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The basic biology of erbB-2 and its participation in colorectal cancers

Abstract: ErbB-2 is one of four cell surface growth factor receptors involved in transmission of signals controlling normal cell growth and differentiation. A range of growth factors serve as ligands, but none is specific for the ErbB-2 receptor. Ligand binding to ErbB-1, ErbB-3 and ErbB-4 induces rapid receptor dimerization, with a marked preference for ErbB-2 as a dimer partner. When ErbB-2 is overexpressed multiple ErbB-2 heterodimers are formed and cell signalling is stronger, resulting in enhanced responsiveness to growth factors and malignant growth. This explains why ErbB-2 overexpression is an indicator of poor prognosis in colorectal cancers and may be predictive of response to treatment. ErbB-2 is a highly specific and promising target for new colon cancer treatments.

Key words: ErbB-2, colorectal cancers, signal transduction, tumour growth.

1. Introduction

The *erbB-2* (*neu, HER-2*) oncogene is located on chromosome 17q11–q22 and encodes a 185 kDa transmembrane glycoprotein which has a structure consistent with a growth factor receptor, due to its similarity to the human epidermal growth factor receptor (EGFR) (RUBIN and YARDEN 2001). The *neu* gene was originally identified in rat neuroectodermal tumours and later its close human relative was isolated (SHIH et al. 1981).

The ErbB-2 receptor has an important role in normal cell growth and differentiation. However, amplification of the *erbB-2* gene leads to overexpression of the receptor, which is linked to the development of many types

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of human cancers including breast, ovarian and those of the gastrointestinal tract (HYNES and STERN 1994; BRYŚ et al. 2001; RUBIN and YARDEN 2001).

Adenocarcinomas of the colon and rectum are one of the major cause of morbidity and mortality for men and women. The pathologic stage of the tumor following surgical resection has long served as the cornerstone for predicting outcome for colorectal cancer (GELB and SCHROCK 1997; ROSS et al. 2001). Thus, considerable recent interest has developed for the evaluation of new prognostic and predictive markers that could be applied to early stage lesions (indicating their propensity for relapse) and the selection of these patients with relatively superficially invasive tumors to receive adjuvant therapy (ROSS et al. 2001).

In colorectal cancer ErbB-2 overexpression appears to be a significant adverse outcome indicator as judged by the current published literature. Either ErbB-2 protein overexpression or gene amplification is associated with a significant percentage of gastrointestinal adenocarcinomas. However, there are data suggesting that ErbB-2 overexpression or amplification is a significant prognostic factor for gastric and colorectal carcinomas. If this prognostic significance is confirmed, strategies designed to employ the marker in therapy selection appear to be warranted.

2. ErbB Family of Rectors and its Ligands

The ErbB family comprises four distinct receptors: EGFR/ErbB-1, ErbB-2/HER-2/neu, ErbB-3/HER-3, ErbB-4/HER-4. In general these cell surface receptors are composed of an extracellular ligand-binding domain, transmebrane part and a cytoplasmic region with tyrosine kinase activity (Fig. 1) (ULLRICH and SCHLESSINGER 1990; KLEIN et al. 2001; TROJANEK 2002). This structure enables signals to be transmitted across the plasma membrane where they activate gene expression and ultimately induce cellular responses such as proliferation. The signal-transducing tyrosine kinase activity of the ErbB receptors is inactive when the receptors are in isolation (YARDEN 2001).

ErbB receptors are activated by a number of ligands, referred to as EGF- related peptide growth factors. These include epidermal growth factor (EGF), amphiregulin (AR) and transforming growth factor α (TGFα) which bind specifically to ErbB-1, betacellulin (BTC), heparin-binding EGF (HB-EGF) and epiregulin (EPR), which exhibit dual specificity in that they

