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Abstract The levels of expression of the four receptors and eleven ligands composing the epidermal growth factor family were measured using immunohistochemical staining in one hundred cases of breast cancer. All of the family were expressed to some degree in some cases; however, individual cases showed a very wide range of expression of the family from essentially none to all the factors at high levels. The highest aggregate level of expression of a receptor was HER2 followed by HER1, then HER3, then HER4. The ligands (including two splice variants of the NRG1 and NRG2 genes) broadly fell into three groups, those with the highest aggregate expression were Epigen, Epiregulin, Neuregulin 1 α , Neuregulin 2 α , Neuregulin 2 β , Neuregulin 4 and TGF α , moderate expression was seen with EGF, Neuregulin 1 β and Neuregulin 3, and relatively low levels of expression were seen of HB-EGF, Betacellulin and Amphiregulin. Statistical analysis using Spearman's Rank Correlation showed a positive correlation of expression between each of the factors. Analysing the data using the Cox Proportional Hazards model showed that, in this dataset, the most powerful predictors of relapse free interval and overall survival were the combined measurement of only Epigen and Neuregulin 4.

Keywords (separated by '-') ErbB - Growth factor - Growth factor receptor - Prognosis - Breast cancer

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2 **The complete family of epidermal growth factor receptors**
3 **and their ligands are co-ordinately expressed in breast cancer**

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5 Philip J. Brown · Colin G. Johnson ·
6 William J. Gullick

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9 **Abstract** The levels of expression of the four receptors
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19 NRG1 and NRG2 genes) broadly fell into three groups,
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Keywords ErbB · Growth factor · 32
Growth factor receptor · Prognosis · Breast cancer 33
34

Introduction 35

The epidermal growth factor family of receptors and 36
ligands consist of four genes encoding receptors and 37
at least eleven genes encoding ligands [1]. Four of the 38
ligands, collectively known as the Neuregulins, are 39
expressed as multiple splice variants [2] and the latest 40
receptor to be discovered, HER4, is made in at least four 41
different forms also due to mRNA splicing [3]. The 42
receptors are stabilised in an active state as homodimers or 43
heterodimers following ligand binding [4]. Exactly, which 44
forms are assembled in vivo is contingent on the repertoire 45
of ligands available in the environment and their relative 46
affinities for each receptor type individually and possibly 47
for preferences for binding to particular dimer pairs. We 48
have attempted previously to construct a computer simu- 49
lation of this process [5] (<http://www.cs.kent.ac.uk/people/rpg/em84/CellApplet1.html>) in which a patch of cell 50
membrane can be populated with different numbers of each 51
receptor type and each of the eleven ligands can be intro- 52
duced to initiate the assembly of the various receptor 53
pairwise combinations. This when run to equilibrium 54
should resemble the state of the system in a simple mem- 55
brane bilayer. 56
57

Overexpression of most, if not all, of the receptors and 58
some of the ligands has been detected in breast cancer 59
biopsies and cell lines. Antibodies or small molecule 60

A1 **Electronic supplementary material** The online version of this
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A3 material, which is available to authorized users.

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61 tyrosine kinase inhibitors have been evaluated targeted to
62 members of the system and some of these have been
63 introduced as clinical treatments for selected patients with
64 some success [6]. It would be helpful, however, to under-
65 stand and predict the activation state of the system in
66 individual patients so that the choice of the available
67 inhibitors can be most precisely made to ensure that
68 appropriate drugs are given and that those that are used can
69 be employed most cost-effectively.

70 Despite nearly 50 years of research and a long term
71 appreciation of the potential importance of this family of
72 molecules in breast and other cancer types as yet there has
73 been no study published to our knowledge that described
74 the expression patterns of the complete family of receptors
75 and ligands in breast cancers at the protein level. Indeed
76 some of the more recently described ligands such as Epigen
77 [7] and Epiregulin [8] have not so far been studied in a
78 series of clinical specimens. We report here using immu-
79 nohistochemical staining a study describing the complete
80 family in one hundred cases of unselected breast cancers.

