IN THE SPOTLIGHT

Understanding the MIG6–EGFR Signaling Axis in Lung Tumorigenesis

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Summary: With multiple clinical trials under way targeting mutant EGFR in patients with lung cancer, Maity and colleagues address important aspects of a MIG6–EGFR signaling axis using genetically engineered mouse models expressing mutated EGFRs on the *MIG6*-deficient background. This study extends our understanding of EGFR regulation by MIG6 and reveals that MIG6 antagonizes tumor formation in mutant EGFR–driven lung adenocarcinoma. *Cancer Discov; 5(5); 472–4. ©2015 AACR.*

See related article by Maity et al., p. 534 (4).

EGFR plays a critical role in primary glandular neoplasia initiation, growth, and dissemination. Multifaceted EGFR signaling orchestrates various cellular processes involved in differentiation, survival, cell-cycle progression, and drug sensitivity via RAS–RAF–MAPK, PI3K–AKT, JAK/STAT, and other pathways. Moreover, extensive cross-talk and transactivation have been observed between EGFR and other receptor tyrosine kinases that modulate the progression of solid cancers. Therefore, dysregulation of downstream EGFR signaling pathway modulators is an important factor when determining tumor sensitivity to EGFR inhibitors.

Mitogen-inducible gene 6 (MIG6; also known as RALT, ERRFI1, or Gene 33), a negative regulator of EGFR, plays an important role in signal attenuation of the EGFR network and subsequent EGFR degradation by blocking the formation of the activating dimer interface through interaction with the kinase domains of EGFR and ERBB2. *Mig6*-knockout mice exhibit hyperactivation of endogenous EGFR, resulting in hyperproliferation and impaired differentiation of pulmonary epithelial cells (1), which may progress to overt glandular adenocarcinoma. In addition, carcinogen-induced tumors in *Mig6*-deficient mice are unusually sensitive to EGFR tyrosine kinase inhibitors (TKI). In non–small cell lung cancer (NSCLC) without known *EGFR* mutations, the response to EGFR-targeted agents is inversely correlated with epithelial-to-mesenchymal transition (EMT; ref. 2). It those cells, MIG6-mediated reduction of EGFR occurs concomitantly with a TGFβ-induced EMT-associated kinase switch of tumor cells to an AKT-activated state, thereby leading to an EGFR-independent phenotype that is refractory to EGFR TKIs (2). Therefore, in wild-type EGFR tumors, MIG6 is considered a tumor suppressor and may be regarded as a molecular marker for indicating the intrinsic EGFR activity. Consequently, MIG6 may have a clinical value for predicting differential response of tumors to EGFR TKIs.

In lung cancer patients and patient-derived xenograft models with wild-type EGFR, a low MIG6:EGFR protein expression ratio was associated with higher gefitinib sensitivity, whereas a higher MIG6:EGFR value was associated with TKI resistance (3). Moreover, a low MIG6:EGFR ratio correlates with a marked increase in progression-free survival for lung cancer patients (3). Another study concluded that the ratio of *MIG6* and *miR200* RNA levels predicts response to erlotinib in directly xenografted primary human lung tumors, regardless of their wild-type EGFR expression levels (2), further supporting the role of MIG6 as a predictive biomarker for TKI sensitivity in the wild-type EGFR setting.

The role of MIG6 in molecular mechanisms underlying mutant EGFR-driven lung adenocarcinoma has not been studied in detail. It is well established that *EGFR* mutation status is a strong predictive factor for anti-EGFR therapy in lung cancer patients. Activating or gain-of-function mutations occurring in exons 19 and 21 result in increased activation of the downstream pathways and increase the sensitivity of the tumor to the EGFR TKIs. Unfortunately, despite the dramatic response to EGFR TKIs, most patients ultimately have a relapse. Mechanisms of acquired resistance to TKIs include EMT, secondary resistant *EGFR* mutations, e.g., T790M, and activating mutations or amplification of EGFR downstream effectors. Therefore, there is an urgent need to elucidate the signaling pathways activated downstream of mutated *EGFR* in TKI-sensitive and TKI-resistant tumors. Recent work by Maity and colleagues (4) has uncovered novel insights into the MIG6–EGFR signaling axis and proposed a model whereby increased MIG6 phosphorylation attenuates its ability to inhibit mutant EGFR.

