POSTER 75

TITLE: Whole Genome Sequencing Reveals Mutational Profiles of Breast Cancer Bone Metastases

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Background:

Breast cancer is the most prevalent cancer affecting women, with about 1 in 8 diagnosed at some point during their lifetime. Of these, about 30% develop Stage IV metastatic disease, with bone as the most common site of metastasis. Bone metastases confer significant morbidity to patients by causing pathologic fracture, pain, nerve compression, and overall decreased survival. Most breast cancers that metastasize to bone are estrogen receptor (ER) positive, and hormonal therapy targeting ER has become a mainstay in treatment. Unfortunately, many tumors go on to develop resistance to endocrine therapy, rendering these therapies ineffective. The cellular mechanisms leading to endocrine therapy resistance are still being studied, but novel mutations in breast cancer cells treated with endocrine therapy may confer resistance. Therefore, genomic analyses of primary and metastatic breast cancer tumors are crucial for the development of novel targeted therapies, as well as for guiding personalized treatment decisions. However, despite being the most common site of breast cancer metastasis, bone metastasis samples are underrepresented in genomic databases due to the challenge of obtaining biopsies and the breakdown of genetic material that occurs during biopsy decalcification.

Purpose:

Our research focuses on characterizing the altered gene mutations between patient-matched primary breast tumors and their respective bone metastases. We aimed to identify clinically actionable genes that can be targeted to overcome endocrine resistance in metastatic breast cancer to bone.

Methods:

Whole genome sequencing was performed on bone metastases and peripheral blood mononuclear cells (PBMC) from five patients treated at our institution. The purified DNA samples were quantified using the Qubit dsDNA BR Kit. For PBMC samples, we extracted DNA from the cryopreserved buffy coats using the DNeasy Blood and Tissue Kit. All samples were then submitted to a specialized genomics 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. For sequencing, 30X coverage was used to obtain greater than 1.6 billion, 150 bp paired-end reads for each tumor sample as well as the PBMC control. The paired end reads were checked for quality, trimmed, and mapped against the human primary assembly genome. Somatic variants were filtered and annotated to create MAF files for single nucleotide variant (SNV) analysis. For copy number variant (CNV) analysis, a pooled reference of per-bin copy number estimates was generated from control PBMC samples. This pooled reference was used to extrapolate gene level gains and losses in bone metastasis samples.

Publicly available whole exome sequencing data collected by MBC Project was also analyzed using the Picard and Firehose pipelines via the Broad Institute. For each MBC Project sample, information containing single mutations, copy number alterations, and deidentified patient information was collected. The number and frequency of SNVs and CNVs was analyzed. Overlapping mutations between the MBC Project samples and our samples were identified. Five paired samples (primary breast tumor sample and corresponding bone metastasis sample from the same patient) were identified in order to isolate bone-specific CNVs.

Results:
Eighteen SNV mutations overlapped between our samples and MBC Project samples, including the estrogen receptor, *ESR1*. Mutations in twelve genes (*PPP1R12B, STUB1, ADGRV1, HIST1H4F, LILRA1, COBLL1, SPIDR, ACBD3, GPBP1, ADGB, TSR3*, and *FRMD6*) overlapped between the most common CNV mutations in our bone metastasis samples and those registered in MBC Project's database. Several of these overlapping genes have regulatory activity at the DNA (*HIST1H4F*), RNA (*GPBP1*), or protein (STUB1, TSR3) levels. Others are involved with double stranded break repair (*SPIDR*) or cytoskeletal structure (*COBLL1*, *FRMD6*). CNVs occurring only in bone metastases, and not in metastases in other locations or in primary breast tumors, were further isolated. Eight CNVs unique to bone metastases were identified (*STUB1, HIST1H4F, COBLL1, SPIDR, ACBD3, GPBP1, TSR3, FRMD6).* Of these, we further identified several genes (*GPBP1, COBLL1)* that may be potential targets for combating endocrine therapy resistance.

Conclusions:
Using genomic data from samples from both our samples and MBC Project, we characterize multiple aberrations that may contribute to breast cancer metastasis to bone. Limitations of this study include a relatively small sample size, with 5 bone metastasis samples from our institution and an additional 8 samples listed from MBC Project. As far as we know, there is no other publicly-available database that specifically has samples of breast cancer metastasis to bone. Therefore, our collection represents a unique and necessary addition to current literature.