

## Biomarkers for EGFR-Antagonist Response: In the Genes and on the Genes!

Hariharan Easwaran and Stephen B. Baylin

Patients with non-small cell lung carcinoma containing epidermal growth factor receptor (EGFR)-activating mutations benefit from EGFR-antagonist therapy. EGFR-antagonist sensitivity is also correlated with epithelial-mesenchymal transition, which Walter and colleagues show to be marked by DNA-methylation changes. If validated, these markers could help identify patients with wild-type EGFR who will benefit from EGFR therapy. *Clin Cancer Res*; 18(8); 2121-3. ©2012 AACR.

In the current issue of *Clinical Cancer Research*, Walter and colleagues (1) report that non-small cell lung cancers (NSCLC) can be stratified into epithelial- and mesenchymal-like tumors based on DNA-methylation markers. Earlier studies indicated that NSCLC tumors with wild-type epidermal growth factor receptor (EGFR) and an epithelial-like gene expression phenotype seem to be sensitive to the EGFR-antagonist erlotinib. Thus, DNA-methylation markers combined with a gene expression panel may help identify NSCLC patients with wild-type EGFR for EGFR-antagonist therapy.

Lung cancer, the majority (84%) of which consists of NSCLC cases, is by far the leading cause of cancer deaths (2). Radiation and chemotherapy have been the first-line choice of treatment for patients with NSCLC, yielding a median survival duration of 7 to 8 months (3). The realization that EGFR is overexpressed in a significant proportion of NSCLCs led to the development of inhibitors that target EGFR activity (3). Although the development of small-molecule EGFR antagonists [also called EGFR tyrosine kinase inhibitors (EGFR-TKI)] resulted in a significant increase in survival, a subset of patients with EGFR-activating mutations were shown to benefit most from the EGFR antagonists [median survival, 25–30 months (3)]. Currently, the EGFR antagonists erlotinib and gefitinib are considered first-line treatments for chemotherapy-naïve advanced disease (4), and second-/third-line treatments for chemotherapy-resistant tumors (5). Aside from the EGFR activating mutation status being the best predictor of response, and KRAS mutations being a negative predictor, there is a lack of markers to predict

responders to EGFR antagonists. Studies using cell lines (6, 7) and patient samples (8) have shown that sensitivity to EGFR antagonists is correlated with epithelial-mesenchymal transition (EMT). This biologic change (Fig. 1) is postulated to be important for the acquisition of stem-like properties in cancers and tumor aggressiveness (9). The observations that epithelial-like tumors are sensitive to EGFR antagonists independently of EGFR-activating mutations, and mesenchymal-like tumors are resistant, can thus contribute to the search for molecular tools that can help identify patients with NSCLC who are most likely to benefit from EGFR-antagonist therapy. Currently, this could enhance the selection of ~13% of NSCLC patients with wild-type EGFR, who seem to be most responsive to these drugs (10).

Molecular tools to distinguish epithelial-like and mesenchymal-like NSCLC tumors could involve the use of immunohistochemistry to identify protein markers for loss of epithelial phenotype [E-cadherin (CDH1),  $\gamma$ -catenin (JUP)] or gain of mesenchymal features [vimentin (VIM), fibronectin (FN1) ref. 8]. Direct mRNA profiling of selected gene panels can also be used to identify EMT (6). However, these tools by themselves may not be optimal due to the low yield and instability of mRNA in formalin-fixed, paraffin-embedded sections, as well as the technical challenges of detecting such protein markers, and in particular precisely quantifying them by immunohistochemistry. These factors limit their use in clinical settings for diagnosis and especially for testing EMT as a predictor of drug response in clinical trials. Techniques to detect gene expression panels from formalin-fixed, paraffin-embedded sections are improving and may become practical to perform in the clinic (11). However, because DNA-based assays are more optimal due to the stability of DNA, Walter and colleagues (1) explore DNA-methylation changes as markers for EMT in their current work.