81 Materials and methods

82 One hundred cases of breast cancer were obtained from
83 Professor Adrian Harris and Dr Russell Leek, Cancer
84 Research UK, Oxford, UK in the form of a tissue array.
85 Ethical approval for use was obtained from Oxfordshire
86 Clinical Research Ethics Committee. The patients were
87 treated by standard protocols, which were updated regu-
88 larly according to national guidelines. ER positive patients
89 received tamoxifen for 5 years, node positive patients
90 under 60 also received 6 cycles of intravenous CMF.
91 Patients treated with wide local excision also received
92 adjuvant radiation therapy. The composition of the patients
93 is described in Supplementary Table 1 including age range,
94 grade, tumour size, ER status, node status, menopausal
95 status, whether treated by chemotherapy or hormonal
96 therapy and follow up. The study was conducted and
97 reported cohering to the guidelines published in McShane,
98 LM, et al. Reporting recommendations for tumour marker
99 prognostic studies. *J Clin Oncol.* 2005 Dec 20; 23(36):
100 9067–9072.

101 The antibodies used were mostly produced in the labo-
102 ratory of Professor Gullick (Table 1). The antibody to EGF
103 was a kind gift of the late Dr Harry Gregory. The anti-
104 bodies to Epigen (Catalogue number AF1127) and to
105 Epiregulin (Catalogue number AF1195) were purchased
106 from R&D Systems, Minneapolis, USA and the antibody to
107 TGF α (Catalogue number GF10) from Calbiochem,
108 San Diego, USA. Immunohistochemical staining was per-
109 formed using the primary antibodies described earlier and
110 the StreptABCcomplex HRP Duet Mouse/Rabbit detection

Table 1 Antibodies used in this study

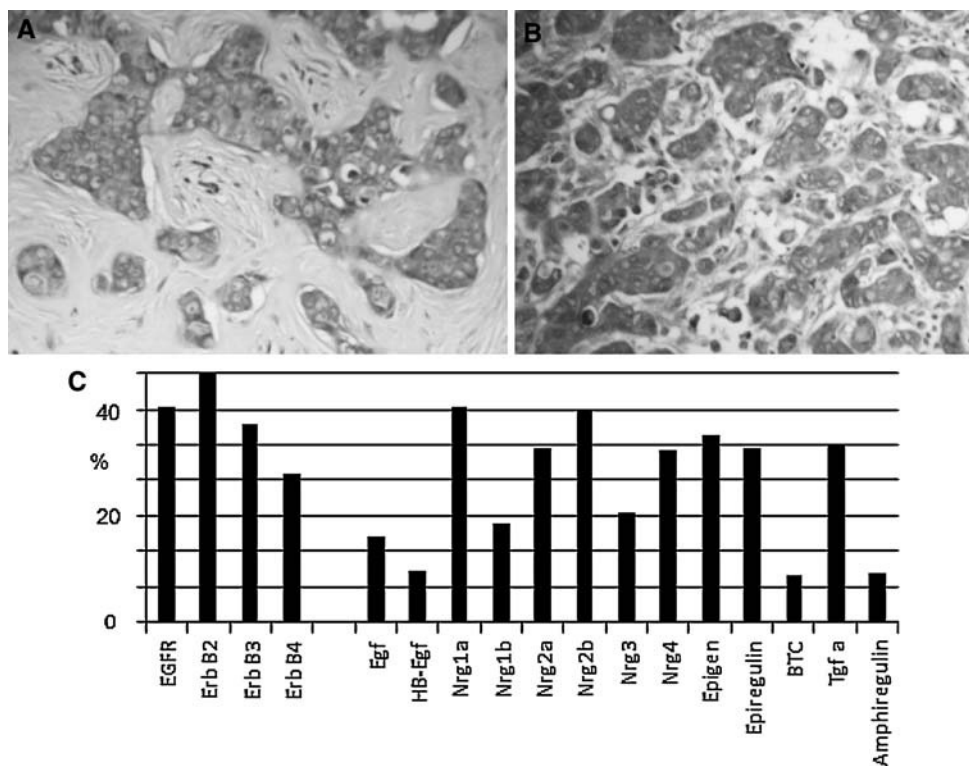
EGF receptor	F4	Mouse mAb	Gullick et al. [9]
HER2	21 N	Rabbit polyclonal	Gullick et al. [10]
HER3	RTJ2	Mouse mAb	Rajkumar et al. [11]
HER4	HFR1	Mouse mAb	Srinivasan et al. [12]
EGF		Rabbit polyclonal	From H Gregory
TGF α	GF10	Mouse mAb	CalBiochem
Amphiregulin	55AR	Rabbit polyclonal	Saeki et al. [13]
HB-EGF	111HB	Rabbit polyclonal	Chobotava et al. [14]
Epigen	AF1127	Goat polyclonal	R&D Systems
Epiregulin	AF1195	Goat Polyclonal	R&D Systems
Betacellulin	97BTC	Rabbit polyclonal	Srinivasan et al. [15]
NRG1 α	76HRG	Rabbit polyclonal	Normanno et al. [16]
NRG1 β	102HRG	Rabbit polyclonal	Srinivasan et al. (15)
NRG2 α	121NRG	Rabbit polyclonal	Dunn et al. [17]
NRG2 β	120NRG	Rabbit polyclonal	Dunn et al. [17]
NRG3	122NRG3	Rabbit polyclonal	Dunn et al. [17]
NRG4	123NRG4	Rabbit polyclonal	Dunn et al. [17]

