

Inflammation, Epithelial to Mesenchymal Transition, and Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitor Resistance

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Inflammation is an important contributor to lung tumor development and progression. In addition, inflammatory signaling may promote epithelial to mesenchymal transition, development of aggressive metastatic tumor phenotypes, and play a role in resistance to targeted therapies. New insights in inflammatory signaling have led to the evaluation of combination therapies that target these specific pathways. In addition to developing the optimal combination of targeted agents, biomarker-based selection of patients who will likely benefit will be critical to the success of this strategy. Here we focus on the potential contribution of inflammatory mediator-induced resistance to epidermal growth factor receptor tyrosine kinase inhibitors.

Key Words: EGFR TK inhibitor, G-protein coupled receptors, Inflammation, Cyclooxygenase-2, PGE2, Epithelial to mesenchymal transition, Drug resistance, NSCLC.

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Targeted therapies for non-small-cell lung cancer (NSCLC), such as epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKI), have become important therapeutic options; however, the overall initial response rate is low and development of resistance is common.^{1,2} It has recently been suggested that resistance to targeted therapies may be overcome by identifying optimal combinations of drugs that target specific molecules.^{3–5}

Although underlying molecular mechanisms are not fully understood, substantial experimental data suggest a contributing role for inflammation in lung carcinogenesis.⁶

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Certain inflammatory mediators also pave the way for epithelial to mesenchymal transition (EMT), the developmental shift from a polarized epithelial phenotype to a highly motile mesenchymal phenotype essential in embryogenesis, organ development, and cancer progression.⁷ Recent work indicates that the progression of EMT, including loss of E-cadherin, may also promote resistance to EGFR TKI in NSCLC. In contrast, restoration of gene expression associated with the epithelial phenotype can sensitize NSCLC cells to targeted therapies.^{8–10} Thus, identifying the specific inflammatory signals governing EMT and EGFR TKI resistance maybe an important step toward expanding response to these agents.

The inflammatory enzyme cyclooxygenase-2 (COX-2) is frequently over-expressed in a variety of malignancies¹¹ and plays a multifaceted role in conferring malignant and metastatic phenotypes.¹² For example, COX-2 expression in NSCLC is associated with apoptosis resistance,¹³ angiogenesis,^{14,15} and metastasis.^{16,17} These tumorigenic effects are, in part, mediated by the COX-2 metabolite, prostaglandin E2 (PGE2), which is abundant in the lung tumor microenvironment. Inflammatory and tumorigenic signaling often converges on the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/Erk) cascade.¹⁸ EGFR transmits mitogenic signals from the cell surface to the nucleus by activating MAPK/Erk (Figure 1), and PGE2 induces rapid Erk phosphorylation in lung cancer cells.¹⁹ Recent studies have shown that PGE2 and other inflammatory mediators derived from neoplastic as well as stromal and immune cells reduce tumor E-cadherin levels via MAPK/Erk-dependent up-regulation of the transcriptional repressors zinc-finger E-box binding homeobox 1 (ZEB-1) and zinc-finger factor Snail homologue 1 (Snail) in NSCLC, whereas COX-2 inhibitors reverse this effect.^{20,21} Loss of E-cadherin is a hallmark of EMT and is also associated with tumor progression and metastasis.^{7,22} High E-cadherin expression or a gene signature associated with a mesenchymal rather than epithelial phenotype is associated with sensitivity to EGFR TKI in NSCLC.^{8–10} Therefore, PGE2 or other inflammatory cytokines in the tumor microenvironment may contribute to EGFR TKI resistance in NSCLC by suppressing E-cadherin expression.

PGE2 exerts its effects through four G-protein coupled receptors (GPCRs) designated E-prostanoid receptors (EP) 1