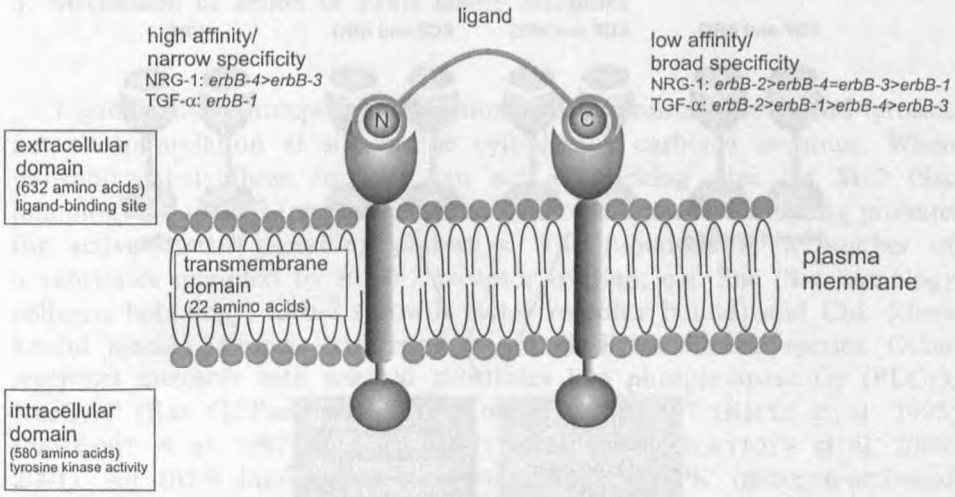

Fig. 1. Structure of the ErbB family receptors and bivalence of EGF-like ligands. The model refers to neuregulin-1 and transforming growth factor α and is based on results described in TZAHAR et al. (1997)

bind both ErbB-1 and ErbB-4, and at least four subfamilies of neuregulins (NRG1-4). NRG1 and NRG2 both bind ErbB-3 and ErbB-4, whereas NRG3 and NRG4 bind ErbB-4 (PELES and YARDEN 1993; KIRSCHBAUM and YARDEN 2000; OLAYIOYE et al. 2000).

Despite the large number of ligands so far identified for ErbB-1, ErbB-3 and ErbB-4, as well as intensive efforts, no direct ligand for ErbB-2 has yet been discovered. ErbB-2 appears to act as a ligand-less receptor. It was stated that ligand binding to ErbB-1 (EGF), ErbB-3 (NRG1) or ErbB-4 (NRG1, BTC) induces the formation of receptor homodimers and ErbB-2 containing heterodimers. In fact, ErbB-2 is the preferred heterodimerization partner for all other ErbB family members and plays a role in the potentiation of ErbB receptor signalling (Fig. 2) (GRAUS-PORTA et al. 1995; TZAHAR et al. 1996, 1997; OLAYIOYE et al. 2000; YARDEN 2001). From ten possible combinations, those heterodimers that contain ErbB-2 are more stable and their signalling is more potent than that of other receptor combinations (TZAHAR et al. 1996; PENUEL et al. 2001).

Multiple ligands differ in their expression patterns and in the ability to form specific homo- and heterodimeric receptor complexes. Due to the latest hypothesis, this latter difference is caused by the existence of two receptor binding sites on each ligand. The model refers to neuregulin-1 and is based on results described by TZAHAR et al. (1997). Neuregulin-1 seems to be a bivalent molecule with two binding sites for ErbB receptors:

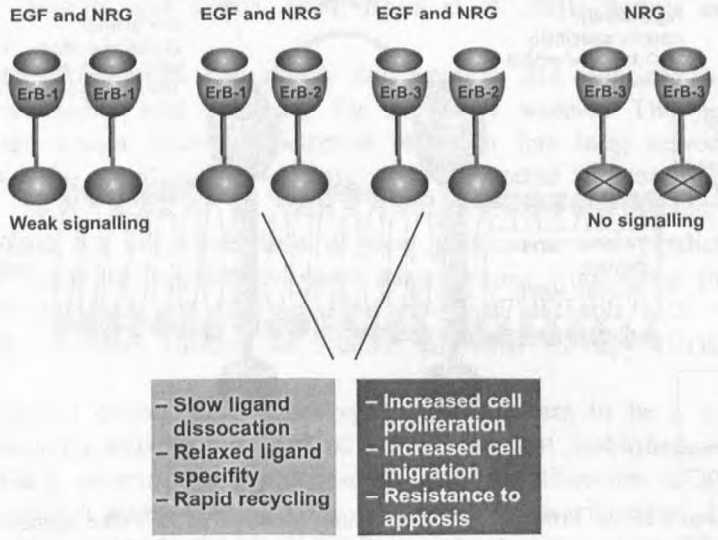

Fig. 2. Signalling by ErbB homodimers in comparison with ErbB-2-containing heterodimers. Binding of a ligand (EGF or NRG) to the extracellular domain of ErbB-1 or ErbB-3 (note inactive kinase marked by a cross) induces homodimer formation. When ErbB-2 is overexpressed, heterodimers form preferentially. Unlike homodimers, which are either inactive (ErbB-3 homodimers) or signal only weakly, ErbB-2-containing heterodimers have attributes that prolong and enhance downstream signalling (white box) and their outputs (grey box)

