Elucidation Of Tumor Clonal Diversity in An AML Drug Resistance Model Using A High Throughput Single Cell Genome Amplification Method

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Introduction

Genomic plasticity within tumors contributes to the cellular heterogeneity, which can drive treatment resistance. However, detection of rare, treatment-resistant progenitors is extremely difficult using either conventional bulk population analysis or when analyzing a few individual cells. The goal of our study was to develop a high-throughput automated workflow that can detect intrinsic and acquired mechanisms of resistance to quizartinib, an FLT-3 inhibitor, in an acute myeloid leukemia (AML) cell line, MOLM-13.

What mechanisms of acquired resistance can we reveal using whole genome single-cell sequencing?

Methods

A “continual” drug dosage resistance model was created by 2-month duration of 2nM quizartinib, replenished every 3 days with fresh media, while a “dose-escalation” model was created by increasing the quizartinib dose weekly by 100 pM increments up to 2 nM. Parental cells were treated in the same manner with vehicle (DMSO). The single-cell whole genome amplification was performed using ResolveDNA (BioSkryb Genomics) with digital cell dispensing (HP D100), digital liquid dispensing (HP D300), and automated library preparation (Agilent Bravo). 184 resistant and 184 parental cells were sequenced by lowpass sequencing (Illumina NextSeq2000). 1,104 were processed at 272plex for whole exome sequencing. Bioinformatics analysis was performed using BaseJumper (BioSkryb Genomics).

Results

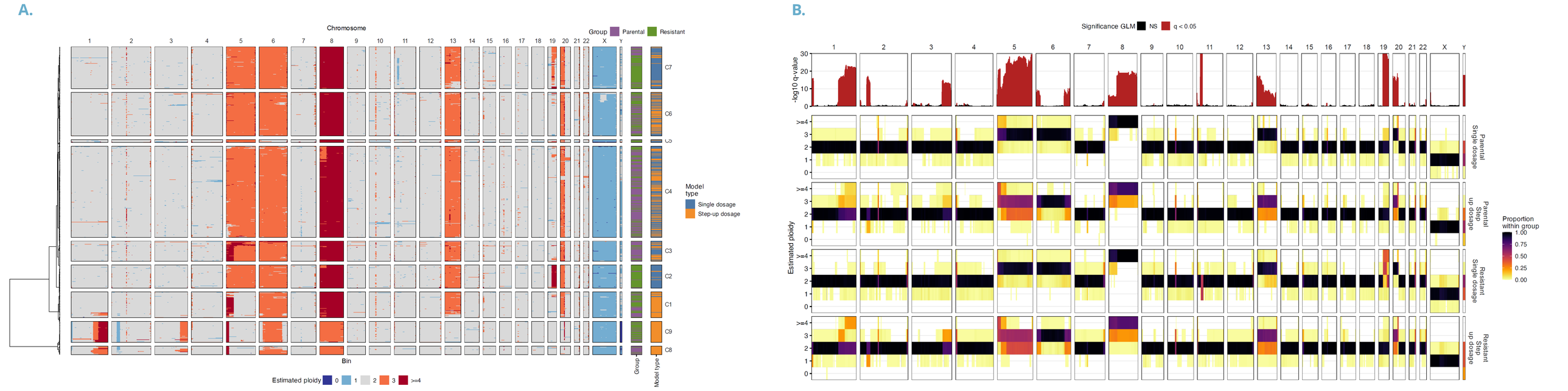

Figure 1: Continual and dose-escalation models can give rise to different ploidy across genomes, potentially supporting unique mechanisms of drug resistance. A. Copy number variation (CNV) analysis of individual parental and resistant MOLM-13 cells in the two models. A subset of dose escalation (step-up dosage) cells cluster in their own subgroup, and include both parental and resistant cells, suggesting these resistant cells arose from existing resisting populations within the parental cell line. **B.** Bulk CNV analysis of parental and resistant MOLM-13 cells in the two models. Ploidy changes across chromosomes are unique between resistant and parental cell lines.

