Deep Whole Genome Sequencing Identifies Recurrent Genomic Alterations in Commonly-Used Breast Cancer Cell Lines and Patient Derived Xenograft Models

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1 **Title page**

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15

16 **Abstract**

17 *Background:* Breast cancer cell lines (BCCLs) and patient-derived xenografts (PDX)
18 are the most frequently used models in breast cancer research. Despite their
19 widespread usage, genome sequencing of these models is incomplete, with previous
20 studies only focusing on targeted gene panels, whole exome or shallow whole genome
21 sequencing. Deep whole genome sequencing is the most sensitive and accurate
22 method to detect single nucleotide variants and indels, gene copy number and
23 structural events such as gene fusions.

24 *Results:* Here we describe deep whole genome sequencing (WGS) of commonly used
25 BCCL and PDX models using the Illumina X10 platform with an average ~ 60x

coverage. We identify novel genomic alterations, including point mutations and genomic rearrangements at base-pair resolution, compared to previously available sequencing data. Through integrative analysis with publicly available functional screening data, we annotate new genomic features likely to be of biological significance. *CSMD1*, previously identified as a tumor suppressor gene in various cancer types, including head and neck, lung and breast cancers, has been identified with deletion in 50% of our PDX models, suggesting an important role in aggressive breast cancers.

Conclusions: Our WGS data provides a comprehensive genome sequencing resource of these models.

- 194 words

Keywords

Breast Cancer Cell Lines, Patient Derived Xenografts, Whole Genome Sequencing, Structural Variants, Non-coding Mutations

Background

Breast cancer cell line (BCCL) models are indispensable tools to study breast cancer biology and heterogeneity. Molecular profiling of BCCLs has generated useful insights into breast cancer subtypes and provides a resources for cancer gene discovery (Kao et al., 2009; Neve et al., 2006). A number of large-scale cancer cell line projects have characterised hundreds of cell lines with whole transcriptome profiling (Klijn et al., 2015), DNA microarray and targeted sequencing (Barretina et al., 2012). These studies provide great resources for cancer cell line studies, however, none of them have performed deep whole genome-wide sequencing analysis of

genetic changes, including mutations and structural variations, on these critical breast cancer models.

Patient derived xenograft (PDX) models, which closely resemble the heterogeneity of clinical BC, are established as important preclinical models (Bruna et al., 2016; DeRose et al., 2011). These breast cancer PDX models have been only characterised by shallow (<1X coverage) whole genome sequencing (WGS) or exome sequencing (Bruna et al., 2016).

WGS is the most sensitive method for detecting structural variants (SVs) and copy number variants (CNVs) and the only method to survey non-coding mutations. WGS is also a more powerful tool compared to exome-seq in detecting exome variants (Belkadi et al., 2015). To fully understand the genomic features of these models, it is important to conduct deep WGS analysis of these models to exhaustively identify complex genomic features of PDX models. In this study, we performed deep WGS to provide a comprehensive resource of genomic events of these important BCCLs and PDX models.

Results

Whole genome sequencing of breast cancer cell line models

According to a PubMed search, the top six studied BCCLs are MCF7, MDAMB231, T47D, SKBR3, MCF10A and MDAMB468, which cover more than 90% of all BCCL associated studies across more than 70,000 publications (Figure S1). Previous studies on these cell lines are restricted to whole-exome or low-pass whole genome sequencing at 0.2X coverage (Ben-David et al., 2018). Here we describe whole genome sequencing (WGS) of these models on the Illumina X10 platform with an average ~ 60x coverage, including two replicates of the most-commonly used MCF7

cell line (~53x and ~78x). Raw reads were mapped to human genome GRCh37 and single nucleotide variants were called using the Issac pipeline (Raczy et al., 2013). WGS is the most accurate way to assess cell line identity (Yu et al., 2015). We have compared the genotyping calls from our WGS data with SNP array from two independent studies previously published (Barretina et al., 2012; Heiser et al., 2012). Correlation analysis of the genotyping calls at the same genomic loci show high concordance between ours and the published data ($R>0.9$) (Figure S2), confirming the identity of all cell lines used in this study. In total, we have identified 3,540,312 to 4,108,844 variants per sample from the WGS data, including SNVs and small indels (Table S1). The majority of variants are present in the dbSNP database (Sherry et al., 2001) (94.0% ~ 96.2%) (Table S1). About 90% of these dbSNP variants have also been reported in the 1000 genome projects (Genomes Project et al., 2012), suggesting most variants in these cell lines models represents common variants in the human population, similar to the previous finding of WGS of the Hela cell line (Landry et al., 2013). However, each of the cell lines has about 5% of total variants that are cell line specific variants (Figure 1A).

