EGFR and NF-κB: partners in cancer

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Oncogenic proteins cooperate to promote tumor development and progression by sustaining cell proliferation, survival and invasiveness. Constitutive epidermal growth factor receptor (EGFR) and nuclear factor κB (NF-κB) activities are seen in multiple solid tumors and combine to provide oncogenic signals to cancer cells. Understanding how these oncogenic pathways are connected is crucial, given their role in intrinsic or acquired resistance to targeted anticancer therapies. We review molecular mechanisms by which both EGFR- and NF-κB-dependent pathways establish positive loops to increase their oncogenic potential. We also describe how NF-κB promotes resistance to EGFR inhibitors.

Constitutive EGFR signaling in solid tumors

Oncogenic proteins (see Glossary) cooperate to efficiently drive tumor development and progression. Cancer cells are indeed characterized by a powerful signaling network showing multiple connections to survive, proliferate and to resist to targeted anticancer therapies. Constitutive signaling from the EGFR receptor (EGFR/HER1/ERBB1), a protein of 170 kDa and a member of the ERBB family of receptor tyrosine kinases (RTKs; Box 1), crucially promotes cell survival, proliferation, and invasiveness [1]. A variety of EGFR peptides trigger EGFR dimerization and phosphorylation of multiple tyrosine residues in its cytoplasmic tail. Those phosphorylated EGFR residues provide docking sites for cytoplasmic SRC homology 2 (SH2) and phosphotyrosine-binding (PTB) domain-containing proteins to specifically trigger PKC, PI3K/AKT/mTOR, SRC, STAT and RAS/RAF/MEK1/ERK1/2 activation (Figure 1) [2,3].

More than 100 EGFR-interacting proteins have been described so far [4]. Among them is growth factor receptor-bound protein 2 (GRB2) which binds to phosphorylated tyrosines 1068, 1086, and 1148. RAS is subsequently activated by phosphorylation, a modification that relies on son of sevenless (SOS). Activated RAS binds to RAF, and this interaction leads to mitogen-activated protein kinase kinase 1 (MEK1) followed by extracellular signal-regulated kinases 1/2 (ERK1/2) phosphorylations [5,6]. RAS activation also relies on the recruitment of the SRC homology domain-containing adaptor protein C (SHC) to phosphorylated EGFR [7]. The p85 regulatory subunit of phosphatidylinositol-3-kinase (PI3K), the kinase SRC, and protein tyrosine phosphatases such as PTP1B, SHP1, and SHP2 also associate with distinct phosphorylated EGFR residues (Figure 1) [8]. EGFR directly or indirectly (through JAK) activates signal transducer and activator of transcription (STAT) members. EGFR phosphorylation also triggers STAT activation through SRC as well as the activation of PI3K that subsequently promotes AKT activation. Activated AKT targets multiple substrates, including mammalian target of rapamycin (mTOR). Phosphoinositide-specific phospholipase Cγ1 (PLCγ1) binds to EGFR through its SH2 domain, becoming activated and hydrolyzing phosphatidylinositol 4,5-bisphosphate to diacylglycerol (DAG) and inositol trisphosphate (IP3). DAG then triggers the activation of serine/threonine kinase protein kinase C (PKC) (Figure 1). NF-κB is also activated through the IKK complex upon EGFR phosphorylation.

Overexpression and activating mutations of EGFR, which have been reported in up to 30% of solid tumors (including breast, colorectal, lung, pancreatic, gastric, head and neck cancer, and glioblastomas), generally correlate with a poor prognosis [9]. A variety of solid tumors, including lung carcinomas, are indeed dependent upon EGFR activation, and this makes them sensitive to EGFR inhibitors such as erlotinib or gefitinib (see [10] for a full description of EGFR inhibitors) [11]. Some patients suffering from lung cancer are highly responsive to gefitinib because of activating EGFR point mutations or in-frame deletions (Figure 1) [12,13]. These genetic alterations

