Identification of Genomic Alterations in Extraskeletal Myxoid Chondrosarcoma

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What is sarcoma?

• Rare, heterogeneous group of musculoskeletal neoplasms
  • <1% of adult cancers, up to 15% of pediatric cancers

• High rates of metastasis to the lungs

• Many sarcoma types show resistance to adjuvant therapy—treatment is wide surgical excision

• Most common sarcomas: osteosarcoma, chondrosarcoma, Ewing sarcoma
Extraskeletal Myxoid Chondrosarcoma (EMC)

• Very rare soft tissue sarcoma subtype

• Neoplasm of uncertain differentiation (WHO, 2002*)

• Caused by chromosomal translocation of NR4A3 gene on chromosome 9q31.1
  • In 70% of cases, fusion with EWSR1, Ewing sarcoma gene (chr22q12.2)
  • In 20% of cases, fusion with TAF15 (chr17q12.2)
  • Also fusion with FUS, TCF12, TFG, etc

• Leads to constitutive NR4A3 gene expression
EMC Patient Presentation

- Typical presentation of EMC: male in 5th or 6th decade of life presenting with mass in proximal extremity
- We collected these samples (primary tumor, lung metastasis, pelvic metastasis, normal blood, normal lung) for Whole Genome Sequencing
EMC primary tumor and metastases on H&E

Primary tumor

Lung Metastasis

Pelvic Metastasis (iliac lymph node)
EWS/22q12 gene fusion product (FISH)

**Orange** = 5’ end of EWSR1 gene

**Green** = 3’ end of EWSR1 gene

Separated **orange** and **green** = gene has been split (and fused with NR4A3)
Aim: Compare genomic structural variants of tumor samples to blood, as well as mutations accumulated with progressive metastases

• Comparisons:
  • P vs BN: Primary tumor vs blood normal
  • LM vs BN: Lung metastasis vs blood normal
  • PM vs BN: Pelvic metastasis vs blood normal
  • LN vs BN: Lung normal vs blood normal (negative control)

• Comparing progress metastases
  • LM vs P: Lung metastasis vs primary tumor
  • PM vs LM: Pelvic metastasis vs lung metastasis
Methods

• DNA was extracted from the primary chondrosarcoma, lung metastasis, pelvic metastasis, normal lung, and peripheral blood mononuclear cells utilizing the QIAamp Fast DNA Tissue Kit

• Samples were then submitted to the UPMC Genome Center for further preparation, sequencing, and analysis

• Libraries were prepared via the Illumina Nextera DNA Flex Library kit and samples were sequenced on the Illumina NovaSeq 6000.
  • 30X coverage was used to obtain greater than 1.6 billion, 150 bp paired-end reads for each sample

• For analysis, the DRAGEN genome pipeline (Edico Genome) was used to map reads to the GRCh38 genome assembly
Methods

• **Mutect2** software (The Broad Institute) was used to identify **single nucleotide polymorphisms (SNPs)**.

• **CNVkit** was used to identify **Copy Number Variants (CNVs)**

• **Structural Variants (SVs)** were identified using Delly v9.1 and **SvABA v11.0** on the Seven Bridges Cancer Genomics Cloud platform
  • Usually >50bps, includes shared patterns of structure variation
  • More difficult to identify larger scale patterns
SNVs found in EMC

P-BN: Primary tumor vs blood
LM-BN: Lung metastasis vs blood
PM-BN: Pelvic metastasis vs blood
LN-BN: Lung normal vs blood (negative control)
SNVs found in EMC

- **FRYL, OR5F1**: play roles in development of sensory neuronal fields
- **IL7R**: interleukin 7 alpha receptor, plays critical role in V(D)J recombination during lymphocyte development
- **UBD**: ubiquitin D/FAT10, may stimulate osteosarcoma, in one study, silencing inhibited metastasis

**P-BN**: Primary tumor vs blood
**LM-BN**: Lung metastasis vs blood
**PM-BN**: Pelvic metastasis vs blood
**LN-BN**: Lung normal vs blood
CNVs in EMC

CNVs in lung metastasis, normal lung, pelvic metastasis, and primary tumor (top to bottom)

Red = copy number increase
Blue = copy number decrease.

**P-BN**: Primary tumor vs blood
**LM-BN**: Lung metastasis vs blood
**PM-BN**: Pelvic metastasis vs blood
**LN-BN**: Lung normal vs blood
SVs in EMC—known transposon

EMC characteristic translocation:

V1: CHROM=chr22, POS=29296336, REF=N, ALT=[N[chr9:99825934[
V2: CHROM=chr22, POS=29296336, REF=N, ALT=[ ]chr9:99825932]N]

We will look for the callers that can identify these translocations
SVs in EMC—shared patterns identified by Delly and SvABA