In MCF7, for example, more than half of the cell line specific variants are located in inter-genic regions and ~ 1/3 are found in introns (Figure 1B). We compared the list of missense mutation in MCF7 to a recent study of multiple strains of MCF7s (Ben-David et al., 2018) (Figure 1C). Most of the missense mutations in (Ben-David et al., 2018) shared between MCF7 strains, therefore likely to come from the founder tumor, were have been identified in this study (20/23), and the concordance with our data increases as the number of mutant MCF7 strains increases (Figure 1D.& 1E). This suggests that the large majority of variants we have identified are real. In addition to the mutations reported in Ben-David et al, our WGS data identified 9,555 additional

missense mutations, 635 of which are not reported in 1000 genome or dbSNP database (Table S2). Sequencing of large cohorts of breast cancer tissue has revealed recurrent mutations in long non-coding genes including *MALAT1* and *NEAT1* (Nik-Zainal et al., 2016). Among all the cancer associated long non-coding genes reported (Quek et al., 2015) ((Gao et al., 2021), Lnc2Cancer v3.0), we identified mutations of *MALAT1*, *HOTAIR* and *ZFAS1* in these cell line models. For example, *Malat1* showed a heterozygous mutation (chr11:65271832 T>C) in MCF7, but not in MDA-MB-231 (Figure S3). The complete list of variants in the non-coding regions in these cell lines models (Table S3) could serve as a useful database for selection of models in non-coding RNA studies.

In addition to the SNVs, we also called SVs and CNVs from the WGS data using different methods, including Breakdancer (Chen et al., 2009) and Delly (Chen et al., 2009) for structural variants; CNVnator (Abyzov, Urban, Snyder, & Gerstein, 2011) and Lumpy (Abyzov et al., 2011) for copy number (see Methods). A summary of SVs and CNVs identified in MCF7 and the other four cell lines is shown in Figure 2A and Figure S4 respectively. In total, we have identified 321 inter-chromosomal SVs, with 38 ~ 108 SVs per cell line (Table S4). We use the GREAT program (McLean et al., 2010) to perform pathway analysis of the SVs events from individual cell lines. As expected, the top enriched pathways are genes in amplified regions previously identified from breast cancer, such as genes like *ZNF217* and *BCAS3* in MCF7 (Hampton et al., 2009); a selected list of luminal genes have also been enriched in the luminal cell lines MCF7 and T47D (Figure S5).

In addition to SVs, we also identified copy number alterations in these cell line models (Table S5). WGS has improved accuracy in detecting CNVs compared to

126 exome-seq due to its uniform coverage (Belkadi et al., 2015). We compared the
 127 CNVs identified from MCF7 with existing data from array-based COSMIC and
 128 shallow WGS from Ben-David et al (Figure 2B, Table S6). There is a poor overlap
 129 between this study and COSMIC, however, a substantial proportion (138/478) of the
 130 CNVs discordant between this study and COSMIC are reported in Ben-David et al
 131 using shallow WGS, suggesting the array-based method missed a lot of CNVs due to
 132 poor coverage. WGS can identify the known key copy number events in each of the
 133 cell lines with fine resolution, including AURKA and MYC amplification, CDKN2A
 134 (Figure 2C) deletion in MCF7, ERBB2 amplification in SKBR3, and CDKN2A
 135 deletion in MDAMD231 (Table S3). Our WGS data can also identify novel CNVs not
 136 reported in either COSMIC or Ben-David et al. *LINC00290*, a long non-coding RNA,
 137 which has been previously reported to undergo copy number loss in a pan-cancer
 138 study (Zack et al, 2013), has a homozygous deletion in MCF7 (Figure 2D).
 139 Furthermore, CNV boundaries were accurately detected by WGS compared to WES,
 140 in many cases with base pair precision (Table S5). For example, in MCF7 cell line,
 141 the boundaries of the homozygous deletion cover only CDKN2A, and the nearby
 142 CDKN2B is in a hemizygous deletion region (Figure 2C). FOXA1 copy number gain
 143 is in a focal amplicon, whereas GATA3 is in a broad copy number gain region about
 144 0.5Mb long & ESR1 copy number gain is towards the 5' end of the gene. (Figure S6).
 145 In order to estimate the functional impact of these variants, we compared our list of
 146 copy number variations with functional screen data from these cell lines (Marcotte et
 147 al., 2016). In MCF7, 33 out of the 445 copy number events harbor at least one of the
 148 breast cancer essential genes from (Marcotte et al., 2016). Interestingly this reveals
 149 coordinated amplification of *ESR1* and its co-factors *NCOA3* and *GATA3* and pioneer
 150 factor *FOXA1*, perhaps explaining the extreme estrogen-sensitivity of this cell line