kit from Dako, Denmark. For detection of Epigen and
Epiregulin rabbit anti-goat biotinylated IgG (Dako) was
used with the kit. Optimisation of the concentration of each
antibody was performed prior to its use on the tissue arrays.
Tumours were scored for intensity of staining by inspection
on an Olympus BX40 microscope with a “double head” by
WJG and EM using a scale of 0 = negative, 1 = weak,
2 = moderate and 3 = strong.

Results

Each antibody detected specifically its cognate protein in a
proportion of cases. Results with antibodies to Epigen and
Epiregulin, which have not previously been measured in
breast cancer, are shown in Fig. 1a and b. In order to assess
the overall expression levels for each protein we summed
the scores for the hundred cases. The highest aggregate
score for the four receptors was for HER2. It should be
noted that this does not reveal heterogeneity of expression
between cases, for instance many previous studies have
reported that about 20% of breast cancers score 3+ for
HER2 but this would not be apparent in this analysis.
However, it does demonstrate, in particular with the
ligands, some of which have not previously been studied,
that there are broad categories of expression present.
Highest scoring ligands included Epigen, Epiregulin,
Neuregulin 1 α , Neuregulin 2 α , Neuregulin 2 β , Neuregulin
4 and TGF α , moderate expression was seen with EGF,
Neuregulin 1 β and Neuregulin 3 and low levels of
expression were seen of HB-EGF, Betacellulin and
Amphiregulin.

Fig. 1 Example of immunostaining of a case of breast cancer with the antibody to Epiregulin (a) and Epigen (b). c Aggregate scores of the ligands and receptors



140 The data obtained was analysed for any associations
 141 between expression of each ligand and receptor with each
 142 of the others using Spearman's Rank Correlation. From the
 143 data in Fig. 2a, it can be seen that all the ligands and
 144 receptors were positively associated. To provide a visual
 145 representation of this large dataset, we have shown the
 146 cases ordered on the ordinate in ascending score for total
 147 ligands (Fig. 2b, left axis, range 0–33) and shown the total
 148 receptor score (range 0–12, right axis). The data reveal a
 149 strong association between increasing total ligand score
 150 and increasing total receptor score. It is also apparent that
 151 there are some cases that essentially lack any receptor or
 152 ligand expression at the cut of value scored while other
 153 cases showed high levels of almost all the ligands and
 154 receptors suggesting very great heterogeneity in the pres-
 155 ence of this highly interactive family of signalling mole-
 156 cules between individual cases.

157 In order to assess the relationship between the expres-
 158 sion of the ligands and receptors and clinical and molecular
 159 variables, the tumours were divided in three ways. First,
 160 they were dichotomised by low and high ligand levels;
 161 second, by low and high receptor levels and finally, by low
 162 and high aggregate ligand and receptor levels. No signifi-
 163 cant associations were found although the strongest rela-
 164 tionship was between receptor levels and tumour size
 165 ($P = 0.06$) (Supplementary Table 2).

