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Secrets of drug resistance in NSCLC exposed by new molecular definition of EMT

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Summary

NSCLC metastasis and drug resistance has been associated with epithelial-to-mesenchymal transition (EMT). This study reports the development of a robust gene expression signature of EMT in NSCLC and reveals new insights into the key molecular events that underlie EMT and drug resistance in NSCLC.

In this issue of *Clinical Cancer Research, Byers et al* report a gene expression signature of epithelial-mesenchymal transition (EMT) that can classify non-small cell lung cancers (NSCLCs) as either epithelial or mesenchymal and demonstrate its utility as a biomarker of response to some targeted therapies used in NSCLC patients (1) (Figure 1).

The management of NSCLC patients has changed dramatically over the past decade because of the identification of molecular drivers of NSCLC and the development of targeted therapies that act against many of these key oncogenic drivers (2, 3). Molecularly targeted therapies used in many NSCLC patients are less toxic and more effective than conventional chemotherapy. This is because NSCLCs that harbor a driver oncogene depend on its activity for their growth such that targeted inhibition of it causes tumor regression with minimal effect in normal cells lacking its expression. Indeed, in the ~10–15% of NSCLC patients with advanced disease whose tumors harbor activating mutations in the kinase domain of the epidermal growth factor receptor (EGFR) the EGFR tyrosine kinase inhibitor (TKI) gefitinib or erlotinib is standard first-line therapy (3, 4). However, EGFR mutant NSCLC patients respond variably to initial EGFR TKI therapy and those who initially respond invariably relapse because of the development of drug resistance (5, 6). Additionally, some patients whose NSCLCs harbor wild-type EGFR also benefit from EGFR TKI treatment. Developing more effective molecular biomarkers of response and resistance to EGFR TKI treatment in both EGFR mutant and EGFR WT NSCLCs is essential to optimize the use of EGFR TKIs in NSCLC patients.

Byers et al shed light on this issue by further investigating the relationship between EMT and drug sensitivity in NSCLC. EMT is a phenotypic manifestation of complex changes in gene expression that include decreased expression of epithelial markers (e.g. E-cadherin) and increased expression of mesenchymal markers (e.g. vimentin) (7). EMT as defined by the analysis of a limited set of epithelial or mesenchymal markers has been observed in a several epithelial cancers, including NSCLCs. EMT has been associated with increased tumor cell proliferation, invasion, migration, and metastasis and in some cases with

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resistance to EGFR inhibitor treatment in NSCLCs (7, 8). Yet, a robust and comprehensive gene expression signature capturing the molecular elements underlying EMT and its association with drug resistance in NSCLC had not been developed. Thus, the relationship between EMT and drug response and the molecular events driving the observed clinical manifestations of EMT in NSCLC have remained incompletely characterized.

Through gene expression profiling in a large panel of NSCLC lines, *Byers et al* defined a signature consisting of 76 genes whose expression most closely correlated with several established markers of EMT, including E-cadherin and vimentin. The authors found that the gene expression classifier composed of the differential expression of these 76 genes could reliably cluster the NSCLC lines into either an epithelial or mesenchymal group. The authors found that cell lines in the mesenchymal group expressed increased levels of EMT markers, such as MMP2, vimentin, and ZEB1. Among the genes increased in mesenchymal lines was the kinase AXL that had been linked previously to EMT in some breast and pancreatic cancers (9, 10). The authors then used a high-throughput proteomics approach to identify differences in protein expression between the cell lines classified by the EMT gene expression signature as either epithelial or mesenchymal. An unsupervised analysis of the proteomic data clustered the cell lines into either the epithelial or mesenchymal group and also confirmed overexpression of AXL in the mesenchymal class. Together, the integrated gene expression and proteomic analysis demonstrated the robust discriminative power of the novel EMT classifier in the NSCLC models.

In a series of elegant *in vitro* experiments, the authors showed that the EMT gene expression signature could be used as a predictive biomarker of resistance to erlotinib and inhibitors of PI3K, AKT and mTOR signaling in a panel of NSCLC cell lines derived from treatment naïve patients. The cell lines classified by the EMT signature as mesenchymal were more resistant to erlotinib and the PI3K pathway inhibitors, but not other targeted agents or cytotoxic chemotherapies, than the cell lines in the epithelial group. In some of the erlotinib-resistant mesenchymal cell lines that had increased expression of EMT markers including AXL, pharmacologic inhibition of AXL was synergistic with erlotinib both *in vitro* and *in vivo*. These effects of combined AXL and EGFR inhibition were observed in some cell lines expressing WT EGFR, suggesting that AXL is a promising therapeutic target to enhance EGFR TKI response in selected EGFR WT NSCLC patients.

