Ligand-Independent EGFR Signaling

Gao Guo, Ke Gong, Bryan Wohlfeld, Kimmo J. Hatanpaa, Dawen Zhao, and Amyn A. Habib

Abstract

Constitutive activation of the EGFR is common in cancer due to EGFR wild-type (EGFRwt) overexpression or the presence of mutant EGFR. Signaling by constitutively active NSCLC EGFR mutants or the EGFRvIII mutant in glioblastoma has been studied extensively and the downstream signals are known. Normally, the EGFRwt is activated when it is exposed to ligand, resulting in activation of canonical signals such as ERK and Akt. The EGFRwt also becomes tyrosine phosphorylated and constitutively activated without ligand when it is overexpressed, but downstream signals are unclear. Recent studies have identified a noncanonical form of signaling triggered by EGFRwt exclusively in the absence of ligand that does not involve ERK or Akt activation but, instead, results in activation of the transcription factor IRF3. The addition of ligand turns off IRF3-dependent transcription and activates ERK and Akt. Thus, the EGFR triggers distinct and mutually exclusive signaling networks, depending on the presence of ligand. Furthermore, noncanonical EGFRwt signaling may influence response to treatment in cancer. Also, there are reports of both synergistic and antagonistic interactions between ligand-dependent EGFRwt and EGFRvIII signaling. Here, we discuss ligand-independent EGFR signal transduction by oncogenic EGFR mutants and EGFRwt, and review the interplay between EGFRwt and EGFRvIII.

Corresponding Author: Amyn A. Habib, The University of Texas Southwestern Medical Center, Dallas, Texas. Phone: 214-645-6240; E-mail: Amyn.Habib@UTSouthwestern.edu

doi: 10.1158/0008-5472.CAN-15-0989

©2015 American Association for Cancer Research.
exclusive in tumors expressing EGFRwt (19, 20). EGFRvIII may have a more focal or limited distribution compared with EGFRwt in GBM (21, 22). However, recent studies suggest that EGFRwt is usually coexpressed in EGFRvIII-expressing tumor cells. Fan and colleagues showed the colocalization of EGFRwt and EGFRvIII in individual tumor cells by immunohistochemistry in GBM samples with antibodies specific for EGFR and EGFRvIII (23), while Puliyanapaddamba and colleagues showed that clones derived from single cells from primary GBM cultures expressed both EGFRwt and EGFRvIII (24). The coexpression of both EGFRwt and EGFRvIII within the same tumor raises the possibility of interactions between the receptors and a number of studies have investigated EGFRwt–EGFRvIII interactions as outlined below. In addition to EGFRvIII, other less common constitutively active EGFR mutants in GBM include the deletion mutant EGFRvIV, a tandem kinase domain duplication in EGFR (TKD-EGFR), and a series of point mutations in the EGFR extracellular domain (10, 20, 25).

Constitute Activation of EGFR Wild-type

Although studies have reported that EGFRwt overexpression results in persistent tyrosine phosphorylation and a constitutive activation of the receptor in the absence of ligand (11), the downstream signals triggered by this constitutive EGFRwt activation have never been clearly described, and the general assumption may have been that constitutive and ligand-induced signals are similar. Ramanarin and colleagues reported that constitutive activation of EGFRwt in glioma cells leads to increased expression of mRNA for 66 genes in the absence of exogenous ligand (26), providing evidence for a constitutive EGFRwt signaling program. In a subsequent study, constitutive versus ligand-induced EGFR signaling was studied in detail, in glioma and breast cancer cells (12). In the absence of ligand, increased expression of the EGFRwt resulted in tyrosine phosphorylation of the EGFR, but did not lead to activation of canonical signals such as ERK and Akt. In addition, ligand-independent EGFR signaling did not result in expression of immediate early genes such as EGR1 and EGR2. Instead, ligand-independent EGFR signaling triggers a noncanonical EGFR pathway that is regulated by activation of the transcription factor IRF3. Thus, EGFR overexpression leads to phosphorylation and activation of IRF3 and transcription of IRF3-dependent downstream genes such as IFT1, IFT27, and TRAIL. An autocrine loop was excluded by the use of cetuximab, which failed to prevent ligand-independent EGFR signaling, and the use of a non–ligand-binding EGFR mutant, EGFRvIII, which also triggered activation of IRF3-dependent target genes, thus excluding an intracellular autocrine activation of the EGFRwt (12). Furthermore, expression of a kinase inactive EGFRwt failed to induce IRF3 activation, and the use of erlotinib blocked ligand-independent EGFR signaling, demonstrating that the kinase activity of the EGFR is required. Interestingly, addition of EGF resulted in a termination of the IRF3-transcriptional program, as demonstrated by a loss of IRF3 phosphorylation, loss of IRF3 transcriptional activity, and downregulation of IRF3-dependent downstream genes IFT1, IFT3, and TRAIL, with a concomitant activation of canonical ERK and Akt signals and induction of immediate early genes EGR1 and EGR2 (12). These data challenge the a priori view that ligand-independent and ligand-dependent EGFR signals are similar and demonstrate that the downstream signals activated by ligand-independent EGFR signaling are noncanonical and distinct from ligand-activated EGFR signaling. Furthermore, this study suggested that ligand-independent and ligand-activated signals are mutually exclusive. These data together suggest that the overexpressed EGFRwt oscillates between two distinct and mutually exclusive modes of signaling, depending on the presence of ligand (Fig. 1A).