166 Kaplan–Meier curves for overall survival (OS) were
 167 generated for all the receptors and ligands based on lack of

168 expression (0) or any level of expression (1–3) (Fig. 3).
 169 Several of the factors have not previously been studied in
 170 breast cancer and thus the dichotomisation of the data was
 171 chosen to ensure as far as possible similar numbers of cases
 172 in each category. HER2 expression would normally be
 173 divided into low (0–2) versus high (3) as this has been
 174 shown previously to give the best discrimination between
 175 good and poor survival but it was considered more
 176 appropriate in this study to maintain consistency within the
 177 analysis. HER2 was separately analysed as a single factor
 178 as low (0–2) versus high (3) and, as expected, high
 179 expression was associated with reduced OS. Analysis of
 180 the survival data using Cox's Proportional Hazards model
 181 identified Epigen and Neuregulin 4 as the factors most
 182 strongly associated with OS.

183 Interestingly, expression of Epigen was positively
 184 associated with improved survival, and NRG expression
 185 was associated with worse OS. Various laboratory studies
 186 have shown that different activation states of the EGF
 187 family may induce either growth or differentiation and thus
 188 in the light of our still imperfect knowledge of the system it
 189 is not unexpected that some factors may have opposite
 190 effects. In further analysis using the model omitting
 191 sequentially the weakest factor (backwards elimination
 192 dropping the factor with the smallest positive or negative
 193 coefficient), the combination of Epigen ($P = 0.003$) and
 194 NRG4 ($P = 0.01$) retained the strongest association with
 195 OS (Table 2). In order to assess the influence of these

