Co-delineating genomic and transcriptomic modes of resistance to MEK inhibition in individual triple negative breast cancer cells

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Abstract

Triple negative breast cancer (TNBC) tumors are frequently driven by MAPK signaling and are initially susceptible to MEK inhibition, yet resistance invariably develops. This resistance occurs, in part, by transcriptional adaptation where signaling is bypassed to reactivate MAPK signaling through a different node, yet resistance can also develop or co-develop through genomic modification. We therefore exploited single-cell ResolveOME™ chemistry to simultaneously capture whole genome single nucleotide variation (SNV), copy number variation (CNV) and full-transcript RNAseq from the same individual cell to define multifaceted contributions to drug resistance and to probe single-cell heterogeneity of these contributions for lineage identification. We employed two TNBC models of resistance to the MEK inhibitor trametinib whereby transcriptional and epigenetic contributions to the resistance have been extensively characterized, yet in which comprehensive assessment of genomic contributions to resistance has not yet been performed. In an epithelial subpopulation of SUM-229PE cells, previous work demonstrated resistance to trametinib coincided with upregulated KRAS expression relative to treatment-naive parental cells and manifested as one of the most statistically significant differentially-expressed genes. The genomic arm of ResolveOME™ and BaseJumper™ analysis software unveiled a genomic lesion, a ~650 kilobase block of differential allelic identity between parental and trametinib-resistant cells encompassing the KRAS gene indicating structural variation at the locus and a candidate genomic mechanism underlying KRAS transcript upregulation and consequential MEK inhibitor resistance. The transcriptomic arm of the ResolveOME™ workflow concurrently validated the upregulation of KRAS and previously known gene sets while defining single-cell heterogeneity within. We also subjected parental and trametinib-resistant SUM-159PT cells, a mesenchymal model of the claudin-low molecular subtype of TNBC, to ResolveOME™ profiling. We observed expression modulation of CEBP family of enhancer factors and SMARC family chromatin remodeling factors, and current efforts are focused on linking resistant cell-specific SNVs proximal to these and other upregulated factors in both models as candidate regulatory variants in the paradigm of epithelial mesenchymal transition (EMT). The unification of genomic and transcriptomic data here uncovered DNA variation underlying the observed transcriptional modulation and provides a framework for ultimately seeking targetable variation in longitudinal primary patient samples.

Methods

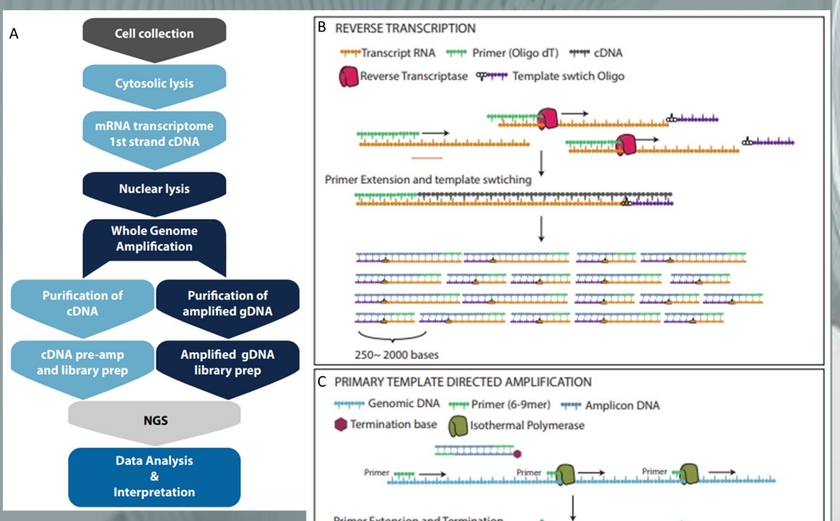
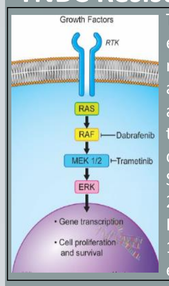


Figure 1. ResolveOME™ workflow enables the integration of first strand cDNA synthesis and genome amplification with downstream separation of the libraries for NGS analysis (A). Isolated single cells undergo cytosolic lysis to permit reverse transcription (1st strand synthesis) of mRNA with a template-switching protocol (B) (1) followed by primary template amplification (PTA) of nuclear genomic DNA. PTA is a quasi-linear strand displacement process, where exonuclease-resistant terminators are incorporated into the reaction to generate small double-stranded amplification products (2). The small amplicons have a lower propensity to serve as templates for further amplification and thus primers are redirected to the main template, decreasing error propagation and resulting in uniform genome coverage (C). The cDNA is isolated from the combined pool and separated from the amplified genomic DNA. Distinct libraries from both the transcriptome and genome fraction are prepared for downstream next generation sequencing and analysis by BaseJumper™ software.