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**Glossary**

- **Erlotinib**: a pharmacological inhibitor that binds in a reversible fashion to the ATP binding site of the EGFR receptor. This EGFR inhibitor showed a survival benefit in the treatment of lung cancer in Phase III clinical trials. Erlotinib is more effective in patients with EGFR activating mutations.
- **Gefitinib**: the first EGFR inhibitor approved for the treatment of non-small cell lung carcinoma. Similarly to erlotinib, this drug binds in a reversible fashion to the ATP-binding site of the EGFR receptor.
- **Oncogenes**: gene candidates coding for proteins involved in tumor development. Many oncogenes are amplified or targeted by activating mutations to act in a genetically dominant manner.
- **Paronychia**: a bacterial or fungal infection of the hand or foot where the nail and skin meet.
- **Polyubiquitination**: a post-translational modification in which several copies of 7-kDa ubiquitin are bound to a protein substrate to create a polyubiquitin chain. This covalent modification involves three sequential enzymatic reactions catalyzed by the E1 (ubiquitin-activating), E2 (ubiquitin-conjugating), and E3 (ubiquitin ligase) enzymes [78].
- **Xerosis**: a skin disease involving the integumentary system. Symptoms include the peeling of the outer skin layer, itching, and skin cracking.
Box 1. ERBB members

The ERBB receptors include the EGF receptor (EGFR, also named HER1), ERBB2 (HER2/Neu), ERBB3 (HER3), and ERBB4 (HER4), and belong to the family of type I receptor tyrosine kinases (RTK) [79]. ERBB receptors are mainly expressed in epithelial, mesenchymal, and neuronal cells, as well as in their progenitors. The receptors share an extracellular ligand-binding domain, a single membrane-spanning region, and a cytoplasmic domain that includes a juxtamembrane domain, a region harboring an intrinsic tyrosine kinase activity, as well as a C-terminal domain [80]. ERBB receptors are bound by EGF-family peptides. These ligands include EGF, transforming growth factor (TGF)-α, amphiregulin (AR), and epigen (EPI) which bind to EGFR, β-cellulin (BTC), heparin-binding EGF (HB-EGF), and epiregulin (EPR) which bind to both EGFR and ERBB4; and neuregulins (NRGs) such as NRG-1 and NRG-2 that are known to bind to both ERBB3 and ERBB4, as well as NRG-3 and NRG-4 acting as ligands for ERBB4 only [79,80]. NRG-1 has several isoforms (type I NRG-1, also named ‘heregulin’, to type VI NRG-1). ERBB2 does not directly bind to any of these peptides, whereas ERBB3 is devoid of any strong kinase activity and only signals when bound to other ERBB members.

target the cytoplasmic domain of EGFR in lung adenocarcinomas, while, in contrast, mutations in glioblastomas showing constitutive EGFR signaling target the extracellular domain of this receptor [14,15]. Exons 18–21 of the tyrosine kinase domain of EGFR harbor all key mutations. About 40% of genetic alterations found in highly-responsive patients are in exon 21, and the most common is L858R. Some in-frame deletions in exon 19 (ΔE746-A750 as well as other deletions in this exon) account for about 46% of the reported EGFR genetic alterations found in highly-responsive patients. Point mutations in exon 18 (G719A, G719S, and G719C) have been described in about 1% of those patients. Less-frequent EGFR mutations underlying drug sensitivity or resistance have been described elsewhere [16,17].

The therapeutic effectiveness of EGFR inhibitors has been disappointing due to the emergence of resistant cancer cells. Virtually all patients who initially respond to EGFR inhibitors become resistant to these drugs as a result of acquired EGFR mutations [18]. The most clinically relevant EGFR mutation found in 50% of the cases showing acquired resistance to EGFR inhibitors (gefitinib and erlotinib) is the T790M mutation located in exon 20 (Figure 1) [19]. This mutation, which is located within the ATP-binding site of the kinase domain, causes steric hindrance for access of the inhibitor to the cleft owing to the bulkiness of the methionine sidechain [20]. The use of irreversible inhibitors of the EGFR kinase activity to treat patients harboring this mutation is an attractive therapeutic approach, and has prompted the search for new EGFR inhibitors that specifically target the EGFR T790M mutation. Several molecules have been identified that are more specific for this mutated EGFR than for the wild type receptor [21].

