Discover Genetic Heterogeneity in Cancer

The Chromium Single Cell CNV Solution

Breast cancer is a complex disease with a high degree of inter- and intra-tumor heterogeneity (1). Clinically, breast cancers are commonly categorized into subtypes based on the expression pattern of the predictive and prognostic biomarkers ER, PR, and HER2. Bulk copy number variation (CNV) patterns have also been used to further sub-categorize tumors but traditional bulk measurements can only recover the average genetic profile and may miss clinically-relevant variants in minor sub-clones (2-5). With the Chromium Single Cell CNV Solution, we demonstrate that single cell resolution is necessary to unmask genetic heterogeneity.

Single Cell CNVs Reveal Tumor Heterogeneity

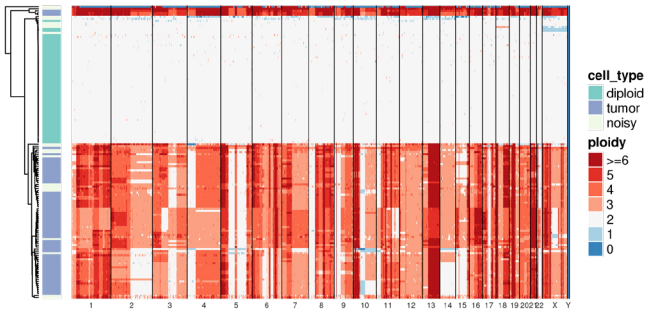


Figure 1

We profiled CNVs at single cell resolution from snap-frozen breast tumor tissue obtained from Bioserve. Using the Chromium Single Cell CNV Solution, we obtained integer-scaled CNV profiles across the genome for each cell (145 cells). We visualized the results as a heatmap. This analysis revealed two distinct populations: a homogeneous diploid population likely representing normal cells (57 cells) and a heterogeneous population displaying CNV amplifications likely corresponding to tumor cells (67 cells). Diploid cells showed a high degree of coverage uniformity with a near-linear relationship between genome fraction and number of reads.

Amplifications of Regions Implicated in Cancer

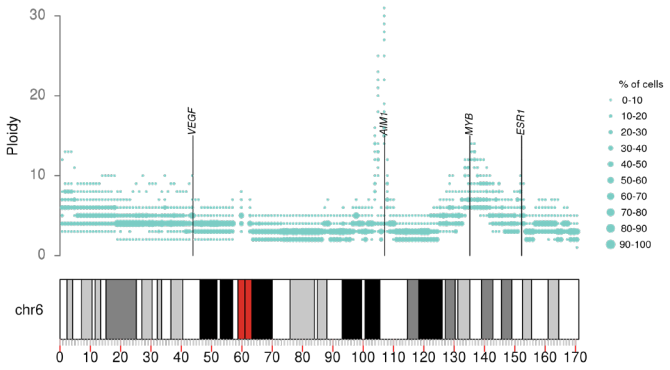
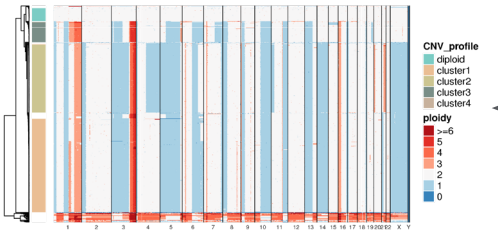


Figure 2

To highlight chromosomal regions with genetic variation, we plotted the observed ploidy in 1-Mb intervals across the genome, varying the point size to reflect fraction of tumor single cells with that ploidy. Chromosome 6 is shown as example. In this tumor, chromosome 6 shows a high degree of variability. The region with the highest gains in ploidy overlaps with a METABRIC driver somatic copy number aberration region (6) and includes the AIM1 gene. The fact that the majority of the tumor cells show a ploidy >15 at the chromosome 6 hyper-amplified regions suggests the possibility of a double minute, though this would need further confirmation with a cytogenetic experiment.

