Single-cell clarity and heterogeneity in copy number profiles in primary synovial & Ewing sarcoma with ResolveDNA™ genomic amplification

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Abstract

Soft tissue sarcomas including Ewing (ES) & synovial (SS) sarcoma represent a diverse set of mesenchymal malignancies frequently driven by translocation events. While well characterized, limited data exist surrounding broader genomic alterations & intra-tumoral heterogeneity. Unfortunately, this precludes personalized prognoses and treatment, with 50-70% of cases resulting in relapse or progression, leading to overall poor clinical outcomes[1-3].

In a collaboration with the Hopp Children's Cancer Center Heidelberg (KiTZ), we utilized the ResolveDNA™ process to assess copy number aberration (CNA) at the single cell level in two soft-tissue sarcoma samples, a putative Ewing sarcoma sample, and a putative synovial sarcoma.

ResolveDNA™ chemistry attenuates amplicon size, redirects amplification to the primary DNA template and avoids exponential copying of amplicons[4]. This results in unprecedented coverage and uniformity, with high SNV precision and sensitivity, allelic balance, and the enhanced ability to accurately call CNA[4].

Single cell analysis in this study demonstrated diverse phenotypes – SS cells harboring highly disrupted genomes, with stark focal, sub-chromosomal gains and losses, and ES cells maintaining a more typical profile, with limited discrete, full-chromosome (CHR) gains and losses.

Whole exome sequencing demonstrated profile differences between the two sample types.

Methods

FACS Sorting

Patient derived cells were obtained and sorted in collaboration with the Hopp Children's Cancer Center Heidelberg. Single cells were deposited into individual wells containing 3uL of BioSkryb Genomic's proprietary cell buffer.

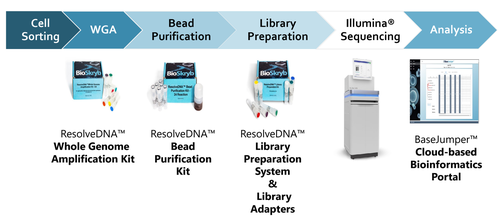
ResolveDNA™

Plates underwent ResolveDNA ™ per manufacturer's instructions. After amplification, libraries were prepared using ResolveDNA™ library amplification & quantified using high sensitivity Qubit and an Agilent tape station. DNA libraries were equimolar pooled & sequenced on an Illumina MiniSeq targeting 2M reads per sample using 2x75 paired end sequencing chemistry. Exome samples were generated using the IDTv2 xGen panel with Kapa fragmentase after ResolveDNA™ library preparation with 12plex multiplexing. A total of 500ng library was input to the fragmentation reaction. All exomes were sequenced targeting 40M reads per sample using 2x150 paired end sequencing chemistry on an Illumina NovaSeq.

Sequencing & Data analysis

Fastq files were analyzed using multiple BaseJumper modules – including CNA and SNV assessments, as well as genome assembly and quality evaluation.

Workflow



Resolve DNA™ Amplification Method

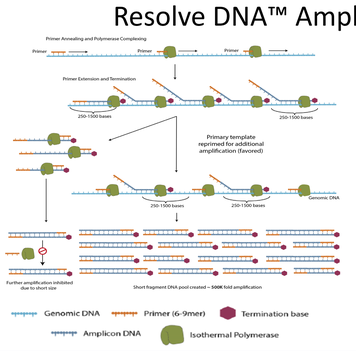


Figure 1: ResolveDNA™ Amplification Method

The ResolveDNA™ amplification method is based on Primary Template-directed Amplification, wherein termination bases are utilized to drive reaction kinetics back to the original template vs. already amplified products[4]. This results in unprecedented uniform genomic coverage, and a significant reduction in amplification-based errors[4].

Resolve DNA™ provides high quality balanced data

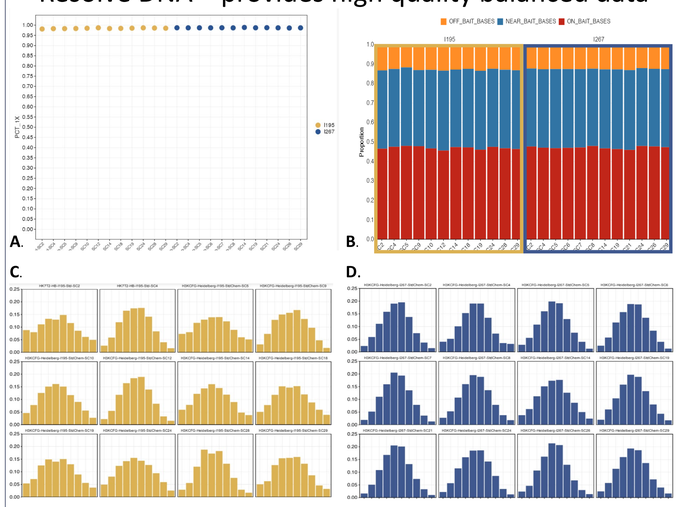


Figure 2: Summary of data quality using ResolveDNA™ whole genome amplification for whole genome (WGS) & whole exome (WES) sequencing using BaseJumper modules