![Figure 1. EGFR-dependent signaling pathways and EGFR mutations in solid tumors. Ligands of EGFR homodimers include EGF, TGF-α, and AR. Examples of proteins recruited on tyrosine-phosphorylated EGFR residues are listed on the left and the most characterized signaling pathways activated upon EGFR phosphorylation are illustrated on the right. The most frequent mutations linked to drug sensitivity and found in drug responders are depicted in green. The most clinically relevant EGFR mutation (T790M) that promotes resistance to EGFR inhibitors is represented in red. Abbreviations: AR, amphiregulin; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; P, phosphorylation; TGF-α, transforming growth factor α; TM, transmembrane.](image-url)
**Box 2. The NF-κB family of transcription factors**

NF-κB proteins, which include RelA (also named p65), RelB, and c-Rel, share a N-terminal Rel homology domain (RHD) that is required for homo- and heterodimerization and for binding to sequence-specific DNA-binding sites in the promoters of ~200 target genes. These NF-κB proteins harbor a C-terminal transactivating domain (TAD). NF-κB proteins also include p50 and p52, which are generated from precursors NF-κB1/p105 and NF-κB2/p100. Both p50 and p52 lack any TAD and therefore rely on other members to drive gene expression of NF-κB-target genes. In unstimulated cells, NF-κB proteins are sequestered in the cytoplasm through binding to inhibitory molecules whose prototype is IκBα [81]. Other inhibitory molecules include p100, p105, IκBβ, and IκBε, as well as BCL3, IκBζ, and IκBNS. IκB proteins as well as p100 and p105 bind to NF-κB dimers through multiple ankyrin repeats. Stimulation with a variety of stimuli, such as proinflammatory cytokines (e.g., TNFα and IL-1β), Toll-like receptor ligands (e.g., lipopolysaccharide (LPS) and double-stranded RNA (dsRNA)), triggers NF-κB activation through the so-called ‘classical’ or ‘canonical’ pathway. This signaling pathway leads to the IKK complex, composed of both kinases IKKα and IKKβ, assembled by the scaffold protein NEMO/IKKγ. The IKK complex phosphorylates IκBα on N-terminal serines, and this triggers its degradative polyubiquitination through the proteasome. NF-κB dimers (mainly p50/p65 and p50/c-Rel) are consequently released and translocated to the nucleus to drive gene transcription of candidates involved in innate immunity, inflammation, proliferation, and survival. Growth factors can also trigger the activation of the IKK complex through signaling pathways described in Figure 2 in main text.

The ‘alternative’ or ‘non-classical’ NF-κB-activating pathway is triggered by cytokines such as BAFF and lymphotoxin-β, and leads to the activation of an IKKα homodimer, which phosphorylates p100. This inhibitory molecule is subsequently processed to generate p52. NF-κB dimers (p52/RelB) move into the nucleus to drive the expression of candidates involved in adaptive immunity, as well as in lymphoid organogenesis. The activation of all NF-κB signaling pathways relies on the sequential phosphorylation of multiple proteins, as well as on the polyubiquitination of key actors through several types of chains, the most characterized being the K48-linked chain, which triggers the degradation of its substrate or both linear and K63-linked chains, which enhance protein–protein interactions [82].

EGFR point mutations are not the only mechanism by which cancer cells are (or become) resistant to EGFR inhibitors. Activation of other RTKs such as ERBB2/HER2 also occurs in cells resistant to cetuximab, an EGFR-targeting monoclonal antibody, which paves the way for the dual inhibition of both EGFR and HER2 to improve the clinical response [22]. Signaling from ERBB3/HER3 is also specifically activated in epithelial malignancies treated with EGFR inhibitors [23–25]. Although HER3 lacks intrinsic kinase activity, it nevertheless strongly activates AKT signaling as a dimer with HER2 [26,27]. Therefore, a variety of pharmacological approaches, including HER3-blocking antibodies, have been recently developed to circumvent resistance [10,28]. It is currently unclear whether the use of multiple ERBB inhibitors is the best approach, or whether other types of inhibitors have to be combined with them. In any case, dissecting all relevant oncogenic pathways is of paramount importance to identify new mechanisms underlying resistance to EGFR inhibitors, and to define the best combination of specific drugs to fight epithelial malignancies. This review will focus on molecular mechanisms by which the transcription factor NF-κB is activated upon EGFR activation, as well as on NF-κB-dependent pathways underlying resistance to EGFR inhibitors.