Spatial Segregation of Clones

Figure 3A



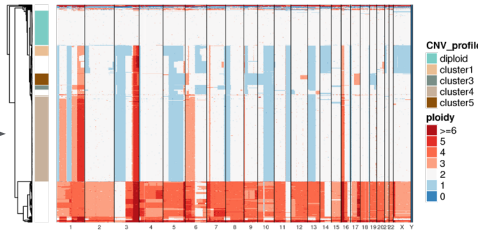


Figure 3B

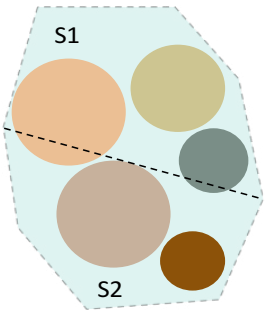
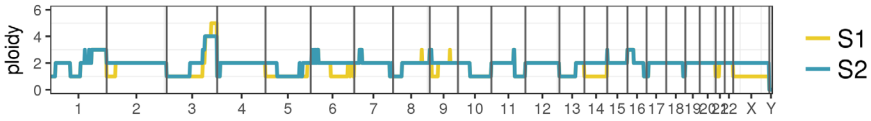


Figure 3C



We analyzed a second breast tumor that we sectioned into 2 sectors (S1 and S2). Using the Chromium Single Cell CNV Solution on each section (S1 – 602 cells, S2 – 671 cells), we identified clusters of cells based on similarity of their CNV profiles (Figure 3A). The proportion of cells in each cluster was markedly different between sectors (Figure 3A, 3B). The heterogeneity of the S1 and S2 tumor regions is revealed only at single cell resolution, since the pseudo-bulk CNV profiles for the two sectors show an overall ploidy of 2 across the genome (Figure 3C).

Conclusions

Using the Chromium Single Cell CNV Solution on breast tumor samples, we demonstrated that tumor heterogeneity can be dissected based on CNV detection empowered by robust genome representation and coverage uniformity. We also showed that the tumor heterogeneity is distributed spatially and is revealed only at single cell resolution. The characterization of tumors at single cell resolution is key to understanding the true nature and distribution of tumor heterogeneity, which will be key to understanding tumor evolution and identifying driver regions and new therapeutic targets.

Resources

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Citations

1. K. K. Shiu *et al.*, DNA amplifications in breast cancer: genotypic-phenotypic correlations. *Future Oncol.* 6, 967-984 (2010).
2. N.R. Bertos, M. Park, Breast cancer - one term, many entities? *J. Clin. Invest.* 121, 3789-3796 (2011).
3. J.S. Beckmann *et al.*, Copy number variants and genetic traits: closer to the resolution of phenotypic to genotypic variability. *J. Nat. Rev. Genet.* 8, 639-646 (2007).
4. K. Polyak, Heterogeneity in breast cancer. *J. Clin. Invest.* 121, 3786-3788 (2011).
5. N. McGranahan, C. Swanton, Clonal heterogeneity and tumor evolution: Past, present, and the future. *Cell* 168, 613-628 (2017).
6. C. Curtis *et al.*, The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. *Nature* 486, 346-352 (2012).

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Spatial Segregation of Clones

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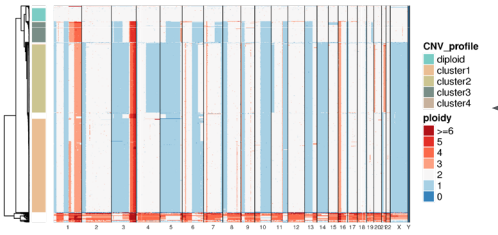

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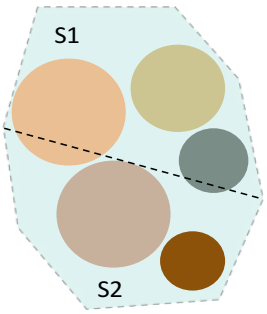

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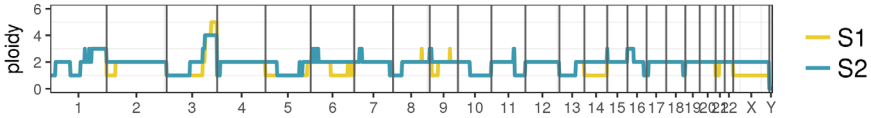

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