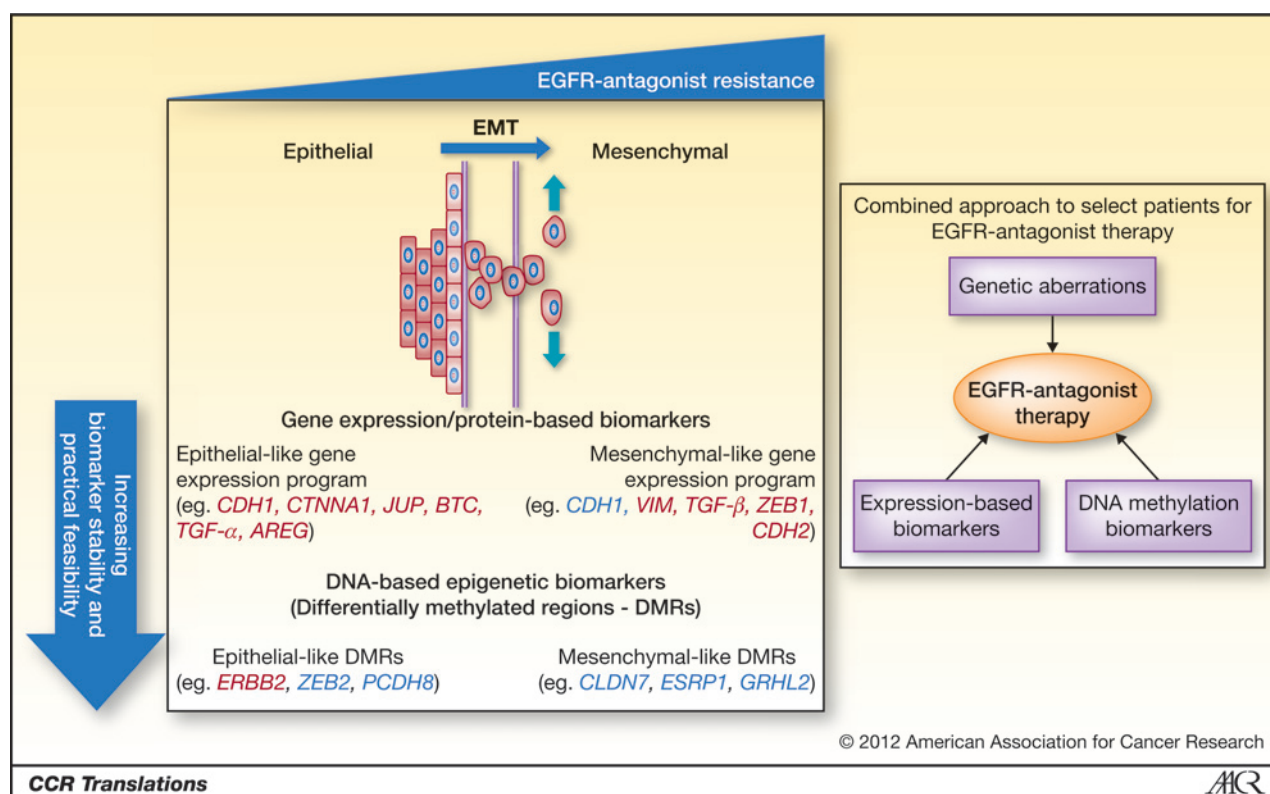
DNA methylation in eukaryotes mainly involves the modification of cytosines to 5-methylcytosine at CpG dyads and is important for the heritable transfer of gene expression programs. The biology of the modified CpG sites

**Authors' Affiliation:** Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine, Baltimore, Maryland

**Corresponding Author:** Hariharan Easwaran, Bunting Blaustein Cancer Center, Suite 541, 1650 Orleans Street, Baltimore, MD 21231. Phone: 410-955-8506; Fax: 410-614-9884; E-mail: heaswar2@jhmi.edu

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**Figure 1.** Epigenetic abnormalities in NSCLC track with EMT and may help predict sensitivity to EGFR-antagonist therapy in patients. A subset of NSCLCs display EMT resulting in aggressive and metastatic tumors (cells depicted as invading the basement membrane shown by the 2 vertical purple lines). Epithelial-like NSCLCs are more sensitive to EGFR-antagonist agents (e.g., erlotinib) compared with mesenchymal-like NSCLCs. These biologic states can potentially be discriminated with the use of gene-expression-based biomarkers (genes shown in red and blue are up- and downregulated, respectively) or DNA-based biomarkers (DMR; genes shown in red and blue are hypo- and hypermethylated, respectively). Because of the stability of DNA-methylation markers, DMRs are practically feasible surrogates for gene-expression-based discrimination of epithelial- versus mesenchymal-like NSCLCs. A combined approach involving DNA-based biomarkers (e.g., genetic aberrations and DNA methylation) and gene-expression-based biomarkers will be key for determining which patients with NSCLC will benefit from EGFR-antagonist therapy (right).