### Molecular mechanisms linking EGFR signaling to NF-κB activation

Growth factors promote NF-κB activation through ERBB members, but the underlying mechanisms are only now starting to be elucidated. The family of NF-κB transcription factors are typically activated by proinflammatory cytokines such as tumor necrosis factor (TNF)α or IL-1β, as well as by Toll-like receptor (TLR) ligands through extremely well defined signaling cascades (Box 2) [29]. Early studies demonstrated that EGF triggers NF-κB activation through the proteasome-mediated degradation of the inhibitory molecule IκBα in estrogen receptor ERα-negative breast cancer cells and in lung cancer-derived cells [30,31]. Heregulin also triggers NF-κB activation through the IKK complex in ERα-negative and ERBB2-positive breast cancer cells [32]. In addition, constitutive EGFR signaling leads to NF-κB activation through IκBα phosphorylation on serines 32 and 36 in prostate cancer cells [33].

Although it is now well established that EGF activates NF-κB through the IKK complex (that includes both catalytic subunits IKKα and IKKβ as well as the scaffold protein NEMO/IKKγ), signaling molecules that link EGFR activation to the IKK complex have only been recently characterized (Figure 2). Distinct pathways have been elucidated in detail, but it remains unclear whether they are activated simultaneously or in a cell specific manner. EGF stimulation in prostate and breast cancer cells, as well as in EGFR-overexpressing glioblastoma-derived cells, triggers PKCε: monoubiquitination at Lys 321 in a PLCγ1-dependent manner [34]. PKCε: monoubiquitination relies on the E3 ligase RINCK1, but not on the linear ubiquitin assembly complex (LUBAC) that includes HOIL-1L and HOIP. Monoubiquitinated PKCε recruits the IKK complex to the plasma domain through a physical interaction with a ubiquitin-binding domain in the zinc finger of NEMO/IKKγ. PKCε then activates NF-κB through IKKβ phosphorylation at Ser 177. This pathway ultimately drives tumor growth by inducing the expression of pyruvate kinase 2 (PKM2), the enzyme involved in the rate-limiting final step of glycolysis. The IKKβ-phosphorylating kinase TGFβ-activating kinase 1 (TAK1) appears to be dispensable for this pathway, meaning that EGF as well as proinflammatory cytokines such as TNFα and IL-1β activate NF-κB through distinct signaling pathways that will nevertheless converge at the IKK complex [34].

EGF-dependent NF-κB activation in some breast and lung cancer-derived cells also relies on the scaffold protein caspase recruitment domain (CARD), membrane-associated guanylate kinase-like domain protein 3 (CARMA3; also referred to as CARD10), and B cell lymphoma protein 10 (BCL-10) [35]. Interestingly, both CARMA3 and BCL-10 also promote GPCR- and PKC-dependent NF-κB activation when complexed with mucosa-associated lymphoid tissue lymphoma translocation gene 1 (MALT1), but it is currently unclear whether MALT1 is actually required in EGF-dependent IKK phosphorylation [36,37]. MALT1, as a subunit of the CBM (CARD10–BCL-10–MALT1) complex, recruits the E3 ligase TRAF6, which forms K63-linked polyubiquitin
chains, to promote IKK activation through TAK1 in T lymphocytes [38]. Because MALT1 and BCL-10 are polyubiquitinated by TRAF6, they could be bound by NEMO/IKKγ in a TAK1-independent manner, a model that fits with the reported dispensable role of TAK1 in EGF-dependent IKKβ phosphorylation [34]. Activation of the CBM complex relies on PKC activation, but the PKC isoform that links EGFR activation to CARMA3 has not been identified. Whether PKCε fulfills this function remains to be demonstrated.

An additional pathway that links EGFR activation to NF-κB involves the guanine nucleotide exchange factor SOS1 [39]. Upon EGF stimulation, SOS1 binds to phosphorylated EGFR through the adaptor protein GRB2, which then triggers RAS activation at the plasma membrane [40]. Interestingly, its GDP–GTP exchange activity, known to be crucial for EGF-dependent MAP kinases activation, is dispensable for NF-κB activation upon EGF stimulation, and this supports the notion that SOS1 may also act as a scaffold protein to transmit oncogenic signals [39]. Nevertheless, signaling molecules that link SOS1 to the IKK complex are totally unknown (Figure 2).

