Tracking tumor evolution one-cell-at-a-time

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ABSTRACT

Accumulating evidence suggests that intra-tumor heterogeneity (ITH) within tumor sub-populations is a major contributor to therapy resistance, with genomically distinct clonal populations exhibiting different therapeutic vulnerabilities. Here we employed single-cell transcriptomics in patient-derived primary cells to understand the impact of phenotypic intra-tumor heterogeneity on drug-induced tumor evolution in oral squamous cell carcinoma.

Main Text

Like any other living being, cancer cells also have the ability to evolve to overcome unfavorable conditions, making tumor evolution as one of the major causes for drug-resistance in the clinic.\(^1\) The notion of tumor evolution was first proposed by Peter Nowell in the 1970s and recently Charles Swanton and colleagues reignited the field by introducing multi-regional tumor sampling.\(^2\) Despite recent advances in our understanding of tumor evolution and intra-tumor heterogeneity, it is still not clear whether it is always the clonal selection of certain sub-populations that lead to drug-resistance or that some cells may stochastically adapt to overcome chemotherapy.\(^3\) Moreover, the impact of phenotypic heterogeneity (as determined by their transcriptomic signatures and hence cell states) on tumor evolution remains underexplored. In the past, one of the biggest obstacles in addressing these questions was the inability of bulk transcriptomic profiling studies to decipher cellular heterogeneity. However, recent advances in single-cell genomics that allow transcriptional profiling of thousands of single cells simultaneously, provide an unprecedented opportunity to understand the role of cellular heterogeneity in the evolution of treatment resistance.\(^4\)

A pertinent challenge that continues to exist in cancer research is to identify the mechanisms of drug resistance that may eventually pave the way for better patient stratification and personalized treatment or precision oncology.\(^5\) Advances in genomic technologies have provided an opportunity to investigate patients’ tumors at the time of presentation and relapse; however these ‘static timepoints’ fail to reveal insights into the dynamic nature of drug-induced tumor evolution. Therefore, we decided to longitudinally model and study tumor evolution using patient-derived tumor cells in the laboratory setting. Recently, we reported the generation of patient-derived primary cells from oral squamous cell carcinoma patients that allowed us to predict treatment response and also prospectively guide treatment in the clinic.\(^6\) We, therefore, employed these patient-derived primary cells to model tumor evolution in order to address how phenotypic heterogeneity impacts how they evolve under the selection pressure of stranded-of-care chemotherapy, Cisplatin.

We generated single-cell RNA-sequencing profiles from \(\sim\)1300 cells representing four evolutionary states of tumors, including primary, metastatic, drug-resistant and relapse (drug-holiday).\(^7\) Our study identified two divergent modes for the emergence of drug-resistance in oral squamous cell carcinoma. We found that cisplatin treatment of phenotypically heterogeneous models comprising both epithelial and mesenchymal cell populations led to ‘Darwinian-selection’ of slow-cycling, quiescent epithelial cells \((\text{Figure-1(a)})\). In contrast, phenotypically homogenous epithelial patient-derived primary cells displayed drug-induced cellular plasticity resulting in a switch in cell identities as observed by the de novo gain of mesenchymal properties \((\text{Figure-1(b)})\). Most importantly we observed similar evolutionary trajectories between patient-derived primary cells, mouse xenograft models and the matched patient tumors, underscoring the power of PDPCs to reliably model tumor evolution in the clinic.

Since cancer stem cell-like properties has been closely linked to drug resistance,\(^8\) we investigated the status of stem-cell genes in single-cell RNA-sequencing clusters. Specifically, SOX2 has been associated with tumor-initiation and drug-resistance in squamous cell carcinoma.\(^9\) Surprisingly, we observed a striking loss of SOX2 expression during cellular plasticity-mediated emergence of drug-resistance. These results were consistent with SOX2 downregulation in the metastatic patient-derived primary cells models. Intriguingly, SOX2-negative cells remained capable of initiating tumors in immuno-compromised mice suggesting a switch to the usage of an alternative stem-cell factor during cellular plasticity. To identify this alternate stem-cell factor performed an RNA-interference-based phenotypic screen of >200 transcription factors and discovered SOX9 as a key determinant of cellular...
plasticity. Identification of SOX2 to SOX9 stem-cell switch during drug-induced epithelial to mesenchymal plasticity was further validated in patient tumors and The Cancer Genome Atlas (TCGA) data.

Most strikingly, we observed similar phenomenon of cellular-plasticity during drug-resistance and lymph node metastasis in patients. These results indicated mechanisms of cellular memory, which may facilitate seamless cellular plasticity. Indeed, when we profiled the chromatin of these cells we observed pre-existing open chromatin marks (H3K4me3) at mesenchymal genes in treatment naïve primary cells as if they were poised for a cell-fate switch, even though they remained transcriptionally silent. Detailed chromatin profiling revealed that the mesenchymal genes in naïve primary cells display a bivalent poised state, notably by the presence of both repressive (H3K27me3) and active (H3K4me3) marks. However, during drug-resistance and metastasis, there was a loss of the repressive mark concomitant with the gain of H3K27Ac-activation mark at these mesenchymal promoters, thus leading to the activation of a mesenchymal-like program in the evolving tumor. Importantly, the naïve cell-specific enhancers and promoters harbored SOX2 motifs, while cellular plasticity associated de novo opening of regulatory elements were enriched for the SOX9 motif. These observations strongly implicated epigenetically modulated cell-fate-switch during tumor evolution.

A synthetic lethal screen for chromatin modifiers and chromatin-targeting drugs led to the identification of BRD4, a histone acetyl transferase (HAT), and its inhibitor JQ1 as the key mediators involved in cellular plasticity associated chromatin opening. Importantly, JQ1 treatment could reverse cisplatin-resistance by inhibiting H3K27ac at mesenchymal genes, such as Vimentin. These results suggest a novel therapeutic opportunity to target tumor evolution by the combinatorial use of epigenetic inhibitors with cytotoxic drugs. This study also highlights that in the absence of epigenetic profiling, potential mechanisms of drug-resistance might remain “hidden in plain sight”. Therefore, including epigenomic profiling studies to genomic and transcriptomic interrogation becomes essential to predict and eventually prevent the evolution of resistance and/or metastasis. Finally, while this proof-of-concept study underscores the power of single-cell genomics in longitudinal models of tumor cell evolution, the next frontier would be to directly apply these principles in the clinical setting to holistically understand the dynamics of drug-induced tumor evolution, both in the context of the tumor as well as its microenvironment.

Funding
This work was supported by the A-star [Core funds].

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