Hiding in Plain Sight: Epigenetic Plasticity in Drug-Induced Tumor Evolution

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ABSTRACT: Cancer is a heterogeneous disease with key differences at the cellular and molecular levels. Acquisition of these differences during the course of tumor development manifests into functional and phenotypic heterogeneity leading to tumor diversity, also referred to as intra-tumor heterogeneity (ITH). Within a tumor, there are subpopulations of cells capable of tumor initiation and maintenance. These cells often exhibit resistance to standard-of-care anti-cancer drugs. However, the role of various subpopulations (clones) in drug resistance remains to be investigated. Moreover, the jury is still out about whether drug resistance is a result of clonal selection of preexisting cells, or the cells acquire resistance by dynamic re-wiring of their epigenome. Therefore, we investigated the drug-induced tumor evolution in patient-derived primary cells of head and neck squamous cell carcinoma. Our data demonstrated the role of a preexisting poised epigenetic state in drug-induced adaptive evolution of tumor cells. Importantly, the combination of chemotherapy and epigenetic inhibitors can prevent/delay drug-induced tumor evolution.

KEYWORDS: cellular reprogramming, epigenetic plasticity, single cell RNA-sea, tumor evolution, drug-resistance

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Game of Clones: You Gain Some, You Lose Some

Intra-tumor heterogeneity (ITH) within cancer subpopulations is a major cause of therapy resistance.[1] Drug resistance can be acquired by preexisting genetic ITH or by epigenetic adaptation into a new environment.[2] Both genetic and epigenetic heterogeneities can drive ITH. However, the dynamics of epigenetic adaptation, consequent ITH, and subsequent drug resistance remain largely unexplored. To identify ways to circumvent resistance and improve patient survival, it is important to understand the underlying mechanisms that drive acquired drug resistance. To date, efforts to understand the basis of therapy resistance have largely focused on uncovering genetic alterations within a tumor population that are selected during drug treatment.[3] However, findings that are more recent have also revealed nongenetic mechanisms of drug resistance.[4,5] Many cancers exhibit marked cell-to-cell variability in gene expression and functional phenotype but lack genetic explanation, suggesting the involvement of epigenetic mechanisms.[6]

We employed 2 phenotypically distinct patient-derived primary cells (PDPCs) from head and neck squamous cell carcinoma (HNSCC) patients (HN120 and HN137) to model drug-induced tumor evolution.[7] PDPCs derived from HN120 primary tumor (HN120Pri) displayed phenotypic homogeneity for epithelial cell state in terms of E-cadherin (ECAD) expression. Cells from HN137 primary tumor (HN137Pri) exhibited phenotypic heterogeneity and consisted of both epithelial (ECAD+) and mesenchymal (VIM+) cells.[8,9] We seeded ~100 cells from each PDPC model in 24 wells of a 384-well plate and examined their evolutionary trajectories every 72 hours over the course of 6 weeks. We observed remarkable divergence in evolutionary trajectories of 2 models. Drug-induced tumor evolution leads to loss of mesenchymal (VIM+) cells in HN137Pri cells and result cisplatin-resistant (CR) population, demonstrating highly epithelial properties. In complete contrast, HN120Pri cells gained mesenchymal phenotype (Vimentin expression) and CR clones exhibited epithelial to mesenchymal (EMT) like properties (ECAD+/VIM+). These results clearly demonstrated that distinct tumor cells could take very divergent paths to acquire drug-resistant properties (Figure 1).

Epigenetically Camouflaged Cell State

One of the most striking observations of our study was the emergence of de novo EMT-like (ECAD+/VIM+) cell state leading to drug-induced adaptive tumor evolution. To understand the mode of this transdifferentiation, we investigated the state of chromatin organization during drug resistance. We hypothesized that in a conceptual framework of the Waddington epigenetic landscape, cancer cells with the preexisting epigenetic state can be tipped into valleys of EMT-like state resulting in the acquisition of drug resistance. We assessed the open chromatin state by profiling H3K4me3 marks in HN120Pri cells and, surprisingly, promoters of EMT-like genes were decorated with H3K4me3 even in the absence of drug treatment. Next, we analyzed the active chromatin mark (H3K27ac) in these cells and acetylation of H3K27 on promoters of EMT-like genes corroborated with the acquisition of drug-resistant (ECAD+/VIM+) phenotype. Our results indicated that the camouflaged (epigenetically poised, transcriptionally inactive) cell state in HN120Pri cells allowed a

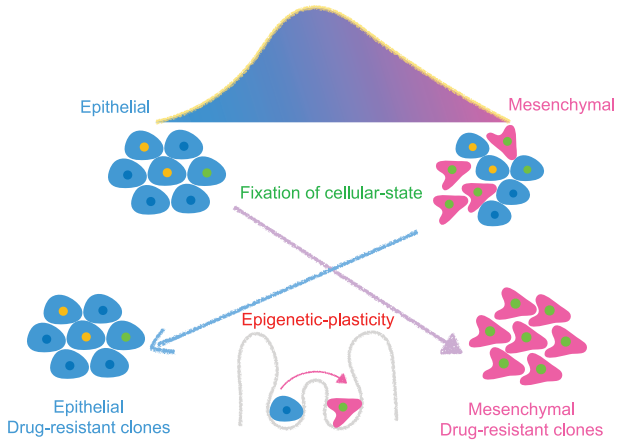

Figure 1. Implication of epigenetic plasticity in drug resistance. Tumor cells exist in the continuous of epithelial to mesenchymal cell states. Cells can acquire drug resistance by Darwinian selection of preexisting cell states (increase epithelial or mesenchymal properties) or by adapting to different cell states (epithelial to mesenchymal). Preexisting poised chromatin state provides a platform for epigenetic plasticity–mediated cellular reprogramming during drug-induced tumor evolution.

drug-induced cell state transition leading to tumor evolution. Because epigenetic reprogramming leads to tumor evolution, we hypothesized that an epigenetic modulator could prevent or delay the emergence of drug resistance. We performed an RNAi-based screening and identified BRD-4 as key epigenetic modulator involved in drug-induced chromatin remodeling. We demonstrated that application of JQ1[10] (BRD-4 inhibitor) can delay the drug-resistant (ECAD+/VIM+) phenotype by preventing H3K27 acetylation at promoters of EMT-genes. These results indicate the importance of including epigenetic inhibitors with the chemotherapeutic drug for combinatorial targeting of tumor evolution.

Future Directions: Toward Intervention

In this proof-of-principle study, we demonstrated the importance of the camouflaged epigenetic state in drug-induced tumor evolution. Moreover, other recent studies also indicate the role of epigenetic plasticity in the acquisition of drug resistance. The next frontier would be directly applying single-cell transcriptomics and epigenomics technologies on longitudinal clinical samples to track drug-induced tumor evolution for timely intervention.[11-13]

Author Contributions

AS wrote the manuscript.

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