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Functional selectivity of EGF family peptide growth factors: Implications for cancer

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ABSTRACT

Breast, prostate, pancreatic, colorectal, lung, and head and neck cancers exploit deregulated signaling by ErbB family receptors and their ligands, EGF family peptide growth factors. EGF family members that bind the same receptor are able to stimulate divergent biological responses both in cell culture and in vivo. This is analogous to the functional selectivity exhibited by ligands for G-protein coupled receptors. Here we review this literature and propose that this functional selectivity of EGF family members is due to distinctions in the conformation of the liganded receptor and subsequent differences in the sites of receptor tyrosine phosphorylation and receptor coupling to signaling effectors. We also discuss the roles of divergent ligand activity in establishing and maintaining malignant phenotypes. Finally, we discuss the potential of mutant EGF family ligands as cancer chemotherapeutics targeted to ErbB receptors.

Contents

1. Introduction

Ligand functional selectivity is defined as divergent ligand activation of signaling pathways through a single receptor. Functionally selective ligands have been identified for the serotonin, opioid, dopamine, vasopressin, and adrenergic receptors (Urban et al., 2007). Ligand binding to these G-protein coupled receptors (GPCRs) results in receptor association with heterotrimeric G-proteins. Ligand functional selectivity appears to be mediated by distinctions in the conformation of liganded receptors and subsequent differential association of the liganded receptors with heterotrimeric G proteins (Urban et al., 2007). The plethora of functionally selective ligands for GPCRs has facilitated the elucidation of the mechanisms by which GPCRs couple to signaling effectors and biological responses. Moreover, functionally selective ligands portend the discovery of GPCR ligands that have therapeutic signaling properties but lack adverse signaling properties (Mailman, 2007).

Like many other receptor kinases, ErbB family receptors have numerous peptide ligands encoded by several distinct genes and by alternatively-spliced transcripts. These peptide ligands exhibit differences in receptor affinity and display exquisite receptor binding specificity. Other factors contribute to ligand specificity, including distinctions in the timing of ligand expression, tissue-specific patterns of ligand expression and differences in post-translational cleavage and processing. Accessory molecules and co-receptors such as heparan sulfate proteoglycans may contribute to ligand specificity by sequestering local high concentrations of these growth factors or by controlling their bioavailability, thereby selectively modulating the duration and/or strength of signaling stimulation by those EGF family

Abbreviations: AR, amphiregulin BTC, betacellulin EGF, epidermal growth factor EGFR, epidermal growth factor receptor EPG, epigen EPR, epiregulin GPCR, G-protein-coupled receptor HB-EGF, heparin-binding EGF-like growth factor MDCK, Madin–Darby canine kidney NRG, neuregulin NSCLC, non-small-cell lung carcinoma PLCγ, phospholipase C gamma PTB, phosphotyrosine-binding SH2, Src-homology domain type 2 TGFα, transforming growth factor alpha.

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members that bind these molecules (Normanno et al., 2001; Jones et al., 2003; Nanba & Higashiyama, 2004; Nishi & Klagsbrun, 2004; Shilo, 2005; Singh & Harris, 2005; Citri & Yarden, 2006; Edwin et al., 2006; Iwamoto & Mekada, 2006; Mahtouk et al., 2006; Ohtsu et al., 2006; Sanderson et al., 2006; Britsch, 2007; Higashiyama et al., 2008).

In addition, peptide ligands that display binding for the same ErbB family receptor have recently been shown to exhibit differences in function. This review will discuss the functional selectivity of ErbB receptor ligands, particularly in the context of the roles that functionally-selective ErbB receptor ligands may play in human malignancies and in the context of the roles that functionally-selective ErbB receptor ligands may play in cancer drug discovery.

The ErbB family of receptor tyrosine kinases includes the epidermal growth factor (EGF) receptor (EGFR/ErbB1/HER1), ErbB2/HER2/Neu, ErbB3/HER3, and ErbB4/HER4. Signaling by ErbB receptors is of principal importance in the control of cell fate, influencing proliferation, survival, or differentiation; hence, deregulated signaling by these receptors plays important roles in human malignancies. Currently, both EGFR and ErbB2 are validated targets for cancer chemotherapeutics (Normanno & Gullick, 2006; Plosker & Keam, 2006) that are being used to treat breast, lung, colorectal, and head and neck cancers. However, the development of resistance to chemotherapeutic agents that target ErbB receptors (Blackhall et al., 2006; Nahta et al., 2006) has spurred continuing investigation into the mechanisms by which ErbB family receptor signaling is regulated and may be deregulated. Combining multiple therapeutics that target ErbB receptors via independent mechanism of action can result in enhanced responses. Moreover, ErbB inhibitors with different mechanisms of action elicit therapeutic responses in tumors that are refractory to treatment with other therapeutics that target ErbB receptors (Storniolo et al., 2005; Geyer et al., 2006; Storniolo et al., 2008; Cameron et al., 2008). Thus, identifying additional agents that can target ErbB family receptors through novel means is an important goal in the treatment of cancer.