These studies have convincingly demonstrated that growth factors promote NF-κB activation through signaling pathways whose initial steps are largely distinct from those triggered by proinflammatory cytokines. These signaling cascades are believed to crucially contribute to tumor development and progression through the expression of NF-κB-dependent genes that promote cell proliferation and survival.

**Crosstalk between EGFR- and NF-κB-dependent pathways through the transcriptional induction of target genes**

While growth factors trigger NF-κB-activating cascades upon binding to ERBB members, the transcriptional induction of some NF-κB target genes also feeds back to impact on EGFR-dependent signaling pathways. In this context, KIAA1199 is transcriptionally induced by NF-κB proteins in transformed keratinocytes as well as in breast cancer-derived cells (Figure 3) [41,42]. The oncogenic human papillomavirus (HPV) also positively regulates KIAA1199 gene transcription through BCL-3 in cervical cancer cells [41]. KIAA1199 promotes EGFR stability by limiting its EGFR-dependent degradation in lysosomes, and therefore positively regulates EGFR signaling [41]. KIAA1199 actually limits semaphorin 3A-dependent cell death by promoting EGFR phosphorylation and as well as EGFR-dependent epithelial–mesenchymal transition (EMT) in cervical cancer cells [41]. As such, KIAA1199 links NF-κB-dependent gene transcription to EGFR signaling to sustain cell survival and invasion [41]. Another example of positive correlation between NF-κB and EGFR activities has been described in head and neck squamous cell carcinomas (HNSCCs) in which IKKα and/or β knockdown significantly decreased...
Therefore, the inhibitors indeed positively signaling, NF-(RIPK1), NF-EGFR transcriptional protein, HPV infection in keratinocytes inhibits CYLD, a ubiquitin C-terminal hydrolase. As a result, the non-degradative K63-linked polyubiquitination of BCL-3, a p50-binding protein, is enhanced, leading to its nuclear translocation. Nuclear BCL-3 drives KIAA1199 gene transcription. KIAA1199 binds to plexin A2 to limit semaphorin 3A-dependent cell death, and also stabilizes EGFR to promote EGFR-dependent SRC and ERK1/2 activation, and subsequent epithelial–mesenchymal transition (EMT) [41].

EGFR mRNA and protein levels [43]. In contrast to this NF-κB signaling pathway that positively regulates EGFR signaling, EGFR expression is negatively regulated at the transcriptional level by receptor-interacting kinase (RIPK1), which is typically activated by proinflammatory and NF-κB-activating cytokines such as TNFα [44]. RIPK1 indeed appears to interfere with Sp1 activity, a transcription factor that promotes EGFR mRNA synthesis [44]. Therefore, multiple feedback loops involving the transcriptional induction of target genes that link both EGFR and NF-κB-dependent pathways have been described, even if they do not systematically lead to the establishment of positive loops.

**NF-κB activation as a mechanism for resistance to EGFR inhibitors**

NF-κB-activating cascades promote resistance to chemotherapy through multiple mechanisms, including the transcriptional induction of multidrug resistance gene-1 (MDR1) in colon cancer cells [45]. Recent studies have also defined mechanisms by which resistance occurs through crosstalk between EGFR- and NF-κB-dependent pathways. The tyrosine kinase FER, known to be activated by EGFR and PDGFR upon ligand engagement, promotes resistance to quinacrine, a drug with antimalarial and anticancer effects, when overexpressed in prostate cancer cells [46–48]. Mechanistically, FER binds to EGFR to enhance its phosphorylation on tyrosine residues, which leads to NF-κB activation through an AKT-independent pathway [46].

An unbiased screen for oncogenic pathways underlying resistance to EGFR inhibitors led to the identification of multiple candidates involved in NF-κB signaling [49]. Indeed, an unbiased short hairpin RNA (shRNA)-based high-throughput screen carried out in lung cancer-derived H1650 cells insensitive to EGFR inhibitors led to the identification of several candidates, many of which act in NF-κB-activating cascades [49]. Consistent with a role of NF-κB in the resistance to EGFR inhibitors, the genetic or pharmacologic inhibition of NF-κB increased the sensitivity to erlotinib in several models of EGFR-mutated lung cancers. Moreover, decreased expression of the inhibitory IκBα protein is associated with resistance to erlotinib and is predictive of worse progression-free survival in patients suffering from lung cancer [49]. Consistent with a role of NF-κB as a mediator of resistance to EGFR inhibitors, quinacrine overcomes resistance to erlotinib, at least by decreasing the level of SSRP1, an active subunit of the
facilitates chromatin transcription (FACT) complex that promotes NF-κB transcriptional activity [50].