A. QC of WGS indicate >90% coverage at 1x or greater for both SS (yellow) and ES sample (blue). **B.** ~90% of reads are on or near target in WES of Synovial (yellow) and Ewing (blue) sarcomas. **C & D.** Estimation of allelic drop out rate of SS (C) and ES (D). **Note:** As these are patient samples, allelic balance was calculated using only high confidence variants that exhibited allelic frequency between 25-75%, that were present 80% or greater cells, and that were previously reported in dBSNP.

Whole Exome Sequencing identifies differences in expression profiles in ES and SS

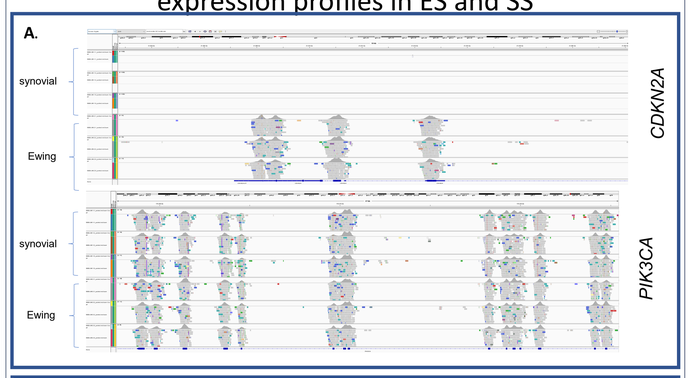


Figure 5: Overview of Differentially Expressed Loci between Ewing Sarcoma and Synovial Sarcoma

A. Loss of *CDKN2A* expression in SS not seen in ES, with no difference in *PIK3CA* expression **B**. Diagnostic translocation between *EWS* and *FLI1 (t11;22)(q24;q12)* in ES. Interestingly the expected *SYT/SSX t(X;18)(p11;q11)* translocation was not identified in SS cells, suggesting this tumor may better represent a radiation induced sarcoma.

Copy number profiles in synovial sarcoma suggest cryptic clones

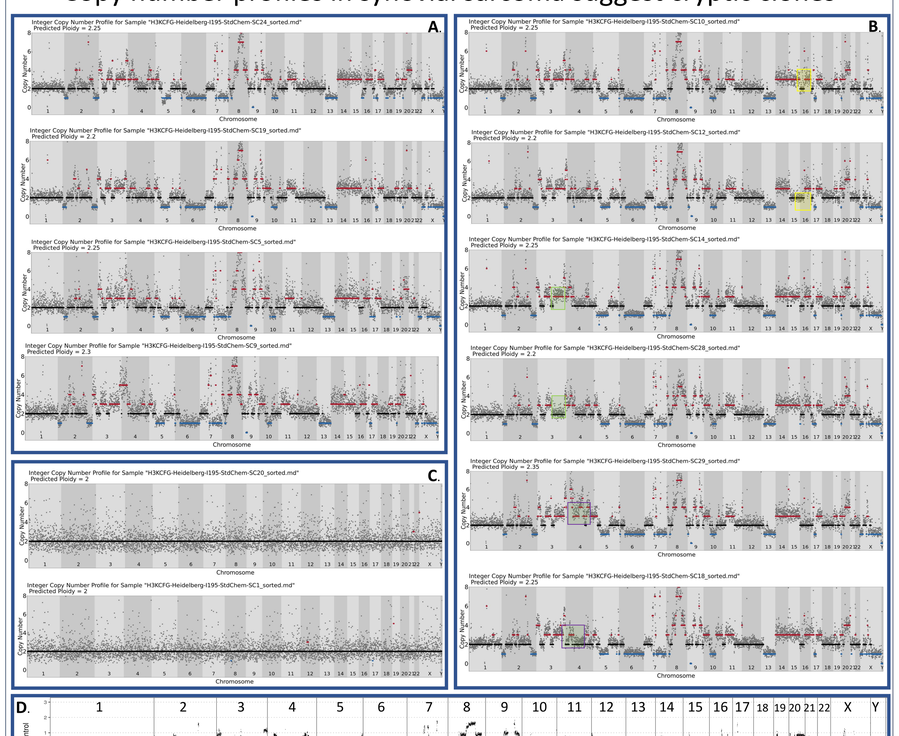


Figure 3: Copy number comparison between single cells and bulk synovial sarcoma

All data are generated using the CNV module of BaseJumper which incorporates the Ginkgo algorithm using a 500kb window size. **A**. WGS copy number profiles (0.4x coverage) of representative single synovial sarcoma cells. **B**. Evidence of potential clonal evolution with 3 distinct copy number profiles (boxes) at ~0.4x coverage. **C.** Evidence of 'flat' copy number profile cells which may represent infiltrating immune cells. **D.** Copy number profiles of the bulk primary tumor (~4x coverage).