a high affinity/narrow specificity site (N-terminal) and a low affinity with broad specificity site (C-terminal). By the high affinity site ligand binds to its specific receptor (Fig. 1). Once the low affinity site is effectively 'immobilized' at the plasma membrane, its operational affinity to potential partners of the dimer is increased. Importantly, the receptor that preferentaially binds to the immobilized low affinity arm of the ligand is ErbB-2. As a result of this heterodimerization, the ErbB-2 receptor is able to participate in signal transduction in the absence of a specific ligand. This preferential binding with ErbB-2 is also enhanced by the overexpression of ErbB-2 in human cancer cells (TZAHAR et al. 1997).

Heterodimer formation is especially important between ErbB-2 and ErbB-3, not only because a high–affinity ligand–binding site is formed but also because ErbB-2 provides an active tyrosine kinase (EC 2.7.1.112) for the catalytically impaired ErbB-3 (GUY et al. 1994). Homodimerization of ErbB-3 or ErbB-4 results in the formation of a low–affinity neuregulin binding site, whereas heterodimerization between ErbB-2 and either ErbB-3 or ErbB-4 is sufficient for the formation of a high–affinity binding site (FITZPATRICK et al. 1998; PANUEL et al. 2001).

3. Mechanism of action of ErbB family receptors

Ligand–induced receptor dimerization leads to transactivation and tyrosine autophosphorylation at site in the cytoplasmic carboxyl terminus. When phosphorylated, these residues can act as docking sites for SH2 (Src homology 2) or PTB (phosphotyrosine binding) domains containing proteins for activation of signalling pathways. The functions of a number of a substrates recruited by ErbB-2 receptor overlap, e.g. Shc (Src homology collagen homology) Grb-2 (growth factor receptor bound) and Chk (checkpoint kinase) interacts with most if not all ErbB dimeric species. Other receptors interacts with selected substrates like phospholipase Cγ (PLCγ), RasGAP (Ras GTPase activating protein) and Grb-7 (RICCI et al. 1995; DANKORT et al. 1997; ZRIHAN-LICHT et al. 1998; OLAYIOYE et al. 2000, 2001). All ErbB family members activate the MAPK (mitogen-activated protein kinase) pathway. Activation of Ras–MAPK pathway is regulated by association of Grb-2 or Shc and phosphorylated ErbB-2. Activation of phosphatidylinositol-3 kinase is regulated by its association with YXXM motifs in the carboxy terminus of ErbB-3 and Cbl (Casitas B-lineage lymphoma) that specifically associates with EGFR, all of which contribute to cell growth and survival. The kinetics and potency of MAP kinase activation exhibit significant dependency on the identity of receptor dimers. Heterodimers are more sustained activators than homodimers, and heterodimers containing ErbB-2 are especially potent in this regard (TZAHAR and YARDEN 1998; PENUEL et al. 2001). ErbB-2–containing heterodimers display increased ligand affinity due to a deacelerated off–rate that can be correlated with prolonged activation of downstream signalling pathways (GROUS-PORTA et al. 1995).

Heterodimers containing ErbB-2 remain at the cell surface for a longer period of time, undergoing endocytosis at lower rate than homodimers do. Furthermore, once the activated complex is internalized ErbB-2/ErbB-1 heterodimers are targeted for recycling, while ErbB-1 homodimers are destined for degradation. The recycling pathway returns receptors to the cell surface, ready for another cycle of activation and augments growth–factor signalling (LENFERINK et al. 1998; PENUEL et al. 2001; YARDEN 2001).

Furthermore, biological responses such as proliferation, morphological differentiation and migration/ invasion are enhanced in cells expressing ErbB-2 (GROUS-PORTA et al. 1995; KARUNAGARAN et al. 1996; SPENCER et al. 2000; OLAYIOYE et al. 2001).

ErbB heterodimerization is a means not only for signal amplification but also for signal diversification. The subset of SH2- and PTB-binding

signalling molecules recruited to an activated receptor are defined by the pattern of phosphorylated tyrosine residues in the C-terminus of the receptor. Based on the finding that the Cbl protein coupled only to EGF- but not to NRG-activated ErbB-1 it is speculated that signal diversification arises at one level by differential transphosphorylation of a given receptor in distinct ErbB dimers (GROUS-PORTA et al. 1995; OLAYIOYE et al. 2000).