The crosstalk between EGFR- and NF-κB-activating cascades is particularly relevant in tumor-initiating cells, which crucially promote drug resistance [51,52]. Integrin α3β3-expressing tumor-initiating cells from breast, lung, and pancreatic carcinomas are resistant to EGFR inhibitors [53]. NF-κB activation contributes to this phenotype because integrin α3β3 drives tumor stemness and resistance to EGFR inhibitors by interacting with Kirsten rat sarcoma viral oncogene homolog GTPase (KRAS) through galectin-3 to promote the sequential activation of both GTPase v-Ral simian leukemia oncogene homolog B GTPase (RALB) and TANK-binding kinase-1 (TBK1), which then targets c-Rel, a NF-κB protein [53]. Interestingly, this pathway does not require the binding of any ligand to integrin α3β3, demonstrating that resistance to EGFR inhibitors can be cell autonomous.

Cell intrinsic mechanisms of resistance to anticancer therapies have also been described in glioblastomas, the most common malignant primary brain cancer of adults. 40–50% of glioblastomas show EGFR gene amplification and/or mutations, which, in both cases, cause constitutive EGFR signaling [15]. The most common activating EGFR mutation (EGFRvIII) results from a deletion of exons 2–7 of the EGFR gene, which causes an in-frame deletion of 267 amino acids from the receptor in its extracellular domain. As a consequence, EGFRvIII cannot bind to any ligand and is constitutively active [14]. EGFRvIII triggers mTORC2 activation, a complex composed of the kinase mTOR bound to unique regulatory proteins, including Rictor and SIN1 [54]. mTORC2 signals to NF-κB through an AKT-independent pathway to promote proliferation, survival, and cisplatin resistance in glioblastomas [55] (Figure 4). Although it is currently unclear whether this pathway is specifically induced in glioma cells resistant to EGFR inhibitors, this study nevertheless shows that NF-κB also acts as a central player in chemotherapy resistance in glioblastoma cells harboring constitutively-active EGFR. EGFRvIII also activates NF-κB in glioma cells by assembling a signaling platform with TNF receptor-associated protein 2 (TRAF2), RIPK1, both the cIAP1 and cIAP2 (cellular inhibitor of apoptosis 1/2) E3 ligases, as well as TAK1 [56]. RIPK1 becomes polyubiquitinated in a K63-linked, non-degradative manner to trigger TAK1 and subsequent IKKβ phosphorylation.

All these pathways occur in a cell autonomous manner, but it is now increasingly obvious that the microenvironment also provides NF-κB-activating signals to promote resistance to EGFR inhibitors. TNFα, mainly synthesized by tumor-associated macrophages upon activation of TLR-dependent pathways, acts as a paracrine signal to trigger NF-κB activation in glioma cells [57]. This pathway involves the activation of the serine/threonine kinase

