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AUTHOR'S VIEW

ETS1 inactivation causes innate drug resistance to EGFR inhibitors

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ABSTRACT

Mutations in epidermal growth factor receptor (EGFR) are found in approximately 10% of lung cancers. Treatment with EGFR inhibitors, although promising, has surprisingly resulted in greater than 90% tumor reduction in only 5% of cases, prompting us to investigate the mechanism of innate drug resistance.

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BCL2L11 (best known as BIM); dual specificity phosphatase 6 (DUSP6); ETS1 transcription factor; epidermal growth factor receptor (EGFR); innate drug resistance; MAPK3 (best known as ERK1); MAPK1 (best known as ERK2); non-small-cell lung cancer (NSCLC); tyrosine kinase inhibitor (TKI)

Lung cancer is the leading cause of cancer death in the US and other countries. Of the 2 major histologic types, small cell lung cancer and non-small cell lung cancer (NSCLC), the latter is by far the more prevalent (~85%). Approximately 14% of NSCLCs harbor mutations in the gene encoding epidermal growth factor receptor (EGFR), a receptor tyrosine kinase (RTK). Despite the fact that treatment of these mutated NSCLCs with EGFR tyrosine kinase inhibitors (TKIs) has shown remarkable progress, a recent randomized Phase 3 clinical trial with erlotinib found tumor reduction greater than 90% in only 5% of patients.[1] Although the remainder also responded, the response was only partial even though they too had TKI-sensitive *EGFR* mutations—either exon 19 deletions or the L858R missense mutation in exon 21. This limited primary response could be attributable to resistance inherent in the tumor cells rather than resistance acquired over the course of treatment.

RTK ligands secreted through paracrine, autocrine, and endocrine mechanisms in the tumor microenvironment are important determinants of therapeutic responses to anticancer kinase inhibitors. Indeed, hepatocyte growth factor (HGF)-mediated activation of the RTK MET is the most likely cause of innate resistance to EGFR TKIs in NSCLC cells.[2] However, the precise contribution of MET activation remains unclear. Given that causes of acquired resistance to EGFR TKIs are multiple and complex, the innate resistance of NSCLC cells may likewise be heterogeneous.

We recently investigated the molecular mechanism by which the RAS/mitogen-activated protein kinase (MAPK) pathway is activated after EGFR inhibition despite blockade of RTK activity in NSCLC cells (Fig. 1).[3] EGFR TKIs suppressed both MAPK and AKT protein kinase pathways for a short time, after which the RAS/MAPK pathway became reactivated. AKT inhibition selectively blocked the transcriptional activation of ETS1, which in turn inhibited its target gene, *dual specificity phosphatase 6* (*DUSP6*), a negative regulator specific for MAPK3 (best known as ERK1) and MAPK1 (best known as ERK2). As a result, ERK1/2 was activated.[4] Furthermore, elevated SRC (best known as c-SRC) stimulated Ras GTP-loading and activated RAF and MEK kinases. These observations suggested that not only ERK1/2, but also AKT activity, is essential to maintain the ETS1 transcription factor in an active state. Therefore, despite high levels of ERK1/2, ETS1 target genes including *DUSP6* and *CCND1*, *CCND3*, and *CCNE2* (best known as *cyclin D1*, *cyclin D3,* and *cyclin E2*) remained suppressed after EGFR inhibition. The reduction in DUSP6 combined with c-SRC to renew activation of the RAS/MAPK pathway, resulting in increased cell survival by accelerating protein turnover of BCL2L11 (best known as BIM).[5] Thus, EGFR TKIs evoked innate drug resistance by preventing AKT activity and inactivating ETS1 function in NSCLC cells.