Using the transgenic mouse models, the authors revealed that genetic ablation of *Mig6* accelerates NSCLC formation in mice expressing Tet-inducible L858R or del746–750 *EGFR* alleles. Significantly, *Mig6*-null mice succumbed to EGFR-driven lung tumors much faster than their wild-type littermates. In the *Mig6*-deficient background, accelerated lung tumor growth was seen early after induction of mutant

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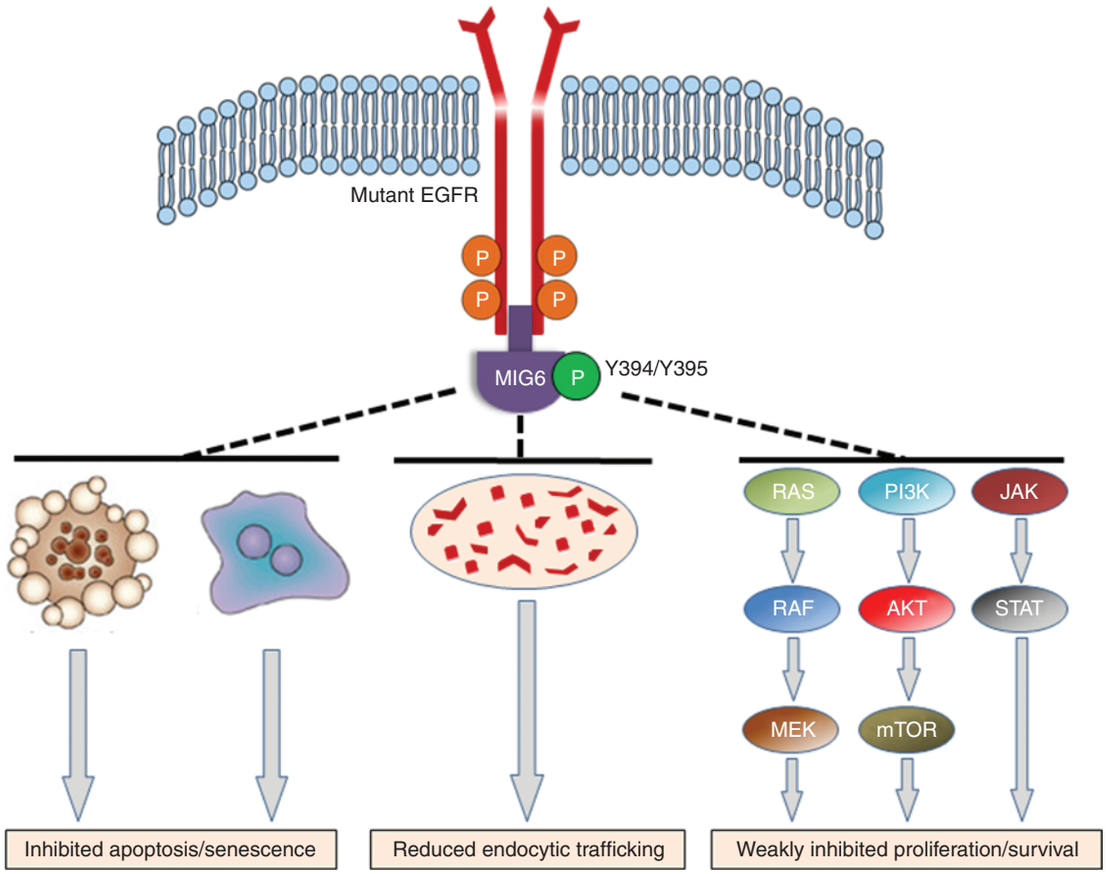

Figure 1. Effects of MIG6 on mutant EGFR inhibition. By constitutively catalyzing phosphorylation of MIG6 at Y394/Y395, mutant EGFR increases MIG6–EGFR binding affinity. In contrast to wild-type EGFR, the increased interaction stabilizes the mutant receptor, because phosphorylated MIG6 fails to induce substantial EGFR clathrin-dependent endocytic trafficking. Utilizing this mechanism, mutant EGFR partially retains its prosurvival and proliferative signaling. Moreover, prolonged retention of MIG6 to mutant EGFR may allow tumor cells to also escape from apoptosis and/or senescence. Although phosphorylation of MIG6 compromises its inhibitory potency, MIG6 still antagonizes tumor formation in the setting of EGFR mutants in transgenic mouse models.