(Table S5). Therefore, this study resolves known copy number events at nucleotide resolution and reports a substantial number of new copy number variants, some with evidence for function in these commonly-used cell line models.

Patient Derived Xenograft models

Bruna et. al. performed shallow WGS (<1X) profiling of 83 breast cancer PDX models and assessed their response to drug treatment (Bruna et al., 2016). Copy number calls were generated at 100kb resolution. Here we have selected six well-established and frequently-used PDX models with two matched blood samples (DeRose et al., 2011) and performed WGS with at least 90x coverage (blood samples were sequenced at 30x coverage)(Table S7) to generate reliable SNP, copy number and structural variant calls. Using the same pipeline as the cell line analysis based on the reads mappable to human genome, we identified 3,435,230 to 4,172,800 variants per sample from the WGS data, including SNVs and small indels (Table S1). Similarly to the cell line data, the majority have been identified in the dbSNP database (Sherry et al., 2001) (95.6% ~ 95.9%) (Table S1). About 84% of these variants identified in dbSNP have also been reported in 1000 genome projects (Genomes Project et al., 2012). Each of the PDXs has about 4% of total variants as PDX specific variants (Figure 3A). In HCI002, more than half of the PDX line specific variants are located in inter-genic regions, followed by variants in introns (Figure 3B). To validate our findings, we performed exome sequencing analysis of selected models and identified There are 12 non-synonymous mutations identified using an independent exome sequencing analysis of these models, all of which have been also identified in our WGS data (Table S8).

Using a stringent cutoff (Minoche et al., 2021), we have created a list of high confidence copy number alterations and structural variants for each of the PDXs (Table S9&S10). Recurrently amplified regions across the PDX models are chromosome 8q in HCI002/005/010/012 (covering MYC, Table S11) and chromosome 7p in HCI004/HCI008 (covering EGFR). Frequent deleted regions harbor classic tumour suppressor genes, for example PTEN in HCI004/HCI010 (Figure 3C, Table S9).

Because PDX tumours probably model more aggressive breast cancers (DeRose et al), we proposed that genomic analysis of PDX models may reveal genes associated with very aggressive disease. Interestingly, the most frequently deleted gene in PDX models, *CSMD1* (in 3 out of our six PDX models), is much more frequently deleted in metastatic breast cancer (14%) than in early disease (4%) (Figure 4A). Low expression of *CSMD1* is also associated with poor survival outcome in the METABRIC early cancer cohort (Figure 4B). Interestingly, we identified copy number loss of *CSMD1* in 23 of 32 PDXs in another PDX sequencing dataset (Bruna et al., 2016). These data suggest that *CSMD1* plays a critical role in suppressing growth or survival of metastatic breast cancers.

Discussion

We have completed whole genome analysis for widely used breast models, including mutations, copy number and SVs. The data has refined previously finding in cell lines such as *AURKA* and *MYC* amplification/*CDKN2A* deletion in MCF7, ERBB2 amplification in SKBR3, and *CDKN2A* deletion in MDAMD231 at nucleotide resolution. Our WGS data also identified novel mutations in non-coding genes and novel CNVs such as a homozygous deletion in *LINC00290*, and will be an important

resource for research in this area. Since WGS is far more accurate in identifying CNVs boundaries, it can provide further insights into well-known cancer associated genes, such as focal amplification of *FOXA1* in MCF7 versus broad copy number gain in *GATA3* and partial gain of *ESR1*. By comprehensively identifying the genomic features of these models, the field can now choose appropriate models to examine the functional significance of genes or pathways of interest.