The authors clinically validated their preclinical observations by examining whether the EMT signature was a predictive biomarker of erlotinib response in EGFR WT (and KRAS WT) NSCLC patients enrolled on the BATTLE-1 trial. Indeed, erlotinib was more effective at controlling the disease in those patients whose NSCLCs were classified by the EMT signature as epithelial compared to mesenchymal, in which increased expression of AXL and its ligand GAS6 was observed. This correlation between the EMT signature and clinical outcomes was predictive and not merely prognostic because there was no association between the EMT signature and outcomes in all clinically evaluable patients enrolled on all treatment arms of the BATTLE-1 trial. These results raise the exciting possibility that the EMT gene signature could be used to predict response to erlotinib in a broad spectrum of NSCLC patients and suggest that inhibition of AXL may enhance responses to erlotinib in some patients with EGFR WT NSCLCs.

The data reported by *Byers et al* compliment recent work that demonstrated that AXL causes acquired EGFR TKI resistance in some EGFR mutant NSCLCs, in some cases in association with an EMT, and that AXL inhibition overcomes resistance to erlotinib in this setting (11). Together, these studies highlight a previously unappreciated and important role for AXL and EMT in regulating response to EGFR inhibitor treatment in NSCLC patients. Prospective

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clinical studies aimed at validating the use of the EMT signature and AXL as predictive biomarkers of drug response and therapeutic targets in NSCLC patients are warranted.

The report by *Byers et al* significantly increases our understanding of the importance of EMT in the response of NSCLCs to targeted therapy and provides rationale for further studies. Though the data show that increased AXL expression is associated with the mesenchymal signature, this study does not directly address the potential mechanisms underlying AXL upregulation. Are there epigenetic alterations that regulate AXL expression in the setting of EMT, and could such epigenetic events be potential therapeutic targets? Are there genomic alterations that contribute to EMT or AXL upregulation in NSCLCs that remain undefined? Furthermore, *Byers et al* found evidence of EMT and increased AXL expression in cells obtained from treatment-naïve patients, while other work showed that AXL upregulation can occur during treatment with EGFR TKIs (11). Is the mechanism of AXL overexpression and the biological functions of AXL identical or different in these distinct contexts? Since emerging data have implicated AXL and EMT in both innate and acquired resistance to erlotinib, how could EMT could be prevented or reversed in NSCLC? Uncovering the answers to these questions should enable us to develop strategies to optimize the efficacy of mechanism-based therapies that enhance outcomes for NSCLC patients broadly.

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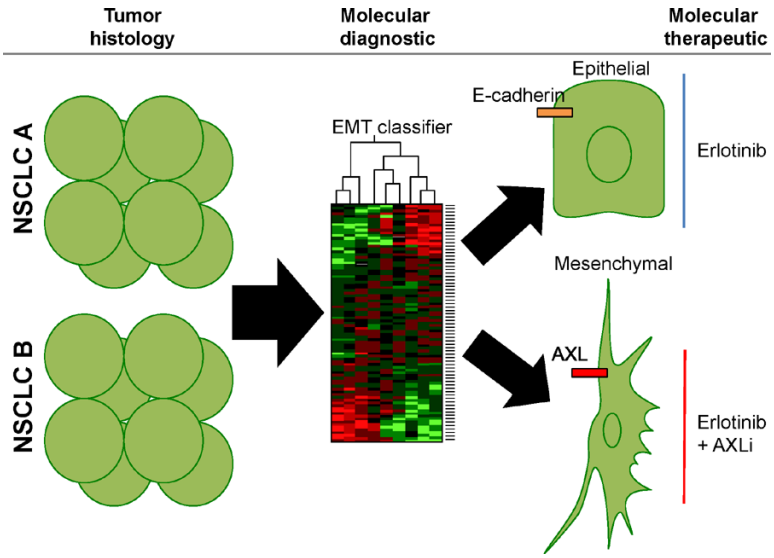

Figure 1.

Diagnostic and therapeutic implications of a molecular classifier of EMT in NSCLC. NSCLC A and NSCLC B appear histologically similar by conventional diagnostic criteria. However, the novel EMT gene expression signature developed by *Byers et al* can reveal critical molecular differences in these tumors that classify them as either epithelial or mesenchymal. This molecular diagnostic classification could be therapeutically important because it predicts response to treatment with selected targeted therapies used in NSCLC patients, including the EGFR TKI erlotinib. Here, NSCLC A is determined to have an epithelial signature, indicating that it is likely to be sensitive to erlotinib monotherapy. In contrast, NSCLC B is determined to have a mesenchymal signature, suggesting that it is likely to be insensitive to erlotinib monotherapy. Some of the EGFR TKI insensitive, mesenchymal NSCLCs harbor overexpression of AXL. The mesenchymal NSCLCs with AXL overexpression could be treated effectively with the combination of an EGFR TKI (erlotinib) and an AXL inhibitor.

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