Results

TNBC Resistance to Trametinib



TNBC (Triple Negative Breast Cancer) is characterized by the lack of expression of estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 and is MAPK pathway driven. Trametinib inhibits MEK 1 and 2 kinase activity and causes decreased cellular proliferation, cell cycle arrest, and increased apoptosis. Trametinib causes an initial potent growth arrest that is overcome by a transcriptional adaptive bypass response. The resistance modes have been extensively characterized for transcriptomic bulk RNA in TNBC cell lines (3). SUM-229PE and SUM-159PT cell lines were developed from primary tumors of TNBC. Resistant SUM-229PE & SUM-159PT cell lines were developed by continual passage in Trametinib (3). Both the Trametinib naïve parental and Trametinib resistant SUM-229PE & SUM-159PT cells were sorted using FACS and subjected to the ResolveOME™ workflow to explore the genomic part in single cells.

SUM-229PE Data

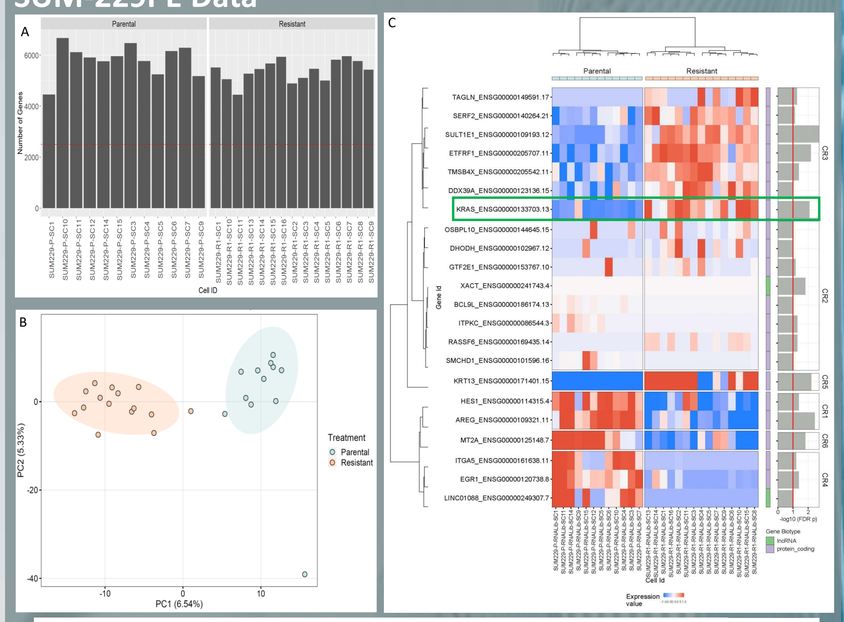


Figure 2: ResolveOME™ transcriptomic analysis in SUM-229PE single cells. The transcriptome analysis detected more than 5000 expressed genes in both the parental and resistant SUM-229PE cells (A). PCA scatterplot showed evident differentiation between genes expressed in parental (turquoise) vs. resistant (salmon) groups of SUM-229PE cells (B). The differential gene expression analysis (heat map) reveals 2 primary blocks between parental (turquoise) and resistant (salmon) single cells, where single cells comprise columns; Gene Symbol/Ensembl transcript ID comprise rows. *KRAS* (highlighted in green) is one of the most highly expressed genes in resistant cells compared to the parental cells (C). 4 genes were differentially expressed between parental and resistant groups (D). *AREG*, a member of the epidermal growth factor family is suppressed in resistant cells which promotes growth of normal epithelial cells and inhibits the growth of cancer cell lines. *KRAS* is one of the most differentially expressed genes between parental and trametinib-resistant in SUM229-PE cells in comparison to the RNA bulk data (D).

