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Histone modification signatures as potential drivers of the mesenchymal-like EGFR-mutant TKI resistant NSCLC cell models.

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Introduction

Activating mutations of epidermal growth factor receptor (EGFR) dictate responsiveness of non–small cell lung cancer (NSCLC) patients to EGFR tyrosine kinase inhibitors (TKIs), including gefitinib and erlotinib. Despite promising initial responses, acquired resistance universally emerges, mediated by the secondary T790M mutation in approximately 60% of patients. NSCLCs with acquired EGFR TKI resistance expressing epithelial-to-mesenchymal transition (EMT) features have also been identified in several clinical studies. Similarly, NSCLC cell lines with EGFR TKI sensitizing mutations develop EMT upon chronic exposure to erlotinib in vitro, and has been extensively used to dissect the genetic and epigenetic changes driving the mesenchymal-like phenotype.

EMT is a transcriptional program observed in normal development and is implicated in tumor progression and metastasis. During the acquisition of drug resistance, neoplastic cells undergo dynamic and reversible transitions between multiple phenotypic states, the extremes of which are defined by the repression and overexpression of epithelial and mesenchymal genes, respectively. This plasticity is enabled by underlying epigenetic switches, depending on complex epigenetic regulatory mechanisms, in particular the induction of histone modifications associated with chromatin. Currently, patients with EGFR TKI resistant tumors presenting EMT are left with no therapeutic options. The understanding of the functional interactions between such EMT transcriptional program and the chromatin remodeling factors will provide druggable targets (e.g., histone modifier enzymes). For this aim, in this work we purpose the identification of the EMT epigenetic signature induced by EGFR TKI resistance in EGFR-mutant cell lines and the analysis of potential histone modulators that dictates EMT.

Methodology

We used EGFR-mutant NSCLC cell lines of HCC827, HCC4006, and NCI-H1975 (ATCC). To generate cell lines resistant to EGFR TKIs, cells were exposed to increasing concentrations of TKIs over 6 months and EMT markers were evaluated to identify and obtain mesenchymal cells as our model of study. To elucidate the epigenetics mechanisms promoting EMT in NSCLCs harboring mutated EGFR, we have performed western blotting to analyze global levels of the tri-methylated histone marks (H3K4me3, H3K9me3, H3K27me3), and Chromatin Immunoprecipitation (ChIP) assays of these histone marks followed by the detection of bona fide epithelial (CDH1, EPCAM, GRHL2, MIR200C) and mesenchymal (Vimentin, ZEB1, TGFβ, NNMT) genes by real time PCR. We have also evaluated by RT-PCR the gene expression of different histone methyltransferases during EGFR inhibition and EMT activation.

Results and conclusions

We observed a differential profile of global histone methylation marks in TKI sensitive epithelial versus TKI resistant mesenchymal NSCLC cells, which indicates that EMT program induces a dramatic epigenetic reprogramming driven by these chromatin modifications. Besides, using ChIP followed by real time PCR, and scoring the enrichment on target EMT genes of H3K4me3, H3K9me3, and H3K27me3 marks, we found a significant enrichment of mesenchymal genes with the activation mark H3K4me3, while repressive marks H3K9me3 and H3K27me3 were enriched in epithelial markers. Interestingly, we also found a cell-specific pattern of the enrichment scores, suggesting that each tumor would have an intrinsic epigenetic background that prone cells to EMT during the acquisition of TKI resistance under drug-induced selection and adaptation. The integration of these data with gene expression analysis and the promoter DNA methylation profiling of both EMT and histone modifying genes will allow the identification of epigenetic EMT drivers, providing druggable targets for overcoming the TKI acquired resistance.

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