Signalling diversity emanating from the ErbB family is generated by the repertoire of ErbB ligands and the combinatorial properties of induced receptor dimers. With the exception of EGF, which is found in many fluids, ErbB ligands generally act over short distances as autocrine or paracrine growth factors. The availability of a specific ligand is, therefore, one way to control its signalling ability. In this respect, ErbB ligands demonstrate distinct expression patterns that are organ- and developmental stage-specific (OLAYIOYE et al. 2000).

4. ErbB-2 receptor and cancer

ErbB-2 is a protein expressed in a variety of tissues of epithelial origin and it plays a fundamental role in cellular proliferation and differentiation during fetal development. In adults, the *erbB-2* gene is present as a single copy and protein overexpression is seen in various cancers including breast, ovarian, colon, uterine, gastric, prostate and adenocarcinoma of the lung (BERNHARD et al. 2002).

ErbB-2 overexpression triggers ligand–independent activation of the kinase domain, apparently as a result of spontaneous dimmer formation. Homodimerization of ErbB-2 can be achieved by mutating a single amino acid residue in the transmembrane domain, leading to constitutive dimerization and activation of this receptor (BARGMANN et al. 1986; OLAYIOYE et al. 1998; PENUEL et al. 2001). Although ErbB-2 homodimers alone may contribute to malignancy, a number of observations suggest that ErbB-2 does indeed cooperate with other ErbB receptors during tumor development (HARARI and YARDEN 2000; OLAYIOYE et al. 2000). Many human tumors that contain ErbB-2 also exhibit autocrine stimulation of ErbB-1 via expression of one of its numerous ligands (SALOMON et al. 1995; DIGIOVANNA et al. 1998). The ability of ErbB-2 to potentate ErbB-1 signalling would provide tumor cells with a more potent growth stimulus and could lead to the activation of additional intracellular pathways. Such cooperation would contribute to the maintenance of increased proliferation rates associated with tumor development.

ErbB-2 and ErbB-4 are also coexpressed in more than 50% of childhood medulloblastomas, and the ErbB-4 ligand NRG1 is found in a significant proportion of the same tumors. Coexpression of ErbB-2 with ErbB-4 can enhance the effects of autocrine or paracrine NRG1 signalling (OLAYIOYE et al. 2000). Another important observation pertaining to ErbB heterodimer collaboration during tumor development is that expression of ErbB-3 is seen in many of the same tumor types that overexpress ErbB-2, including breast, bladder and melanoma (GILBERTSON et al. 1997).

ErbB-2 and ErbB-3 function together to stimulate mitogenic signalling networks. This in turn contributes to uncontrolled tumor cell proliferation by a mechanism involving deregulation of the G1–S transition through modulation of the activation status of the essential G1–S regulator cyclin E–dependent kinase 2 (NEVE et al. 2000; OLAYIOYE et al. 2000).

The relatively low, normal expression of ErbB-2 level is enhanced in several types of carcinomas. For example $25 \div 35\%$ of ovarian carcinomas show overexpression of ErbB-2 and survival of patients with ErbB-2 overexpression is significantly shorter than survival of non-overexpressors (TZAHAR and YARDEN 1998). Lung cancer is another example. Unlike squamous lung carcinomas, where the significance of overexpression is still un settled, $30 \div 35\%$ of lung adenocarcinomas display overexpression, which correlates with shorter patient survival time (KERN et al. 1990; TATEISHI 1991; LOPEZ-GUERRERO et al. 1999). Overexpression of ErbB-2 due to gene amplification occurs in about $20 \div 30\%$ of breast tumors but particularly ($\sim 90\%$) in comedo forms of ductal carcinoma *in situ* (DCIS), a malignant ductal carcinoma that has not progressed beyond the basement membrane barrier (BARNES et al. 1992). Even after progression to invasive disease, a correlation with the DCIS subtype and ErbB2 overexpression is maintained. Importantly, high ErbB-2 level predicts lower disease-free and overall survival in both lymph node negative and particularly in lymph node positive tumors, indicating a functional role of ErbB-2 in breast cancer (BARNES et al. 1992; LIU et al. 1992; TZAHAR and YARDEN 1998; DOWSETT M. 2000; HARARI and YARDEN 2000).

Other types of carcinomas that show variable overexpresion are those of the kidney (SELLI et al. 1997), bladder (IMAI et al. 1995; RAJKUMAR et al. 1996; TANNAPFEL et al. 1996), salivary gland (SKALOVA et al. 2001), prostate (AGUS et al. 2000; SANCHEZ et al. 2002) and the pancreas (SATOH et al. 1993; KOKA et al. 2002). However, the prognostic significance of overexpression in these tumors is not as clear as in breast, ovarian and lung cancers.