- **Delly**
  - **P1**: Chromosome 10 deletion at pos 64490851 + chromosome 19 deletion at pos 4713002 + t(9;22)(q22;q12. 2)
  - **P2**: t(9;22)(q22;q12. 2) – slightly different end position
- **SvABA**
  - **P3**: Chromosome 10 deletion at pos 64490851 + t(9;22)(q22;q12. 2)
  - **P4**: alterations in chromosomes 2, 4, 10 and Y

```plaintext
P4
Record(CHROM=chr10, POS=41860834, REF=N, ALT=[chr10:41861175])
Record(CHROM=chr10, POS=41865670, REF=N, ALT=[chr10:41866405])
Record(CHROM=chr10, POS=41869802, REF=N, ALT=[chr10:41870088])
Record(CHROM=chr2, POS=89827889, REF=N, ALT=[chr2:89828200])
Record(CHROM=chr2, POS=89828578, REF=N, ALT=[chr2:89828815])
Record(CHROM=chr2, POS=89830931, REF=N, ALT=[chr2:89831498])
Record(CHROM=chr2, POS=89840297, REF=N, ALT=[chr2:89840669])
Record(CHROM=chr2, POS=90382058, REF=N, ALT=[chr2:90385988])
Record(CHROM=chr2, POS=90385077, REF=N, ALT=[chr2:90390312])
Record(CHROM=chr2, POS=90380661, REF=N, ALT=[chr2:90398259])
Record(CHROM=chr2, POS=90380680, REF=N, ALT=[chr2:90398776])
Record(CHROM=chr2, POS=90399006, REF=N, ALT=[chr2:90399238])
Record(CHROM=chr4, POS=49092674, REF=N, ALT=[chr4:49092960])
Record(CHROM=chr4, POS=49093225, REF=N, ALT=[chr4:49093550])
Record(CHROM=chr4, POS=49097147, REF=N, ALT=[chr4:49097440])
Record(CHROM=chr8, POS=2517778, REF=N, ALT=[chr8:KI270821v1_al000])
Record(CHROM=chrY, POS=11295282, REF=N, ALT=[chrY:11295746])
Record(CHROM=chrY, POS=11297914, REF=N, ALT=[chrY:11298352])
Record(CHROM=chrY, POS=11299106, REF=N, ALT=[chrY:11299320])
Record(CHROM=chrY, POS=11299350, REF=N, ALT=[chrY:11299699])
Record(CHROM=chrY, POS=11302830, REF=N, ALT=[chrY:11303186])
Record(CHROM=chrY, POS=11305271, REF=N, ALT=[chrY:11305589])
```
SVs in EMC

Delly

SvABA

LM-PT: lung met vs primary tumor
PT-LN: primary tumor vs lung met
LM-LN: lung met vs normal lung
PT-BN: primary tumor vs bone
LM-BN: lung met vs blood

Plots created using SB’s Conseca app
SVs in EMC

V1, V2 (known translocations) are present in primary tumor, lung met, and not normal lung or blood.

Plots created using SB’s Conseca app

LM-PT: lung met vs primary tumor
PT-LN: primary tumor vs lung met
LM-LN: lung met vs normal lung
PT-BN: primary tumor vs bone
LM-BN: lung met vs blood
SVs in EMC

Delly

SvABA

P2 (slightly shifted translocation) present in lung met but not primary tumor or blood

LM-PT: lung met vs primary tumor
PT-LN: primary tumor vs lung met
LM-LN: lung met vs normal lung
PT-BN: primary tumor vs bone
LM-BN: lung met vs blood

Plots created using SB’s Conseca app
SVs in EMC

Delly

SvABA

Plots created using SB’s Conseca app

P4 (complex pattern with SVs in chr 2, 4, 10, and Y) present in lung met but not primary tumor or normal lung

LM-PT: lung met vs primary tumor
PT-LN: primary tumor vs lung met
LM-LN: lung met vs normal lung
PT-BN: primary tumor vs bone
LM-BN: lung met vs blood
SVs deeper dive: Primary Tumor

![Graph showing structural variants across different chromosomes]
SVs deeper dive: Primary Tumor

BND = break end point
DEL = deletion
INV = inversion
DUP = duplication

Normalized by chromosome length
SVs deeper dive: Lung Metastasis

![Graph showing structural variants across chromosomes.]

- **Background**
- **Hypothesis**
- **Methods**
- **Results**
- **Conclusions**
- **Future Directions**
SVs deeper dive: Lung Metastasis

BND = break end point
DEL = deletion
INV = inversion
DUP = duplication

Normalized by chromosome length
Methods

• **Mutect2** software (The Broad Institute) was used to identify single nucleotide polymorphisms (SNPs).
  
  → SNPs in FRYL, OR5F1 (neuronal sensory development), IL7R (possible targetable biomarker), UBD (may stimulate osteosarcoma metastasis)

• **CNVkit** was used to identify Copy Number Variants (CNVs)
  
  → Copy number increases in chromosomes 1 and 8, copy number decreases in chromosomes 6 and 10 in tumor samples compared to blood

• **Structural Variants (SVs)** were identified using Delly v9.1 and **SvABA v11.0** on the Seven Bridges Cancer Genomics Cloud platform
  • Usually >50bps, includes shared patterns of structure variation
  • More difficult to identify larger scale patterns
  
  → Patterns identified in lung met but not primary tumor or control samples (P2, P4)
  → Chromosomes 8, 10, and 19 have increased numbers of SVs, especially in lung met
Future Directions

- Further explore shared structural patterns identified through Delly and SvABA and specific genes impacted by these structural variants
- Add pelvic metastasis comparison data to study mutational progression of metastasis
- Identify targetable biomarkers or molecular profile for this patient’s EMC
Conclusions

• We must expand our very small database of EMC knowledge

• No predictive factors currently exist to predict metastatic disease and inform the use of systemic treatment
  • Is it possible to identify therapeutic markers that will predict if EMC will eventually metastasize in its indolent course?

• Molecular profiling of EMC can help us develop targeted, personalized treatment for individuals
Thank you!

- Musculoskeletal Oncology Lab
  - Kurt Weiss, MD
  - Rebecca Watters, PhD
  - Tanya Heim, MS
  - Bill Li, MD
  - Brittany Royes, MS
  - Murali Kovvur
  - Nerone Douglas
  - Luke Carlson
  - Maggie Gajda
  - Andrew Frear

- Pathology images: Ivy John, MD

- Cancer Bioinformatics Services: Anish Chakka, Uma Chandran

- UPMC Hillman Cancer Center and Tissue and Research Pathology/Pitt Biospecimen Core

- Shadyside Hospital Foundation

- Seven Bridges
  - Poro Burman
  - David Roberson
  - Ana Damljanovic