Copy number profiles in ES demonstrate more clonal phenotype

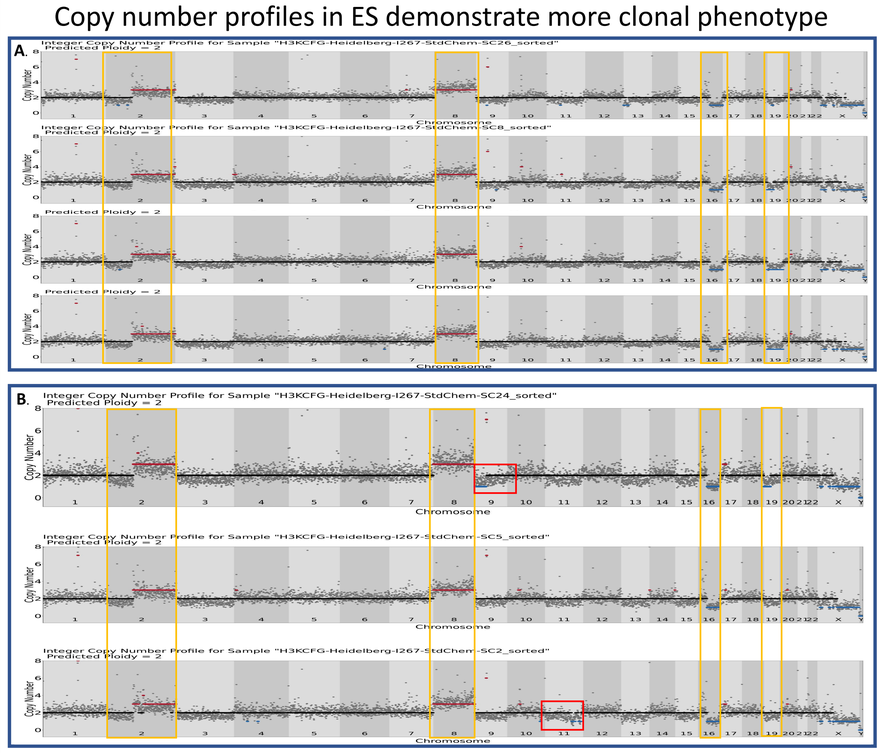


Figure 4: Copy number comparison between single cells and bulk Ewing Sarcoma

All data generated using the CNV module of BaseJumper which incorporates the Gingko algorithm at 500kb window size. **A.** WGS copy number profiles (0.4x coverage) of single ES cells with minimal genome disruptions aside from gains of CHR 2 & 8 with losses of CHR 16 & 19 (yellow boxes). **B.** Evidence of minor additional genomic alterations in a few single cells (red boxes) indicating cell-to-cell heterogeneity within a mostly clonal population.

Results

Single cell analysis of ES demonstrated significant clonality with minimal CNA. All cells harbor gains on CHRs 2 and 8, & losses of 16. Greater than 50% of cells demonstrate loss of CHR 19. Unlike synovial sarcoma, minimal numbers of cells showed additional alterations. Those that did exist were focal gains on CHR 10 (n=4/12 cells), and losses on CHRs 9 and 11 (n=1 cell each).

Strikingly, copy number aberrations in the synovial sarcoma cells were numerous, with focal points of large-scale amplification and losses, including at the *MYC* locus. Intriguingly, single-cell heterogeneity was notable in multiple sub-chromosomal regions including 3q, 4p and 16q each seen in at least two independent cells. The expected SS18:SSX translocation was not identified in this patient, suggesting that these cells may have originated from a secondary sarcoma as opposed to the originally diagnosed synovial sarcoma.

Summary

Sarcomas represent a group of mesenchymal tumors frequently driven by translocation events[1]. They can be difficult to treat, often resulting in recurrence and metastasis[1-3]. Typically, these tumors have been thought of as being highly clonal, with minimal disruption of the genome. The ability to identify rare instances of sub-clonal populations of tumor cells is likely to improve prognostic outcomes in these diseases.

The heterogeneity observed in this study demonstrates the power of single cell vs bulk analysis. Here we reveal the nature of genomic instability and aid in the characterization of sarcoma subtypes at unprecedented levels of resolution. Despite the anticipated clonality of ES cells, evidence of sub-clonal heterogeneity can be seen.

The ability to detect these rare events are particularly crucial to understand disease development and progression in orphan sarcomas, especially those with complex genome rearrangements and/or poor clinical outcome. Further studies, including transcriptomic evaluation are ongoing.

References & Acknowledgments

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