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**Figure 4.** Mechanisms by which NF-κB promotes resistance to chemotherapy in glioblastomas. (A) The production of TNFα by macrophages promotes resistance to EGFR inhibitors through NF-κB activation in glioma cells. Tumor-associated macrophages sense dying cells and pathogen-associated molecular patterns (PAMPs) through TLRs to trigger IKK-dependent NF-κB and ERK1/2 activation. These pathways promote TNFα synthesis, which acts in a paracrine fashion to activate NF-κB through TNFR1-dependent aPKC phosphorylation on residues 403/410 in glioma cells [57]. aPKC, whose activation requires the scaffold protein p62, triggers IKKβ (not illustrated) and IκBα phosphorylation to promote the nuclear translocation of NF-κB subunits p50 and p65. This signaling cascade ultimately induces the expression of proinflammatory cytokines (TNFα, IL-1β, IL-8), which establish a positive loop to further activate NF-κB in glioma cells. It is important to note that aPKC activation also occurs upon EGFR activation, but the scaffold protein involved in this pathway is Par6 and the target genes downstream of this cascade are not proinflammatory cytokines but EGFR-induced candidates such as CD44 and the matrix metalloproteinase MMP9. Whether, and how, these candidates precisely promote resistance to EGFR inhibitors in glioma cells remains to be clarified. (B) A second pathway underlying resistance to chemotherapy involves an EGFRvIII-dependent (but Akt-independent) signaling pathway that leads to mTORC2 activation in glioma cells. Signaling from the EGFRvIII mutant triggers mTORC2 phosphorylation, a pathway negatively regulated by PTEN. mTORC2 activation ultimately targets IκBα phosphorylation and NF-κB activation through a poorly defined signaling cascade, which will ultimately drive transcription of NF-κB target genes such as Bcl-xl, cyclin D1, and IL-6. EGFRvIII expression also promotes cell survival in glioma cells through K63-linked polyubiquitination of RIPK1 by c-IAPs bound to TRAF2. This signaling complex culminates in NF-κB activation through TAK1-dependent IKKβ phosphorylation [56].
atypical protein kinase C (aPKC) bound to the scaffold protein p62 to induce the expression of proinflammatory cytokines through NF-κB. Interestingly, another pool of aPKC interacting with the scaffold protein Par6 is specifically activated upon EGF stimulation, and drives the expression of another set of genes such as CD44 and MMP9 (Figure 4). Targeting aPKC activity causes tumor regression in EGFR kinase inhibitor-resistant glioblastoma [57].

Combining EGFR and NF-κB inhibitors: the way to go?
The demonstration that NF-κB contributes to resistance to EGFR inhibitors suggests that the simultaneous inhibition of both EGFR and NF-κB activity might be a therapeutic strategy to circumvent resistance. While tempting, it is currently unclear whether patients would benefit from this combination. Preclinical studies to treat HNSCCs have been conducted to address this issue because both EGFR and NF-κB activity are enhanced in a high percentage of these malignancies [58]. The EGFR-targeting cetuximab used in combination with radiation prolonged overall survival but failed to reduce the incidence of metastasis, and the response rate with cetuximab administered alone is not above 13% [59]. One mechanism underlying resistance to cetuximab results from the expression of the EGFRvIII mutant, which has a truncated ligand-binding domain, and signals to Lyn, a member of the SRC family of kinases and to STAT3 in up to 42% cases of HNSCC [60,61]. Although the EGFR inhibitor gefitinib showed some response in clinical trials of HNSCC by decreasing EGFR, MEK1, and NF-κB p65 phosphorylation, it failed to interfere with these oncogenic pathways in nonresponder patients [62]. Therefore, there was a need to combine EGFR inhibitors with other targeted therapies.

Bortezomib, a proteasome inhibitor, triggers cell apoptosis by blocking NF-κB activation through IκBα stabilization [63]. However, bortezomib showed limited clinical efficacy in HNSCC because NF-κB activation through the noncanonical pathway remained unchanged [64,65]. Moreover, bortezomib did not impact on other prosurvival pathways driven by STAT3 and ERK1/2 phosphorylation [64]. Therefore, the combination of bortezomib with EGFR inhibitors was expected to improve the clinical response. A Phase I clinical trial was carried out with patients suffering from HNSCC to assess preclinical evidence for combining the EGFR inhibitor cetuximab with bortezomib and radiation therapy [66]. This combination showed unexpectedly early progression due to EGFR stabilization, increased prosurvival signaling, and enhanced cytokine expression. Bortezomib attenuated the cytotoxic effects of cetuximab and radiation by limiting EGFR degradation through the proteasome [66]. Proteasome inhibition actually enhanced MAPK, AKT, and STAT3 prosurvival pathways through both EGFR-dependent and -independent mechanisms [67]. These studies highlighted the need to design new combinatorial approaches by using a more-specific NF-κB inhibitor than bortezomib, which blocks the degradation of IκBα as well as of numerous other proteins, including key oncogenic products [68]. Interestingly, both IKKα and IKKβ are aberrantly activated in HNSCC, act as mediators of NF-κB activation, and enhance EGFR signaling in HNSCC [43]. Consistently, wedelactone, a dual IKKα/β inhibitor, more effectively inhibited NF-κB activation than MLN120b, a specific IKkB inhibitor [43,69]. Moreover, 17-DMAG, a geldanamycin derivative that inhibits the heat shock protein 90 kDa (HSP90), a molecular chaperone involved in both IKKα/β- and EGFR-dependent signaling cascades, was more effective at triggering cell death than MLN120b alone [43,70]. Therefore, these data demonstrated why specific IKKκ inhibitors have not led to expected efficacies in preclinical studies, and suggest that combining dual IKKκ and EGFR inhibitors would be more successful, at least for epithelial malignancies.