To our knowledge, our report was the first to highlight ETS1 transcription factor at the juncture of the AKT and MAPK pathways and to reveal its contribution to innate resistance to EGFR TKIs (Fig. 1).[3] We demonstrated that synthesis of ETS1 and transactivation of its target genes require AKT and ERK1/2 kinase activities. For this reason, although ERK1 and ERK2 were more active after EGFR inhibition, they could not transactivate ETS1 target genes. The outcome of persistent inactivation

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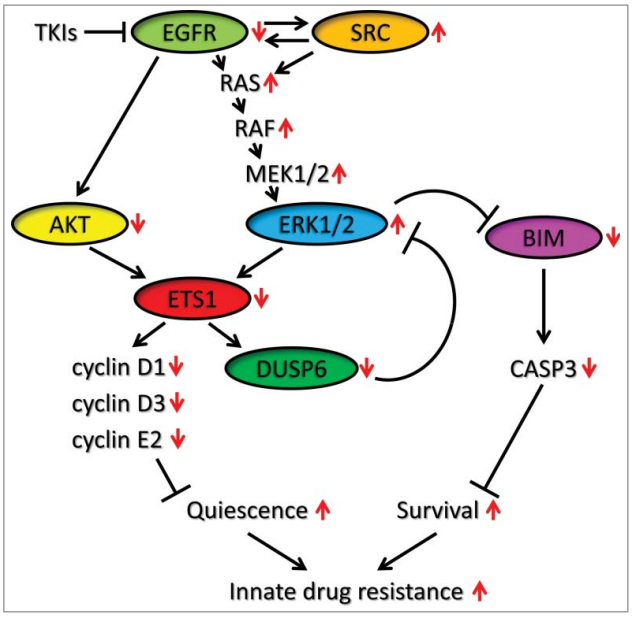

Figure 1. Molecular mechanism of EGFR inhibition and innate resistance to EGFR inhibitors in NSCLC cells with EGFR mutations. Increases and decreases in the activity/expression of signaling molecules or biological outcomes resulting from EGFR inhibition are indicated by red arrows. EGFR, epidermal growth factor receptor; NSCLC, non-small cell lung cancer.

of ETS1 is quiescence and survival of the NSCLC cells; quiescence is triggered by reduction of the cyclins and cell survival is enhanced when sustained activation of ERK1/2 after DUSP6 inhibition accelerates BIM protein turnover.[4,5] Thus, our insights into the regulation of ETS1 target genes reveal a new point of convergence of AKT and MAPK signaling and elucidate a previously unidentified aspect of the innate resistance to EGFR TKIs in the absence of growth factors, without activation of RTKs.

We found that addition of a MEK inhibitor enhances programmed cell death by rewiring apoptotic signaling.[3] Therefore, we can reduce the probability of emergent resistance to EGFR TKIs in NSCLCs by combined TKI and MEK inhibitor treatment. Recent reports from other laboratories have also proposed this novel combined therapy, which is thought to be effective not only in innate resistance, but also in acquired resistance with T790M second-site EGFR mutation.[6,7] In fact, ERK1/2 reactivation was similarly observed with DUSP6 reduction after L858R/T790M EGFR-selective inhibition in originally TKI-resistant NSCLC cells.[8] Thus, whether drug resistance is innate or acquired, our study has provided a compelling rationale for combination treatment in EGFR-mutated NSCLCs.

Given that EGFR TKIs synergize with MEK inhibitors in EGFR-dependent NSCLCs for growth inhibition and emergence of drug resistance, the question arises of why administration of single-agent MEK inhibitor is rarely considered. One possible explanation could be that in past clinical trials to test MEK inhibitors, NSCLC patients were selected on the basis of *KRAS* (best known as *K-RAS*) status and not *EGFR* mutations in their tumors, and thus no objective responses were found in the cohort.[9,10] If the researchers had chosen an EGFR-mutated subset the results might have been different.[7]

In summary, our study demonstrated that the discrepant responses to EGFR TKIs among NSCLC tumors harboring *EGFR* mutations could be attributable to innate drug resistance. We found that EGFR inhibition evokes innate resistance by preventing AKT activity and thus inactivating ETS1 function. The result is paradoxical ERK1/2 activation. Because we found that addition of a MEK inhibitor enhances programmed cell death by rewiring apoptotic signaling, we may be able to reduce the probability of emergent resistance to EGFR TKIs by combined TKI and MEK inhibitor treatment. A randomized double-blind trial is necessary before this novel therapy can be integrated into the management of EGFR-mutated NSCLCs in the clinical setting.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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