylation after HGR stimulation remains unclear. HER3 activation mediated by homodimer formation is unlikely in light of the HER3-attenuated kinase activity of HER3. Rather, an unidentified molecule with tyrosine kinase activity may be involved in HER3 activation.

HER4 Activation Depends on the Cellular Context

HER4 protein was detected in only four of the 10 cell lines tested; two of these cell lines, OVCAR3 and OAW42, showed similar levels of overexpression but differential responsiveness to HRG stimulation. Indeed, HER4 was activated by HRG in OVCAR3 but to a very low extent in OAW42 cells, which revealed a faint band of the phosphorylated HER4 only after long exposure (Fig. 3B, lane 12). In HER4-transfected CHO cells, HER4 undergoes tyrosine phosphorylation in response to HRG through homodimerization [Plowman et al., 1993b]. In addition, all the isoforms of NDF under different conditions were found to activate HER4 in two breast carcinoma cells MCF7 and MCF10A [Beerli et al., 1995]. The lack of responsiveness of HER4 to its ligand in some ovarian carcinoma cell lines such as OAW42 lacking HER3 might reflect the key role of HER3/HER4 heterodimers in HRG-induced activation. Although we cannot exclude the presence of a defective HER4 receptor, it seems more likely that HRG signaling is still poorly understood and may involve molecules outside the currently known EGFR family or other cytoplasmic enzymes.

MAPK Activation Kinetics Differs According to Inter-Receptor Interactions Induced by EGF and HRG

The differences in receptor activation are reflected in the downstream signaling pathway to MAPK. Indeed, although activation of EGFR family members led to MAPK activation in all ovarian cancer cell lines examined, the kinetics of activation depended on the kind of receptor complexes induced in each cell line. Either

transactivation between EGFR/HER2 in response to EGF or the presence and activation of both HER3 and HER4 appears to be responsible for prolonged MAPK activation. In contrast, activation of EGFR homodimers or of HER3 in the absence of HER4 leads to a transient MAPK activation. In our study the induction of transient or sustained MAPK signals clearly correlates with induction of proliferation; but only sustained activation leads to proliferation.

EGFR/HER2 Heterodimers and HER4 Transmit a Mitogenic Signal When Activated by EGF and HRG, Respectively

Analysis of EGF and HRG mitogenic activity on the ovarian carcinoma cell lines definitively revealed distinct roles of the complexes induced. In particular, it was possible to distinguish between HER3 and HER4 signaling: HER4 was sufficient to induce a growth signal in the cells even in the absence of HER3 expression, whereas HER3 alone did not induce DNA synthesis even when highly phosphorylated as in IGROV1 cells. The signal transmitted by HER3, unlike the HER4 proliferative signal, appears to be related to adhesion. Consisting with this notion is the recent report that HRG stimulation of breast carcinoma cell line SKBr3, stimulated fibronectin degradation [Hijazi et al., 1994]. We should notice that [^{3}H]-thymidine incorporation was high in control cell lines grown in serum free media for 2 days, suggesting an autocrine loop for growth factors.

In conclusion, our study finds that: 1) the coexpression of EGFR and HER2 generally favors their transactivation induced by EGF and leads to a potent mitogenic signal, although heterodimer formation is not a general role; 2) HER3 and HER4 do not cross-talk with EGFR and HER2 upon stimulation with HRG; 3) HER4 activation by HRG leads to cell growth both when activated alone or with HER3; and 4) HER3 activation by HRG induces changes in cell morphology.

The findings reported here contribute important insight into tissue-specific characteristics of EGFR family mediated oncogenic signals which will be valuable for the development of target specific intervention therapies in ovarian cancer.

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