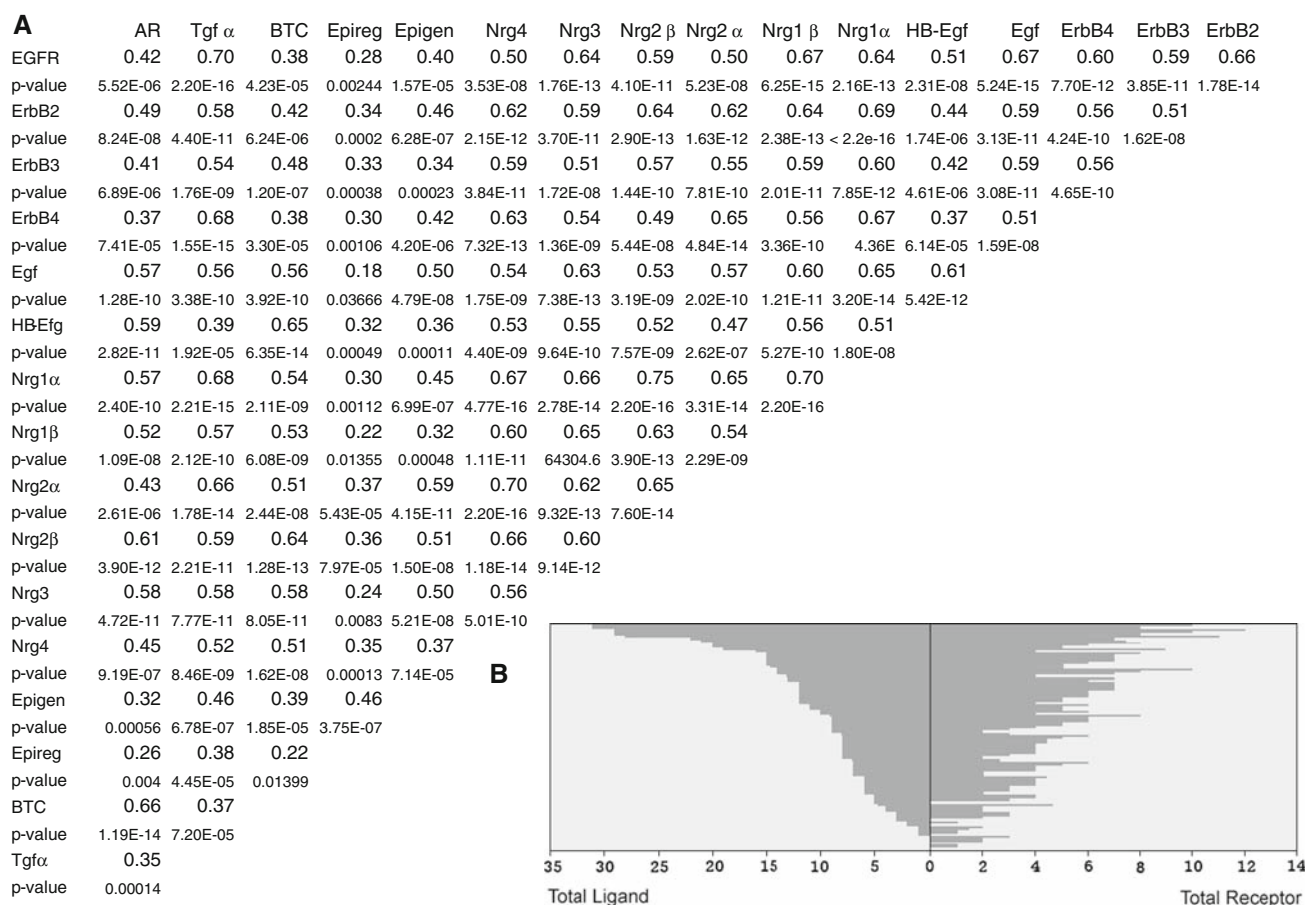
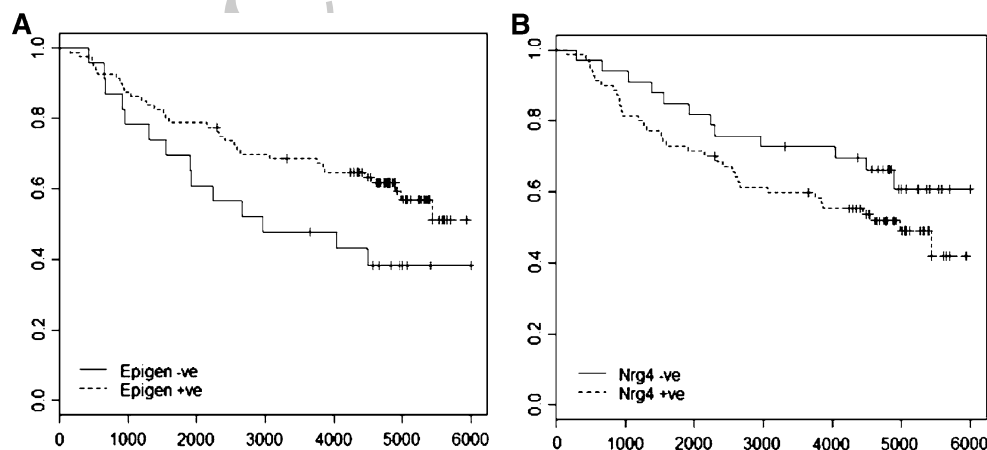


Fig. 2 **a** Spearman's rank correlation analysis of all ligands and receptors. **b** Cases were ordered on the ordinate in increasing ligand score (*left*) and associated receptor scores (*right*)

Fig. 3 Kaplan Meier charts showing the survival (days) of the patients based on the level of expression (0 vs. 1–3) of Epigen (*left*) and NRG4 (*right*)



196 factors in a more molecularly homogeneous group of cases
 197 and to see if there were any major effects of treatment, the
 198 oestrogen receptor positive cases were analysed separately.
 199 Again positive expression of Epigen was associated with
 200 good OS ($P = 0.0092$), but NRG2 α became the other
 201 predictive factor ($P = 0.0057$). The dataset was only
 202 hundred cases (although 1,700 data points were acquired
 203 for the 17 factors measured) and further studies on larger

datasets would be required to confirm or refute these
 apparent relationships.

Discussion

Each ligand and each receptor were expressed at a range of
 levels in a proportion of cases of breast cancer in this study.