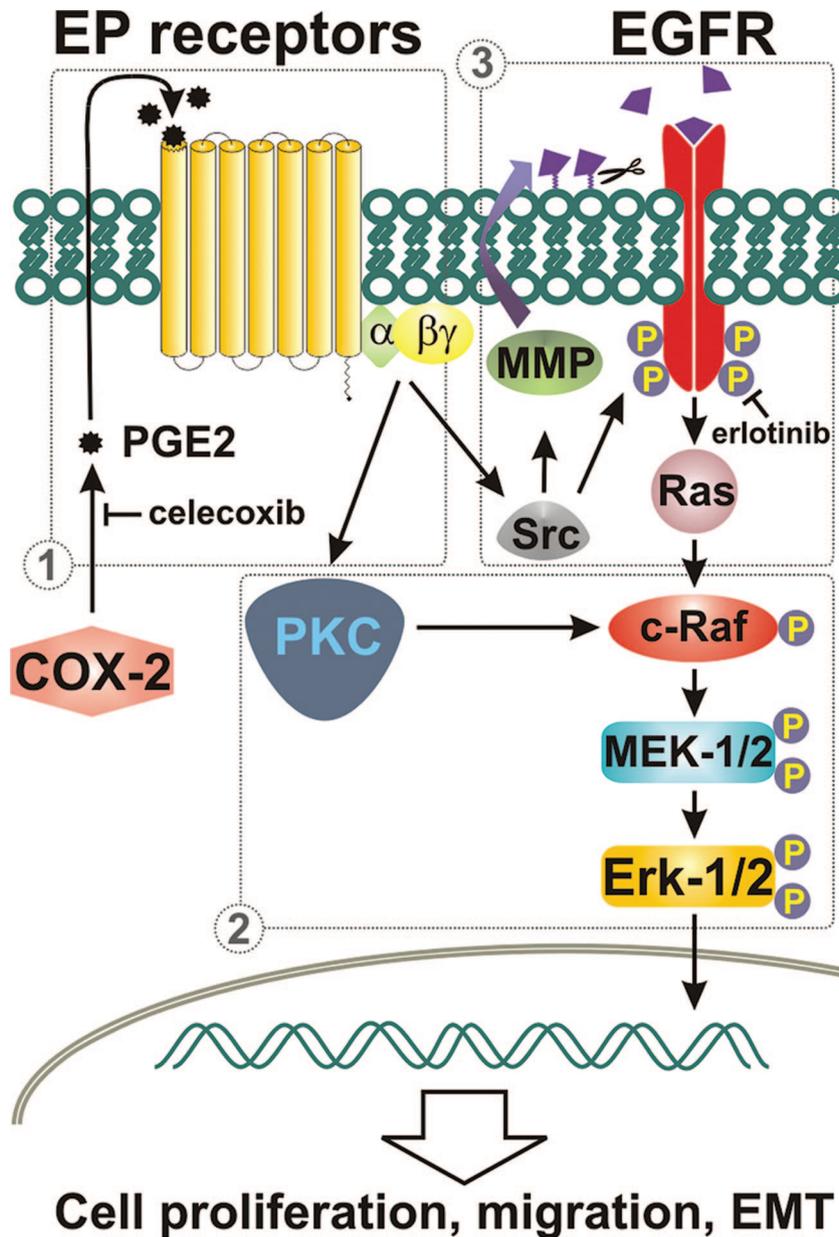


FIGURE 1. Mechanisms of EGFR-dependent and independent MAPK/Erk pathway activation by PGE2. Epidermal growth factor receptor (EGFR) belongs to the tyrosine kinase receptors family and is a major regulator of epithelial cell growth and proliferation. It also plays an important role in tumorigenesis by promoting cancer cell proliferation, invasion, and metastasis. The MAPK/Erk signaling module is located downstream of EGFR and is a critical effector of mitogenic signaling. PGE2 is a major COX-2 metabolite abundantly present in the cancer microenvironment that exerts its effects through four G-protein coupled receptors designated as EP1, EP2, EP3, and EP4 (area 1). PGE2 signaling can promote cell proliferation, migration, and EMT by directly activating intracellular mitogenic pathways or by stimulating proteolytic release of extracellular growth factor receptor ligands that activate the mitogenic cell-surface receptors. In NSCLC, PGE2-induced MAPK/Erk activation occurs by the intracellular pathway and is therefore EGFR-independent, encouraging resistance to EGFR TKI (area 2). In contrast, in colon cancer, PGE2 can induce EGFR ligand release, activating the receptor to increase MAPK/Erk signaling through a mechanism that is sensitive to EGFR inhibition (area 3). Here the scissors indicate MMP-induced release of cell membrane-bound EGFR ligands that can occur in colon cancer. Inhibition of COX-2 abrogates both EGFR-dependent and independent PGE2-induced MAPK/Erk activation in NSCLC (area 1). COX-2: cyclooxygenase-2 (selectively inhibited by COX-2 inhibitors such as celecoxib); PGE2: prostaglandin E2; EP: E-prostanoid receptors; α , β , and γ , G-proteins: components of the G-protein coupled receptors; EGFR, epidermal growth factor receptor (P indicates tyrosines phosphorylated upon receptor activation; this process is blocked by tyrosine kinase inhibitors such as erlotinib); Ras: Ras small guanosine triphosphatase; Raf: Raf serine/threonine kinase; MEK: MAPK/Erk kinase; Erk: extracellular signal-regulated kinase; PKC: protein kinase C; MMP: matrix metalloproteinase; EMT: epithelial to mesenchymal transition.