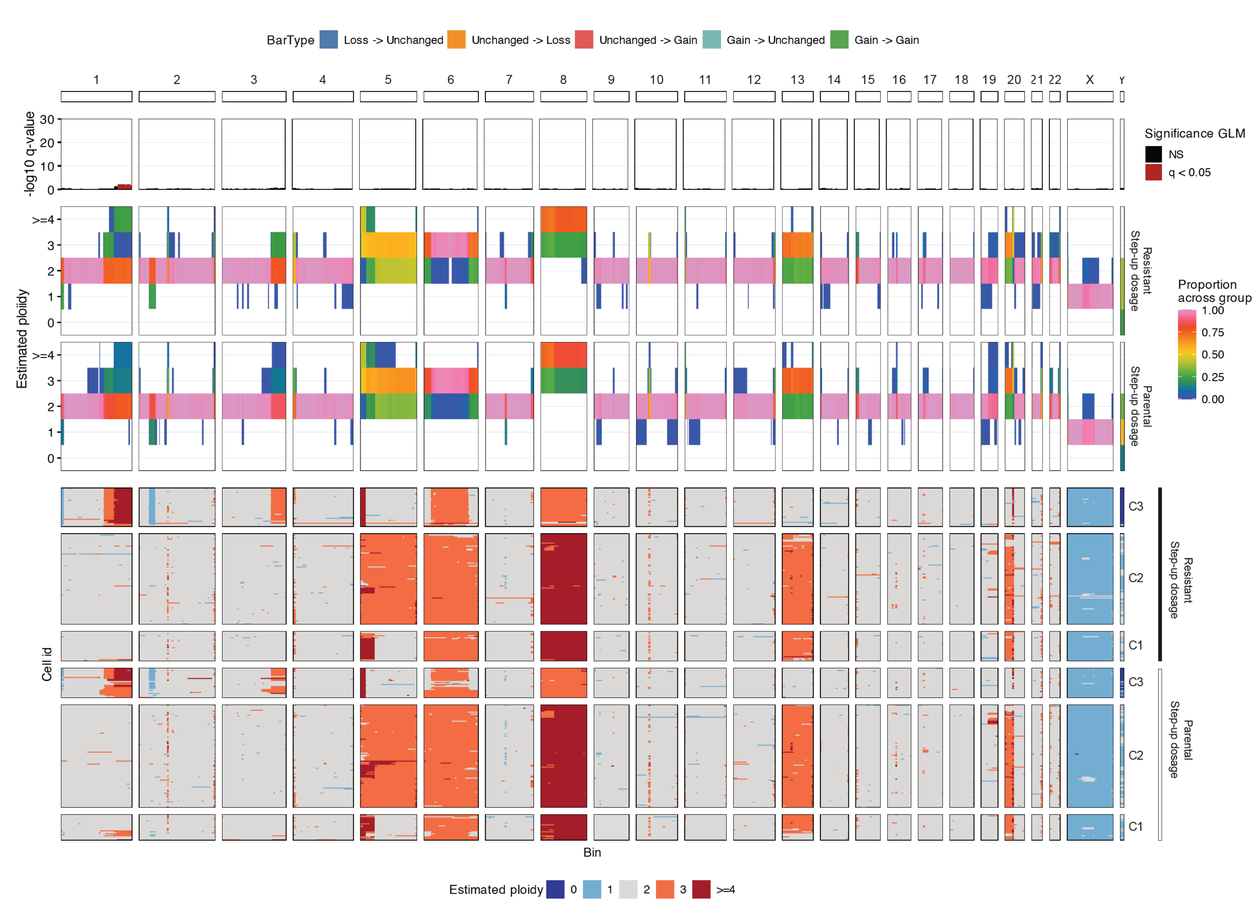

Figure 2: Comparison of parental and resistant cell CNV in the dose escalation model. Copy number variation (CNV) analysis of individual parental and resistant MOLM-13 cells in the dose escalation (step-up dosage) model.