Does NF-κB play any role in the toxicity of EGFR inhibitors?
Although NF-κB activation limits the clinical response to EGFR inhibitors, it is currently unclear whether the side effects commonly reported with these drugs would still occur by targeting NF-κB. Cutaneous side effects are often described in patients treated with EGFR inhibitors (monoclonal antibodies or small-molecule inhibitors) [71,72]. They include hair loss, acneiform eruption, paronychia, and xerosis. Mechanistically, these symptoms result from the inflammation-prone response of keratinocytes to early infiltration of macrophages and mast cells into the skin, as well as from an increased percentage of circulating granulocytes and platelets, but decreased percentage of lymphocytes in the plasma [73,74]. The genetic inactivation of EGFR in the epidermis mimics those symptoms, suggesting that EGFR expression maintains skin immune homeostasis [73]. EGFR-deficient keratinocytes overexpress a variety of chemokines and cytokines (such as IL-1β, TNFα, and IL-6) that are known to be regulated by NF-κB-dependent pathways. However, the genetic inactivation of single proinflammatory pathways (e.g., TNFR1/R2, Myd88) did not reverse the induction and maintenance of the skin phenotype in the EGFR-deficient mouse model [73,74]. Therefore, it is unlikely that targeting NF-κB alone will improve cutaneous toxicity seen with EGFR inhibitors.

Concluding remarks and future perspectives
Recent studies have elucidated the molecular mechanisms by which targeted therapies lead to resistance in epithelial malignancies displaying constitutive signaling from ERBB receptors. The simultaneous administration of specific inhibitors is the most logical approach to treat tumors with intrinsic or acquired resistance. Combining ERBB and NF-κB inhibitors is very promising, given the key role of NF-κB in tumors resistant to EGFR inhibitors; however, key issues remain unresolved (Box 3). New irreversible EGFR inhibitors are currently being tested to treat lung tumors harboring the T790M mutation. Given the genomic plasticity of many aggressive solid tumors, including lung carcinomas, it is likely that new oncogenic mutations and/or activation of signaling pathways will occur in patients treated with these irreversible EGFR inhibitors. Whether NF-κB activation contributes or not to this resistance remains to be clarified. In addition, it is currently unclear which NF-κB inhibitor should be used in combination with ERBB inhibitors for the best clinical response. A variety of
IKK inhibitors have been developed over the past 15 years, and some have been used in clinical trials [75–77]. However, none of them have been approved so far, at least because some doubts on the interest of targeting IKKβ itself were raised [77]. As recent studies carried out in HNSCC indicated that specific IKKβ inhibitors do not totally block NF-κB activation, it is likely that other NF-κB inhibitors will need to be tested in combination with ERBB inhibitors in all epithelial malignancies showing constitutive signaling from ERBB receptors [43]. Being over-specific (i.e., by targeting only IKKβ) may not be the best strategy. Finally, combining EGFR or HER2 inhibitors with HER3 blocking antibodies to circumvent resistance hold promise for the future, but it is also likely that some cancer cells will ultimately escape from this therapeutic strategy. If so, the potential role of NF-κB in these resistant cells will need to be explored.

Acknowledgments

Our laboratory is supported by grants from the Fonds National de la Recherche Scientifique (FNRS), TELEVIE, the University of Liege [Concerted Research Action Program (BIO-ACET) and Fonds Spéciaux (C-11/03)], the Centre Anti-Cancéreux, the Leon Fredericq Fondation (University of Liege), as well as by WELBIO. A.C. is Senior Research Associate at the FNRS.

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