to 4 (Figure 1, area 1).²³ Recent studies indicate that GPCRs can activate the MAPK/Erk cascade in either a receptor tyrosine kinase (RTK)-dependent or independent manner (summarized in Refs. 24–26). Activation of EGFR signaling by PGE2 in lung cancer appears to occur via intracellular cross-talk between EP receptors and signaling modules downstream of EGFR such as MAPK/Erk (Figure 1, area 2).¹⁹ This is in contrast to the pathways defined in colon cancer in which PGE2 stimulates proteolytic release of EGFR ligands^{27,28} or intracellular src-dependent signaling²⁹ (Figure 1, area 3). Thus, in NSCLC, intracellular cross-talk between PGE2 and MAPK/Erk may directly contribute to EGFR TKI resistance. Indeed, PGE2 encourages resistance to the antiproliferative effects of the EGFR TKI erlotinib, and this can be overcome by concurrent targeting of the COX-2 pathway in NSCLC cell lines.¹⁹ These data provide a strong rationale for simultaneously targeting EGFR and COX-2 for lung cancer treatment.³⁰

Clinical trials targeting EGFR and COX-2 have recently been reported. Gadgeel et al. conducted a Phase II trial evaluating combined therapy with the EGFR TKI, gefitinib, and celecoxib in platinum-refractory NSCLC patients, and found that response rates and survival with the combination therapy were similar to those observed with gefitinib alone. Patients receiving combination therapies were treated with gefitinib 250 mg daily and celecoxib 400 mg twice daily.³¹ O'Byrne et al. reported a disease control rate of 35% in relapsed, metastatic NSCLC patients treated with escalating doses of rofecoxib (12.5, 25, or 50 mg/d) combined with gefitinib (250 mg/d). The combination therapy provided disease control rates equivalent to that expected for EGFR TKI single agent therapy.³² These two studies may not have employed the optimal biologic dose to inhibit COX-2. Reckamp et al reported a phase I trial evaluating escalating doses of celecoxib (200–800 mg twice daily) in combination with the fixed dose of erlotinib (150 mg/d) in late stage NSCLC patients and established the optimal biologic dose of celecoxib to be 600 mg twice daily, defined as the maximal decrease in urinary prostaglandin E metabolite.³³ This study revealed an acceptable toxicity profile with the combination therapy and demonstrated a disease control rate above that expected for erlotinib alone. Based on these results, a phase II trial has been recently opened assessing combination therapy with celecoxib at 600 mg twice daily and erlotinib versus single agent erlotinib.

Signaling via other RTKs such as hepatocyte growth factor receptor (MET) and vascular endothelial growth factor receptor may increase tumor COX-2 expression.^{34–37} MET amplification has recently been implicated in acquired EGFR TKI resistance in NSCLC.² Up-regulation of COX-2 expression by RTK may provide additional mitogenic stimuli via the inflammation-dependent mechanisms described above. In addition, MET,³⁸ VEGF,³⁹ and other RTK^{40,41} pathways may be activated by cross-talk with GPCRs. Detailed molecular mechanisms underlying these effects are currently under investigation.

Inflammatory and RTK signaling pathways form complex networks with multiple overlapping modules. PGE2/EGFR cross-signaling has been one of the most extensively studied relationships between these pathways. The results of

preclinical studies support the hypothesis that simultaneous targeting of COX-2 and EGFR pathways may improve response in certain NSCLC patients. Thus far, this concept has been difficult to translate into clinical benefit. However, discovery of biomarkers that predict response to this approach may improve outcomes by allowing selection of patients who are most likely to benefit.^{42–44}

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