The other reason of ErbB-2 changes observed in cancers seems to be connected with polymorphism at codons 654 and 655. EHSANI et al. (1993)

described a G-to-A polymorphism of the *erbB*-2 gene resulting in a variation from valine to isoleucine. The ile-ile, ile-val and val-val configurations of codons 654 and 655 had frequencies of 0.782, 0.206 and 0.012, respectively. In a population-based case control study of the val655-to-ile polimorphism XIE et al. (2000) found that the val allele was associated with an increased risk of breast cancer, particularly among younger woman.

Recently there has been a flurry of interest in the relationship between ErbB-2 and colon cancer. Normal colonic mucosa is negative for ErbB-2, but significant number of benign lesions and adenocarcinomas overexpressed this protein. Adenocarcinoma were significantly more positive than benign lesions. The ErbB-2 rate expression show significant correlation with the epithelial abnormality degree and clinical parameters including Dukes' classification and relapse-free and postoperative survival period (KAPITANOVIC et al. 1997). Colon cancer metastatic to liver or lymph nodes has higher levels of ErbB-2 (SAEKI et al. 1995).

In our studies *erbB*-2 mRNA expression was identified in 51.5% of colorectal carcinoma samples (17/33) and in 29% of normal colonic mucosa (6/21) (Fig. 3). Obtained results suggest also that the *erbB*-2 mRNA expression in colorectal cancer cells is correlated with the progression of the disease. The expression of the *erbB*-2 mRNA was found in 1/2 of colon cancer cases at stage A, in 2/11 at stage B, in 7/13 at stage C and 7/7 at stage D. However no differences were found in the level of *erbB*-2 mRNA expression and tumor size.

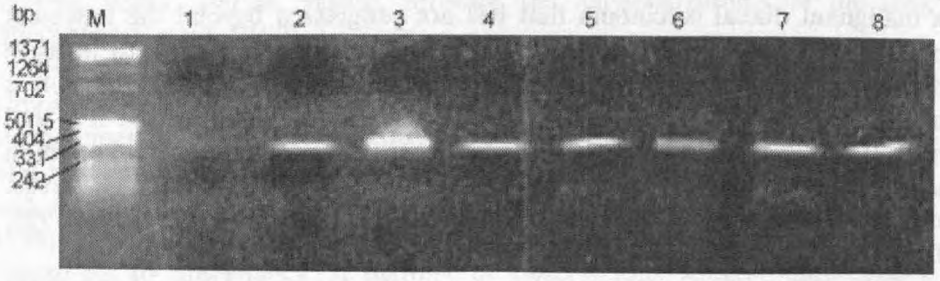

Fig. 3. Representative RT–PCR analysis of the *erbB*-2 expression in colorectal cancer (lanes $1 \div 5$) and normal colonic mucosa (lanes $6 \div 8$). Total RNA was isolated from normal and tumor specimens according to CHOMCZYNSKI and SACCHI (1978), reverse transcribed using RNA PCR Kit ver.2.1 (Takara Shuzo Co., Ltd., Kyoto, Japan) and amplified with specific set of primers (5'- AACTGCACCCACTCCTGTGT-3'; 5'-CAGGGATCCAGATGCCCTTG-3') according to WORTHYLAKE et al. (1997). Amplification products were separated on the 1.5% TBE–agarose gel and visualized by ethidium bromide. M, molecular weight markers; bp, base pairs

5. Conclusions

ErbB-2 is being investigated as a target for cancer therapy since is frequently overexpressed in human tumors and often confers a more aggressive clinical course. Its localization at the cell surface makes it an easy target to access. A wide range of therapeutic strategies targeting many tumors that overexpress ErbB-2 have been investigated, including tyrosine kinase inhibitors, antisense approaches designed to downregulate expression of the *erbB-2* gene, and immunization to actively boost anti-ErbB-2 responses. In addition, selective targeting can be achieved using monoclonal antibodies directed against the extracellular domain of the ErbB-2 protein (De POTTER et al. 1995). Therefore, monoclonal antibodies are the potential greatest advance in the treatment of tumors overexpressiong ErbB-2. Elucidation of the role of ErbB-2 in cell growth should allow the mechanism of action of monoclonal antibodies to be determined and should help optimize treatment of aggressive ErbB-2-positive tumors.

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