Members of the EGF family of peptide growth factors serve as agonists for ErbB family receptors. They include EGF, transforming growth factor-alpha (TGFα), amphiregulin (AR), betacellulin (BTC), heparin-binding EGF-like growth factor (HB-EGF), epiregulin (EPR), epigen (EPG), and the neuregulins (NRGs) (Fig. 1) (Kinugasa et al., 2004; Kochupurakkal et al., 2005; Normanno et al., 2005). Collectively, these agonists regulate the activity of the four ErbB family receptors, each of which appears to make a unique set of contributions to a complicated signaling network (Citri & Yarden, 2006). Tumor cell expression of some EGF family members, most notably TGFα, AR, and HB-EGF, is associated with poorer patient prognosis or resistance to chemotherapeutics (Gee et al., 2005; Ishikawa et al., 2005; Celikel et al., 2007; Ritter et al., 2007; Wang et al., 2007; Eckstein et al., 2008).

Ligand binding causes the homo- and/or heterodimerization of ErbB receptors, leading to the activation of their intracellular tyrosine kinase domains. As a result, ligand binding causes ErbB receptor phosphorylation on cytoplasmic tyrosine residues. This provides a mechanism for coupling to downstream signaling proteins via Src homology-2 (SH2) and phosphotyrosine binding (PTB) domains, which both recognize specific sets of tyrosine phosphorylation sites (Riese & Stern, 1998; Citri & Yarden, 2006; Warren & Landgraf, 2006). The EGF family ligands exhibit a complex pattern of interactions with the four ErbB family receptors; for example, EGFR can bind eight different EGF family members (Fig. 2) and Neuregulin 2beta (NRG2β) binds EGFR, ErbB3, and ErbB4 (Fig. 2). Given that ErbB2 lacks a EGF family ligand, ErbB3 lacks kinase activity, and the four ErbB receptor display distinct patterns of coupling to signaling effectors (Citri & Yarden, 2006), differences in the

A

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                    10        20        30        40        50        60
          123456789012345678901234567890123456789012345678901234567890
EGF     - NSDSECPLSHDGYCLHDGVCMYIEALD---KYACNCVVGYI----GERCQYRDLKWWELR
TGFα    - SHFNDCPDSHTQFCFH-GTCRFLVQED---KPACVCHSGYV----GARCEHADLLAVVAA
AR      - KKKNPCNAEFQNFCIH-GECKYIEHLE---AVTCKCQQEYF----GERCGEKSMKTHSMI
HB-EGF  - KKRDPCLRKYKDFCIH-GECKYVKELR---APSCICHPGYH----GERCHGLSLPVENRL
BTC     - GHFSRCPKQYKHYCIK-GRCRFVV-AE-Q-TPSCVCDEGYI----GARCERVDLFYLRGD
EPR     - QVITKCSSDMNGYCLH-GGCIYLVDMS-Q-N-YCRCEVGYT----GVRCEHFFLTVHQPL
EPG     - KFSHLCLEDHNSYCIN-GACAFHHELE---KAICRCFTGYT----GERCEHLTLTSYAVD

                    10        20        30        40        50        60
          123456789012345678901234567890123456789012345678901234567890
NRG1α   - SHLVKCAEKEKTFCVNGGECFMVKDLSNPSRYLCKCQPGFT----GARCTENVPMKVQNQ
NRG1β   - SHLVKCAEKEKTFCVNGGECFMVKDLSNPSRYLCKCPNEFT----GDRCQNYVMASFYKH
NRG2α   - GHARKCNETAKSYCVNGGVCYYIEGI-NQ--LSCKCPNGFF----GQRCLEKLPLRLYMP
NRG2β   - GHARKCNETAKSYCVNGGVCYYIEGI-NQ--LSCKCPVGYT----GDRCQQFAMVNFSKA
NRG3    - EHFKPCRDKDLAYCLNDGECFVIETLTGSHKH-CRCKEGYQ----GVRCDQFLPKTDSIL
NRG4    - DHEEPCGPSHKSFCLNGGLCYVIPTI--PSPF-CRCVENYT----GARCEEVFLPGSSIQ
NRG5    - EHHIPCPEHYNGFCMH-GKCEH--SINMQ-EPSCRCDAGYT----GQHCEKKDYSVLYVV
NRG6    - SCRSVCDLFPS-YCHNGGQCYLVE---NIGAF-CRCNTQDYIWHKGMRCESIITDFQVMC
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B

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          43    45
NRG2α   - C  L  E  K  L  P  L  R  L  Y  M  P
NRG2β   - C  Q  Q  F  A  M  V  N  F  S  K  A
```

Fig. 1. Amino acid sequence of the EGF homology domain of selected EGF family peptide growth factors. (**A**) Underlined are the six conserved cysteine residues that form the three intramolecular disulfide bridges present in the mature ligands. Selected EGF family peptide growth factors include EGF (NCBI Protein database # NP_001954), TGFα (#AAA61159), AR (#AAA51781), HB-EGF (AAA35956), BTC (#AAB25452), EPR (#BAA22146), EPG (#Q6UW88), NRG1α (#NP_039258), NRG1β (#ABR13844), NRG2α (#NP_004874), NRG2β (#NP_053584), NRG3 (#NP_001010848), NRG4 (#AAH17568), NRG5 (#BAA90820), and NRG6 (#AAC69612). NRG5 is also known as tomoregulin and NRG6 is also known as neuroglycan-C. (**B**) Carboxyl-terminal NRG2 residues that regulate differences in ligand potency, receptor affinity (residue 43), and intrinsic activity (residue 45) are noted.