Table 2 Cox proportional hazard results for overall survival

	Coeff.	P
EGFR	-0.6397	0.240
ErbB2	-0.0235	0.970
ErbB3	0.0812	0.870
ErbB4	0.2826	0.600
Egf	-0.1251	0.800
HBEgf	0.2006	0.720
Nrg1a	-0.7294	0.200
Nrg1b	0.2555	0.570
Nrg2a	0.2613	0.660
Nrg2b	0.8110	0.260
Nrg3	-0.5034	0.270
Nrg4	1.0819	0.062
Epigen	-1.1589	0.019
Epiregulin	0.7059	0.230
BTC	0.3027	0.600
Tgfa	-0.3076	0.570
Amphiregulin	-0.6340	0.230

209 Statistical analysis of the data revealed a strong associate
210 between the expression of any member of the family and
211 all other members. Although breast cancer is acknowl-
212 edged, both clinically and by analysis of molecular factors,
213 to be a heterogeneous disease it is still perhaps surprising
214 how different the composition of the factors between cases
215 were. In some individuals (at the precision of measurement
216 available from simple immunostaining), there were essen-
217 tially no ligands or receptors present. In other individuals,
218 all the receptors and essentially all the ligands were present
219 at the highest quartile of measurement. This suggests that
220 the family may be, in some cases, relatively unimportant
221 whereas in others it clearly has the potential to be an
222 important influence on cell activity. This may also reflect a
223 sensitivity or lack of sensitivity to drugs designed to inhibit
224 this system.

225 Individual receptors and ligands were, in some cases,
226 associated negatively or positively with shorter relapse free
227 interval or survival. This was not unexpected as some
228 ligands are known to provoke increased rates of cell growth
229 while others appear to stimulate differentiation. Using the
230 Cox's Proportional Hazards model, we show that a combi-
231 nation of Epigen and Neuregulin 4 in this series of cases
232 together gives the greatest separation of aggressive from
233 indolent disease. This result could not be predicted as we
234 are currently unaware of their individual activities in any
235 detail nor their effect on the balance between growth on the
236 one hand and differentiation on the other. It is likely,
237 however, that measuring a subset of the family may allow
238 prediction of the natural history of the disease in some
239 cases. Here two factors emerged, but further test datasets

would be required to determine whether this was general- 240
isable. The Neuregulins are produced as multiple splice 241
variants for instance, five have so far been identified as 242
products of the NRG4 gene [18] and these have very dif- 243
ferent destinations within or without the cell and as such 244
may also have different functions. The antibodies used here 245
to the ligands (where known) are directed to the EGF-like 246
sequence which is shared by all the so far reported splice 247
variants and should thus detect the sum of the expressed 248
gene products. The use of reagents which can discriminate 249
between the splice variants may give a better ability to 250
predict their involvement and influence in the disease. 251

The use of computer simulations of the EGF system has 252
been an area of considerable study as we have a reasonable 253
knowledge of its constituents and some understanding of 254
how they function. It may be in the future that a "reading" 255
of the family of receptors and ligands (or a subset of them) 256
may be able to more accurately predict prognosis and, 257
more importantly, select patients for treatment with par- 258
ticular combinations of signal transduction inhibitor drugs. 259