The underlying mechanism may be a switch of EGFR-associated proteins with ligand (Fig. 1A; ref. 12). Thus, in the absence of ligand, the EGFR forms a ternary complex that includes IRF3 and its upstream kinase TBK1. It was proposed that IRF3 activation resulted from the increased association of IRF3 and TBK1 induced by the presence of EGFRwt. The addition of EGF resulted in a dissolution of the EGFR–IRF3–TBK1 complex and a loss of IRF3 transcriptional activity. The ligand-activated EGFR now forms a complex with Shc. The changes in EGFR-associated proteins in response to ligand are likely a consequence of the altered conformation of the ligand-activated receptor, but this awaits experimental confirmation. Initial studies indicate that cells overexpressing EGFR are more resistant to virus-induced cell death in the absence of ligand (12), raising the possibility that ligand-independent EGFR signaling may confer a survival advantage during the clonal evolution of tumors. Similarly, EGFR-overexpressing glioma cells are more resistant to chemotherapy with temozolomide in the absence of ligand (12).

Constitutive EGFR signaling may be activated in cancer. A recent study found that about 60% of GBM tumors that express high levels of the EGFR had low levels of TGFα (12). Interestingly, there is a statistically significant inverse correlation between expression of TGFα and levels of IRF3-dependent genes IFT27 and IFT1, suggesting that in EGFR-overexpressing tumors with high levels of TGFα and hence ligand activation of EGFR, IRF3 is not activated. Conversely, in EGFR-overexpressing tumors in which TGFα was low, levels of IFT1 and IFT27 were high, suggesting that noncanonical EGFR signaling is activated. Other studies have also suggested EGFR overexpression may occur without TGFα expression in GBMs (13) or reported that a significant subset of EGFR-overexpressing GBMs do not have a high level of EGFR phosphorylation, suggesting a paucity of ligand in GBM (27). Similar results have been found in lung cancer, in which estimates of low or absent ligand expression in high EGFR-expressing cancers range from 25% to 32% (14, 15). Similarly, in a breast cancer study, about 48% of EGFR positive tumors were positive for TGFα (16). However, while TGFα is a major EGFR ligand in cancer, there are a number of other known EGFR ligands. These include EGF, HB-EGF, amphiregulin, betacellulin, and epigen (28). Thus, determination of the ligand status of EGFR-overexpressing tumors will require an assessment of multiple ligands.

EGFRvIII is a constitutively active mutant in GBM

EGFR gene amplification and overexpression are a striking feature of GBM, observed in about 40% to 50% of GBMs (20), but are rare in low-grade gliomas, suggesting a causal role for aberrant EGFR signaling in the pathogenesis of GBM. A specific EGFR mutant (EGFR Type III, EGFRvIII, de2-7, ΔEGFR) can be detected in about one third of GBMs (20). EGFRvIII is generated from a deletion of exons 2 to 7 of the EGFR gene, which results in an in-frame deletion of 267 amino acids from the extracellular...
domain of the receptor. EGFRvIII is unable to bind ligand and signals constitutively.

EGFRvIII has a greater oncogenic potential compared with EGFRwt (29), and a significant effort has been focused on investigating what makes EGFRvIII more tumorigenic compared with EGFRwt. A detailed discussion of EGFRvIII downstream signaling can be found in previous reviews (19, 20). EGFRvIII downstream signaling is clearly distinct from EGFRwt signaling in a number of ways. First, EGFRvIII expression is sufficient to induce a ligand-independent continuous activation of Ras (30), ERK (31), and PI3K–Akt (20, 32). EGFRwt expression fails to activate these canonical signals unless ligand is added, even when the EGFR is overexpressed, and ligand-activated signals are limited in time. Expression of EGFRvIII in U87MG cells leads to an increase in Bcl-XL and resistance to apoptotic cell death in response to chemotherapy (33). Other studies have reported an important role for JNK activation in EGFRvIII signaling (34). Additional effector mechanisms used by EGFRvIII include Dock190–Rac1 activation (35), downregulation of miR-9 and upregulation of its target FOXP1 (36), and alternative splicing of Max leading to glycolytic growth of tumors (37).