EGFRs and was associated with increased EGFR–MAPK signaling, reflecting a substantial role for MIG6 during early stages of mutant EGFR-driven tumor growth. These observations suggest that MIG6 antagonizes lung tumorigenesis driven by *EGFR* mutations, in the same way it restrains lung tumorigenesis in wild-type *EGFR* models (1). Interestingly, MIG6 was also shown to be a haploinsufficient tumor suppressor in *EGFR* del746–750 mice. This is an exciting finding, which suggests that mutant EGFRs may dampen the MIG6-induced inhibitory effect by downregulation of MIG6 protein levels, similar to the regulation of a classic tumor suppressor. Decreased *MIG6* RNA and protein expression levels were observed in various human solid tumors, including lung tumors, and were shown to correlate with reduced overall survival. Recent studies in patients with lung cancer harboring wild-type EGFR concluded that absolute MIG6 levels can influence EMT and sensitivity of the tumors to EGFR TKIs (2), raising the question of whether the differential MIG6 expression correlates with initiation, progression, and EGFR-TKI response in the context of mutant EGFRs. These questions remain beyond the scope of the current work and warrant further clinical studies to address these important issues.

In their previous attempt to identify differences between signaling events downstream of wild-type and mutant EGFR, Guha and colleagues (5) discovered that MIG6 was hyperphosphorylated in human bronchial epithelial cells harboring *EGFR* mutations that confer TKI sensitivity, and mapped Y394 as a major MIG6 phosphotyrosine site in *EGFR*-mutant cell lines. Although MIG6 phosphorylation on Y394/Y395 (6, 7) and elevated level of *MIG6* RNA in *EGFR*-mutant lung adenocarcinoma cell lines (8) has been previously described, the significance of increased phosphorylation at these sites in an *EGFR*-mutant background remains largely unknown. In this study (4), the authors have further examined the functional consequences of constitutive MIG6 phosphorylation in *EGFR*-mutant lung adenocarcinoma cells. Through a series of comprehensive phosphoproteomic and genetic experiments, the authors discovered that phosphorylation at Y394/Y395 is inhibited by erlotinib in TKI-sensitive del746–750, but not in TKI-resistant $EGFR^{L858R+T790M}$ cells, confirming previously

reported evidence that MIG6 is a direct target of mutant EGFR (7). The Y394/Y395 site is located within the EGFR-binding region of MIG6 (spanning between residues 323 and 411). Enzymologic studies with purified proteins have shown that MIG6 phosphorylation affects its binding to EGFR and subsequent regulation of EGFR kinase activity by MIG6 (7, 9). Based on the mutational analysis of the above residues, the authors show that tyrosine phosphorylation of MIG6 seems to modulate both stability of mutant EGFR and its affinity. The authors report that phosphorylated MIG6 binds more tightly to mutant EGFR, but in contrast to wild-type receptor, the increased interaction does not direct mutant EGFR to the degradation pathway and enhances its stability. Based on these observations, the authors put forth a model in which enhanced phosphorylation of MIG6 at Y394/Y395 by mutant EGFRs expressed in NSCLC allows these mutant receptors to partially circumvent negative regulation by MIG6 (Fig. 1). Although there is prior evidence that MIG6 tyrosine phosphorylation weakens its ability to inhibit purified near full-length EGFR (9), the authors revealed that despite modulation of EGFR stability, MIG6 still antagonizes tumor formation in the setting of EGFR mutants in transgenic mouse models. Although the exact mechanisms are not yet clear, these observations unveil a conceptually novel mechanism for the biologic regulation of mutant EGFR signaling.

Growing evidence supported by recent studies shows that MIG6 may also exert its tumor-suppressor function by promoting proapoptotic signaling (10, 11). These observations suggest that besides unleashing cell proliferation, loss of MIG6 or increased phosho-MIG6 binding to mutant EGFR could also allow incipient tumor cells to escape from apoptosis (Fig. 1). Therefore, quantification of apoptotic cells at early points post-EGFR induction would be a desirable addition to our understanding of MIG6 tumor-suppressor function. Significantly, although this study proposes an intriguing possibility that phosphorylated MIG6 may exert an oncogenic function by stabilizing mutant EGFR and consequently enhancing its oncogenic potency, residual inhibitory activity of MIG6 is still tumor suppressive, because *MIG6* deficiency reduces survival of mouse models due to accelerated tumorigenesis.

Although a number of elements in this study have been previously identified, significant new discoveries by Maity and colleagues (4) connect multiple elements in a novel manner, and provide a convincing case that activating mutations in the EGFR tyrosine kinase domain may disrupt MIG6 suppressive function via increased phosphorylation of MIG6 itself, which causes a reduction of MIG6 ability to inhibit EGFR catalytic activity and promote EGFR downregulation. These findings extend our understanding of MIG6 in EGFR regulation, but additional studies will unravel the molecular mechanisms of its role in various oncogenic pathways and response to EGFR blockade by either small-molecule–targeted therapy or monoclonal antibodies. It is now clear that MIG6 is an important and perhaps key regulator of anti–EGFR-directed therapies, and significant investment in further defining its status as a predictive biomarker in patient samples and clinical studies is warranted.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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