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References

- Stein RA, Staros JV (2006) Insights into the evolution of the 265
ErbB receptor family and their ligands from sequence analysis. 266
BMC Evol Biol 6:79 267
- Hayes NV, Gullick WJ (2008) The Neuregulin family of genes 268
and their multiple splice variants in breast cancer. *J Mammary 269
Gland Biol Neoplasia* 13:205–214 270
- Sundvall M, Iijin K, Kilpinen S, Sara H, Kallioniemi OP, Elenius 271
K (2008) Role of ErbB4 in breast cancer. *J Mammary Gland Biol 272
Neoplasia* 13:259–268 273
- Lemmon MA (2008) Ligand-induced receptor dimerisation. *Exp 274
Cell Res Oct 31st Epub* 275
- Johnson CG, Goldman JP, Gullick WJ (2004) Simulating intra- 276
cellular processes using object-oriented computational modelling. 277
Prog Biophys Mol Biol 86:379–406 278
- Scaltriti M, Baselga J (2006) The epidermal growth factor 279
receptor pathway: a model for targeted therapy. *Clin Cancer Res 280
12:5268–5272* 281
- Kochupurakkai BS, Harari D, Di-Segni A, Maik-Rachline G, 282
Lyass L, Gur G, Kerber G, Citri A, Lavi S, Eilam R, Chalifa- 283
Caspi V, Eshhar Z, Pikarsky E, Pinkas-Kramarski R, Bacuss SS, 284
Yarden Y (2005) Epigen the last ligand of ErbB receptors, reveals 285
intricate relationships between affinity and mitogenicity. *J Biol 286
Chem* 280:8503–8512 287
- Toyoda H, Komurasaki T, Uchida D, Takayama Y, Isobe T, 288
Okuyama Y, Hanada K (1995) Epiregulin: A novel epidermal 289
growth factor with mitogenic activity for rat primary hepacytes. 290
J Biol Chem 270:7495–7500 291
- Gullick WJ, Marsden JJ, Whittle N, Ward B, Bobrow L, 292
Waterfield MD (1986) Expression of epidermal growth factor 293
receptors on cervical, ovarian and vulval carcinomas. *Cancer Res 294
46:285–292* 295

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297
298
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300
301
302
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304
305
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310
311
312
313
314
315
316
10. Gullick WJ, Berger MS, Bennett PLP, Rothbard JB, Waterfield MD (1987) Expression of the c-erbB-2 protein in normal and transformed cells. *Int J Cancer* 40:246–254
 11. Rajkumar T, Majhi U, Malligarjuna V, Shantha V, Gullick WJ (1995) Prevalence of c-erbB-3 expression in squamous cell carcinomas of the cervix as determined by the monoclonal antibody RTJ-2. *Int J Oncol* 6:105–109
 12. Srinivasan R, Poulosom R, Hurst HC, Gullick WJ (1998) Expression of the HER4/c-erbB-4 protein and mRNA in normal human fetal and adult tissues and in a survey of nine solid tumour types. *J Pathol* 185:236–245
 13. Saeki T, Qi C-F, Johnson G, Gullick WJ, Tahara E, Normanno N, Ciardiello F, Kenney N, Stromberg K, Salomon DS (1992) Differential immunohistochemical detection of amphiregulin and cripto in normal and malignant human colon. *Cancer Res* 52:3467–3473
 14. Chobotava K, Spyropolou I, Carver J, Manek S, Heath JK, Gullick WJ, Barlow DH, And SargentIL, Mardon HJ (2002) Heparin binding epidermal growth factor and its receptors mediate implantation of the human blastocyst. *Mech Dev* 119:137–144
 15. Srinivasan R, Benton E, McCormick F, Gullick WJ (1999) Expression of the c-erbB-3/HER-3 and c-erbB-4/HER-4 growth factor receptors and their ligands, neuregulin-1 alpha, neuregulin-1 beta and Betacellulin, in normal endometrium and endometrial cancer. *Clin Cancer Res* 5:2877–2883
 16. Normanno N, Qi C-F, Gullick WJ, Persico G, Yarden Y, Wen D, Plowman G, Kenny N, Johnson G, Kim N, Brandt R, Soarez I, Dickson RB, Salomon DS (1993) Expression of Amphiregulin, cripto-1 and Heregulin alpha in human breast cancer cells. *Int J Oncol* 2:903–911
 17. Dunn M, Sinha P, Campbell R, Blackburn E, Levinson N, Rampaul R, Bates T, Humphreys S, Gullick W (2004) Co-expression of Neuregulin 1, 2, 3 and 4 in human breast cancer. *J Pathol* 203:672–680
 18. Hayes NVL, Blackburn E, Smart LV, Boyle M, Russell G, Frost T, Morgan B, Baines AJ, Gullick WJ (2007) Identification and characterisation of novel spliced variants of NRG4 in prostate cancer. *Clin Cancer Res* 13:3147–3155
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