Aberrant EGFR signaling is an important mechanism of NF-κB activation in GBM (38, 39). EGFRvIII is reported to induce NF-κB activation and higher expression levels of the proangiogenic factor IL8 in glioma (40). There may be multiple mechanisms used by EGFRvIII to activate NF-κB. EGFRvIII has been reported to activate NF-κB via mTORC2 kinase (41).
Another study reported that EGFRvIII activated NF-κB by an RIP1 kinase-dependent mechanism that includes formation of a signaling platform, including EGFRvIII, RIP1, NEMO, and TAK1 (24). Importantly, several studies have indicated that NF-κB activation is required for EGFRvIII-mediated oncogenicity in glioma models (24, 40, 41).

EGFRvIII activates the receptor tyrosine kinase Met, and combined inhibition of EGFRvIII and Met resulted in enhanced cytotoxicity of EGFRvIII-overexpressing compared with inhibition of either receptor alone, indicating a synergistic effect of EGFRvIII and Met (42–44).

Interactions between constitutively activated and ligand-activated EGF receptors

EGFRvIII is usually coexpressed with EGFRwt in GBM, and the question of whether EGFRwt influences EGFRvIII signaling and vice versa has been the subject of a number of studies.

Synergistic interactions

Luwor and colleagues showed that expression of EGFRvIII in BaF3/3 cells promoted their proliferation and found that coexpression of EGFRwt enhanced this effect. They proposed that EGFRvIII heterodimerizes with EGFRwt, resulting in transphosphorylation of EGFRwt (45), but did not find increased ligand-activated EGFRwt-mediated transphosphorylation of EGFRvIII when both receptors were overexpressed (45). Recently, two studies proposed a role for EGFRwt in the activation of EGFRvIII. Li and colleagues proposed that EGFRwt played a key role in the constitutive activation of EGFRvIII by forming a complex with EGFRvIII and promoting its dimerization. Thus, increasing EGFRwt levels increased EGFRvIII-mediated oncogenicity in an orthotopic model, while decreasing EGFRwt significantly attenuated the oncogenicity of EGFRvIII (46). The authors did not find evidence for a direct transphosphorylation of EGFRvIII by EGFRwt and, in agreement with a previous study (47), found that EGFRwt failed to phosphorylate a kinase dead EGFRvIII mutant, with or without ligand (46). Li and colleagues suggested that EGFRwt constitutively facilitated the dimerization of EGFRvIII, leading to autophosphorylation and activation of EGFRvIII (46). EGFRvIII induced the expression of NF-κB, a ligand for EGFRwt, suggesting a role for EGFRvIII in the activation of EGFRwt (26; 46). In another study, Fan and colleagues also found that coexpression of EGFRwt and EGFRvIII promoted tumor growth in vivo. They proposed a direct transphosphorylation of EGFRvIII by ligand-activated EGFRwt, leading to an enhanced activation of downstream STAT signaling and increased tumorigenicity (23). The EGFR kinase domain is allosterically activated in an asymmetric dimer (48), with one monomer acting as the activator kinase and another as the receiver kinase. Fan and colleagues used an elegant experimental approach by generating receptor-impaired and activator-impaired EGFRwt and EGFRvIII mutants to demonstrate that EGFRvIII is a substrate for EGFRwt (23). Although there are some differences in the proposed mechanisms by which EGFRwt mediates activation of EGFRvIII, which may reflect differences in the experimental approach, EGFRwt levels, and/or cell type-specific differences, the data suggest that EGFRwt plays an important role in EGFRvIII activation. In addition to these interactions, synergism between EGFRvIII and EGFRwt was suggested by an earlier study that found an EGFRvIII-specific induction of an eight-gene signature in glioma cells. Three of these genes were ligands for EGFRwt, suggesting that EGFRvIII signaling generated an autocrine loop in these cells (26). EGFRvIII-induced expression of HB-EGF is biologically significant, and HB-EGF overexpression was shown to accelerate EGFRvIII-induced tumorigenicity, while HB-EGF silencing attenuated EGFRvIII-mediated tumorigenicity in an orthotopic model (46). Another study has reported a biologically significant paracrine loop triggered by EGFRvIII that activates EGFRwt via induction of IL6 (22).