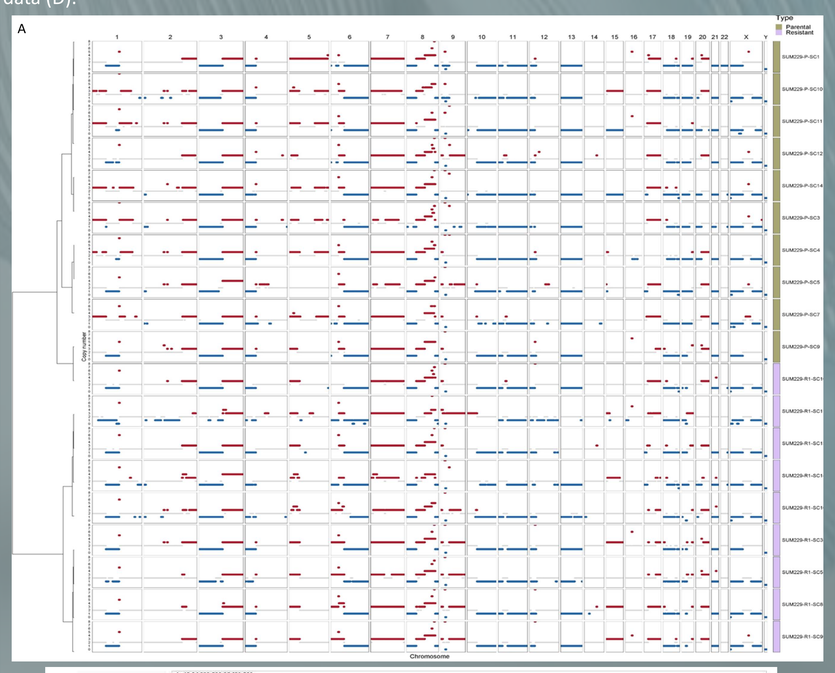


Figure 3: ResolveOME™ genotypic analysis in SUM-229PE single cells. CNV-based heatmap depicting the gains and loses across SUM-229PE cells. Red highlighted regions represent gains (Ploidy > 2) and regions in blue losses (Ploidy < 2). From the panel B, ResolveOME™ also identified a ~650 kb block of differential allelic identity indicative of structural variation between parental vs. resistant groups in accordance with the previous RNA bulk data (4).

Results

SUM-159PT Data

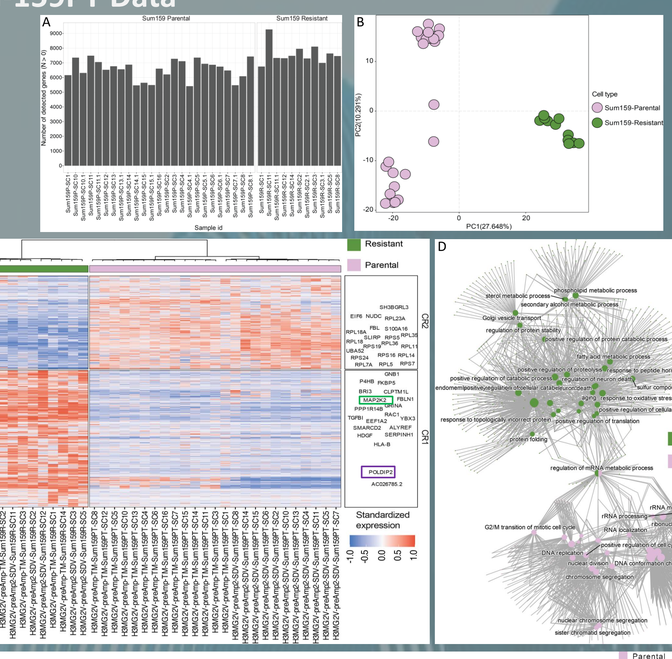


Figure 4: ResolveOME™ genotypic and transcriptomic analysis in SUM-159PT single cells. The transcriptome analysis detected more than 5000 expressed genes in both the parental and resistant SUM-159PT cells (A). PCA projection showed evident differentiation between genes expressed in parental vs. resistant groups of SUM-159 cells (B). Heatmap representing statistically significant different genes (False discovery rate-adjusted p-value < 0.05) between SUM-159PT parental and resistant cells. In the right region of the plot, the top 40 most statistically significant different genes across both cell types were depicted (C). Network of the top 30 statistically significant (q < 0.1) gene ontology terms contained the clusters (CR2 and CR1) from the heatmap of statistically significant genes differentiating SUM-159PT parental and resistant cells (D). Polymerase delta-interacting protein 2 (*POLDIP2*) and *MAP2K2 (*MEK1 protein kinase maker), is upregulated in SUM-159 resistant cells (purple) compared to the parental cells. Prior research showed the knockdown of *POLDIP2* suppresses tumor growth and invasion capacity linked to unfavorable transformation ability in non-small cell lung cancer (5). CNV-based heatmap depicting the gains and loses across SUM-159PT cells. Red highlighted regions represent gains (Ploidy > 2) and regions in blue losses (Ploidy < 2). In the x-axis we represent regions across chromosomes, in the y-axis we represent estimated ploidies (0-8) for each given cell (E). Resistant SUM-159PT cells, exhibited chromosomal gains at 3q (blue), 6p (green), and 17q (purple) and chromosomal loss at 14 (orange). Chromosomal gain at 17q corresponds to the *POLDIP2* gene at 17q11.2. Similarly, chromosomal gain at 3q corresponds to the *PIK3CA* gene. SUM-159PT cells upon trametinib treatment showed recruited *PIK3R1* at loci harboring MEK inhibitor-induced super-enhancers from previous bulk RNA ChIP-seq data (3).

Summary

- ResolveOME™ multi-omic workflow enables the coupling single-cell genomic and transcriptomic information needed to define the molecular mechanisms of oncogenesis and drug resistance in TNBC.
- In trametinib-resistant SUM-229PE cells, ResolveOME™ reveals elevated levels of *KRAS* transcript and copy number gain at 12p compared to the treatment naïve parental cells.
- Structural variation at the *KRAS* locus and acquired overexpression of KRAS in SUM-229PE cells resistant to trametinib may be a combinatorial mechanism contributing to the emergence of drug resistance.
- The upregulation of *POLDIP2* and *MAP2K2* genes in TNBC drug resistant cell line SUM-159PT might be the drivers for transcriptional adaptation to reactivate cellular proliferation.

References

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