is critical for considering cancer biomarkers. Thus, ~85% to 90% of the CpG-rich islands located in proximal gene promoters normally do not harbor DNA methylation, whereas such regions located elsewhere, as well as many non-CpG-rich regions, may be variably DNA methylated among normal cell types. Hypermethylation of promoter CpG islands occurs commonly in cancers but not in normal cells; thus, such cancer-specific hypermethylation has a high potential for aiding in the development of biomarker strategies. Walter and colleagues discuss previous observations that during EMT in a breast tumorigenesis model system, silencing of epithelial marker genes, such as E-cadherin, is associated with cancer-specific *de novo* DNA methylation of CpG sites (12). They hypothesize that EMT-associated DNA-methylation changes might also occur in NSCLC cell lines and tumors and provide differential methylation patterns that could be useful for identifying epithelial-like versus mesenchymal-like tumors, and their associations with erlotinib sensitivity. Using global DNA-methylation profiling of a panel of 69 NSCLC cell lines that were classified into epithelial- versus mesenchymal-like types based on a previously defined gene expression panel (6), Walter and colleagues propose an underlying epigenetic

signature that efficiently identifies most epithelial- and mesenchymal-like NSCLC cell lines. The signature involves differentially methylated regions (DMR) that consist of 549 loci, based on DNA-methylation profiling of the cell lines. Of importance, some of the DMRs are in CpG islands of genes involved in EMT, indicating that these are bona fide classifiers of underlying biology. Using a select group of DMRs (methylation-based classifier), Walter and colleagues were able to efficiently classify cell lines into epithelial and mesenchymal phenotypes, as well as to determine erlotinib sensitivity. They then compared a 13-gene expression panel with their methylation-based classifier, which separated the epithelial versus mesenchymal phenotype in a panel of 31 primary NSCLC samples. Thus, by using a range of common DNA-methylation techniques from pyrosequencing, quantitative fluorescent methylation-specific PCR (qMSP, a highly sensitive and quantitative PCR assay), and global DNA-methylation profiling, and then investigating a larger panel of primary tumor specimens, the authors derived 2 markers for the epithelial phenotype: (i) hypomethylation of CpGs at a putative, intergenic, *ERBB2* enhancer region; and (ii) hypermethylation of a CpG-island promoter of the EMT regulator *ZEB2*.

Where do these initial results of Walter and colleagues leave us? They are important for thinking about a number of future directions. First, the biologic significance of these authors' findings should prompt further studies to dissect how the altered DNA-methylation patterns, and other epigenetic changes that may accompany them, might function to hold NSCLC into subgroups of epithelial- versus mesenchymal-like phenotypes. Such work should include an attempt to define the mechanisms, such as altered expression of *ZEB2*, that control these phenotypes and specify altered sensitivity to EGFR antagonists and other drugs. Second, further studies should hone the methylation-marker approach for actual use in the clinic. The study presented here by Walter and colleagues sets the stage for exploring the EMT and including DNA-methylation profiling in future prospective studies. In all cancer types and virtually every tumor, hundreds of genes have altered DNA-methylation patterns; the best markers among these would be those that exhibit the most cancer-specific changes, such as abnormal promoter CpG-island methylation. The data acquired by Walter and colleagues should be mined further to provide an even more robust marker panel to eliminate problems with normal cell background signals. The detection of such marker panels, probably combined with the detection of key genetic and expression changes (Fig. 1) in both tumor samples and serum DNA (as a noninvasive approach), must be achieved in larger validation studies to show clinical efficacy in patients with NSCLC.

Finally, we are in an age in which epigenetic therapy is gaining much attention for its potential to reverse abnormal

DNA-methylation and chromatin patterns that underlie abnormal cancer gene expression. Our group recently showed this potential in patients with advanced, chemorefractory NSCLC and the value of using promoter DNA hypermethylation changes as markers to predict which patients are most likely to derive the most benefit from particular therapies (13). Might such therapies be useful for altering sensitivity to the therapies studied by Walter and colleagues? Over the past decade, many studies have highlighted the potential of using DNA-methylation changes in cancer to devise biomarker and therapy strategies. The work of Walter and colleagues provides yet another important example. The time is a rich one for taking these concepts ever closer to use in actual clinical management.

#### Disclosure of Potential Conflicts of Interest

S.B. Baylin is a consultant to and serves on the advisory board of Constellation Pharmaceuticals, Aztec Pharmaceuticals, and MDx Health. MSP is licensed to MDxHealth in agreement with Johns Hopkins University, and S.B. Baylin and Johns Hopkins University are entitled to royalty shares received from sales. No other potential conflicts of interest were disclosed.

#### Authors' Contributions

**Conception and design:** H. Easwaran, S.B. Baylin

**Writing, review, and/or revision of the manuscript:** H. Easwaran, S.B. Baylin

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