Antagonistic interactions

There are two reports of antagonistic interactions between EGFRwt and EGFRvIII (Fig. 1B). EGFRvIII-mediated activation of NF-κB in glioma cells is abolished by treating cells with EGF. Thus, it was demonstrated that inducible or constitutive stable expression of EGFRvIII induced NF-κB activation that was abolished by activation of coexpressed EGFRwt receptor with ligand (24). Overexpression of EGFRwt was not required for this antagonistic effect. EGFRvIII induces the K63-linked ubiquitination of RIP1 and formation of an EGFRvIII–RIP1–NEMO–TAK1 signaling complex that mediates NF-κB activation and promotes proliferation of tumor cells in vivo (24). Addition of EGF results in a loss of this signaling platform and a loss of NF-κB activity. RIP1 is known to have a role in both cell survival and cell death (49). In the context of EGFRvIII signaling, activation of EGFRwt results in a loss of RIP1 ubiquitination and its dissociation from EGFRvIII, NEMO, and TAK1. RIP1 now becomes associated with FADD and caspase-8 and leads to an EGF- and RIP1-dependent death of glioma cells (Fig. 1B; ref. 24). EGFRwt activation has also been reported to antagonize EGFRvIII-mediated activation of Met in glioma cells. Thus, EGFRvIII expression results in Met phosphorylation and activation. Addition of EGF to EGFRvIII-expressing cells results in activation of coexpressed EGFRwt and a rapid loss of Met phosphorylation and activation (Fig. 1B; ref. 50). EGFRvIII becomes associated with Met, and addition of EGF leads to a loss of the EGFRvIII-Met association, suggesting a possible mechanism for the loss of EGFRvIII-induced Met activation (50). Furthermore, the loss of Met activation may sensitize cells to chemotherapy (50).

These studies suggest that interactions between the ligand-dependent EGFRwt receptor and the ligand-independent EGFRvIII receptor are multimodal, frequent, and biologically significant.

Concluding Comments

The finding that constitutive and ligand-dependent EGFR signaling are distinct and that EGFR signaling in cancer is bimodal may prove relevant to clinical practice. The observation that EGFR-overexpressing cells are more sensitive to chemotherapy in the presence of ligand is potentially important in stratification of patients with EGFR-overexpressing tumors. The presence or level of expression of EGFR ligand(s) in a particular tumor has largely been ignored in clinical practice. If EGFR signaling in cancer is indeed bimodal, determination of the EGFR ligand status of a particular tumor may help to predict response to chemotherapy. The newly identified signaling networks that are triggered exclusively by ligand-independent EGFRwt signaling may provide new targets for treatment. In addition, new information about interactions between ligand-
independent EGFR mutants and ligand-activated EGFRwt may identify new approaches and new targets for combinatorial treatment. An intriguing example is the finding that ligand-activated EGFRwt can direct the RIP1 kinase cell death switch. Thus, when EGFRvIII is expressed, RIP1 is ubiquitinated and acts in a prosurvival role by activating NF-κB. When the EGFRwt is overexpressed in the same cells and activated with ligand, the RIP1 switch is turned into a cell death mode (24). Thus, there are data that ligand-activated EGFR may render the cell more vulnerable to cell death, an observation that could be exploited for treatment. Future studies will examine the biological significance and therapeutic implications of the new insights into EGFR signaling in cancer.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Disclaimer
The contents of this article do not represent the views of the Department of Veterans Affairs or the United States Government.

Grant Support
This work was supported in part by a Merit Review Award from the Departments of Veterans Affairs [I01BX002559-01], by NIH grant R01 NS062080 (AA.Habib), and by R01CA194578-01 (D.Zhao)

Received April 13, 2015; revised May 5, 2015; accepted May 6, 2015; published online First August 17, 2015.

References

8. Sharma SV, Bell DW, Settleman J, Haber DA. Epidermal growth factor receptor mutations in lung adenocarcinoma. Lab Invest 2014;94:129

Downloaded from cancerres.aacrjournals.org on April 15, 2017. © 2015 American Association for Cancer Research.
Ligand-Independent EGFR Signaling


Cancer Res 2015;75:3436-3441. Published OnlineFirst August 17, 2015.

Updated version  Access the most recent version of this article at:
doi:10.1158/0008-5472.CAN-15-0989

Cited articles  This article cites 50 articles, 19 of which you can access for free at:
http://cancerres.aacrjournals.org/content/75/17/3436.full.html#ref-list-1

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.