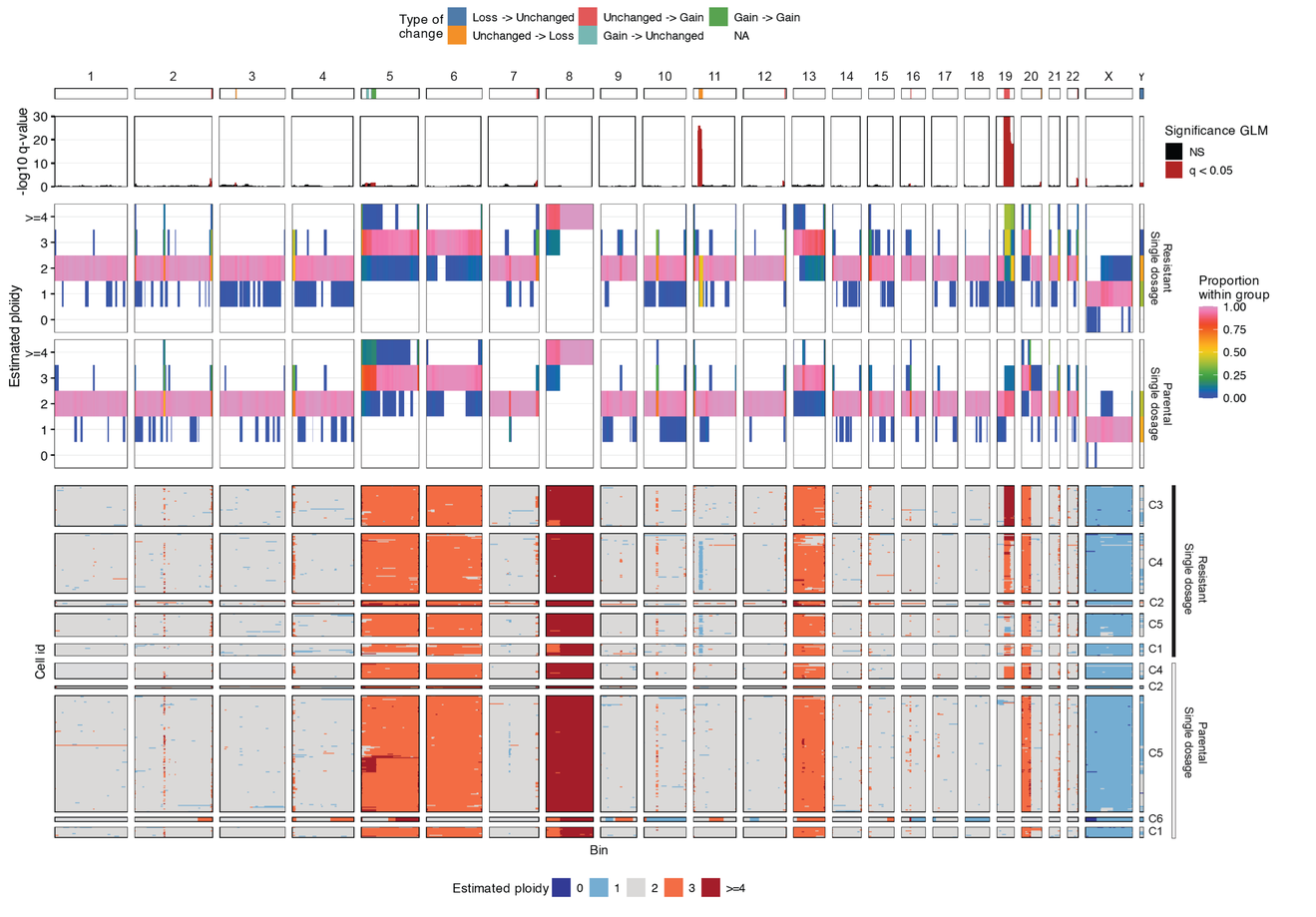

Figure 3: Comparison of parental and resistant cell CNV in the continual dosage model. Copy number variation (CNV) analysis of individual parental and resistant MOLM-13 cells in the continual (single dosage) model.

Conclusions

- Detection of a *FLT3* secondary mutation in the treatment-naïve cell population demonstrates the diversity within treatment naïve cells and highlights the role of natural selection during drug treatment driving resistance.
- The distinct mechanisms of acquired therapy resistance between models shed light on the marked genomic plasticity resulting from varying modes of selection pressure.

References

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