Genomic studies of PDX may help us identify genomic features associated with very poor prognosis, as growth in a PDX acts as a ‘filter’ to enrich for the most aggressive tumours and cells that otherwise occur at low patient frequency in unselected breast cancer cell populations. In support of this, we found recurrent deletion of *CSMD1* in the PDXs models and its associated with metastatic disease and survival. Further extension of this idea to more PDX models may reveal further drivers of aggressive disease. The WGS data in this study is a valuable resource for other genomics studies to map to, for instance, CHIP-Seq or RNA-Seq studies to focus on non-coding and regulatory regions.

Conclusions

We have applied deep whole genome sequencing (WGS) of commonly used cell lines and PDX models using the Illumina X10 platform with an average ~ 60x coverage.

We show that this resource can be used to identify novel genomic alterations, including point mutations and genomic rearrangements, compared to previously available sequencing data.

Specific outcomes include:

- A comprehensive list of point mutations, copy number and structural variants for cell lines and PDX models.
- Integrative analysis with publicly available functional screening data identifies new genomic features of biological significance.

- CSMD1, a known tumour suppressor gene, identified with deletion in 50% of our PDX models, suggesting an important role in aggressive breast cancers.
- Raw data and processed data will be publicly available.

Methods

Sample acquisition and preparation

The MCF7 cell line was originally obtained from Michigan Cancer Foundation, the MCF10A from Brugge Lab, Boston, the MDAMB231 from EG&G Mason RI Worcester MA, and the MDAMB468, SKBR3 and T47D were from ATCC. Cells were cultured in their standard media to expand and frozen cell pellets used for DNA extraction. Breast Cancer PDX models were obtained from Alana Welm, and were expanded in house using techniques previously reported in DeRose et al 2010.

DNA extraction

The DNeasy Blood & Tissue Kit (Qiagen) was used for DNA isolation from about 25mg frozen tumor, according to the manufacturer's recommendations. RNase A (Qiagen) was used to obtain RNA-free genomic DNA. Only isolated DNA with A260/280 and A260/230 ratios above 1.8 and proven to be high quality by gel electrophoresis were used for sequencing.

Whole Genome Sequencing analysis of cell lines and PDX

The five cancer cell lines MCF7, MDAMB231, T47D, SKBR3, MDAMB468 and one non-malignant cell line MCF10A and six PDX models were submitted for sequencing with at least 60x coverage. Raw reads were mapped to human genome GRCh37 using Issac aligner from Illumina and called point mutations and small indels by Issac. Copy number alterations from the cell lines were estimated from cn.mops (R

package), and CNVnator (Abyzov et al., 2011). Structural variants were computed from Breakdancer (Chen et al., 2009), Delly (Chen et al., 2009) and Lumpy (Abyzov et al., 2011). Structural variants were annotated and visualized using ClinSV (Minoche et al., 2021) and IGV (Robinson et al., 2011). Raw sequencing files (fastqs) and variants (vcfs) are deposited at European Genome Archive. Data access will need to be submitted Data Access Committee EGAC00001001974.

Cell line identity check using SNP array

These common cell lines have been profiled by Affymetrix SNP 6.0 array previously in multiple studies. Raw cel files were downloaded from the Heiser et al and CCLE and were analysed by affymetrix genotyping console software. Correlation analysis between the genotyping calls between array and WGS data to confirm cell line identity.

Ethical Approval and Consent to participate

All required ethic approvals for investigations of the patient derived xenograft mouse models have been received for A/P Alex Swarbrick's lab.

Consent for publication

All authors have read the manuscript and provided consent for publication.

Availability of supporting data

All data analysed in this study are included in this article and its supplementary information files. The raw sequencing data supporting these findings are available at

European Genome Archive (Data access will need to be submitted Data Access Committee EGAC00001001974).

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

N.D and A.S conceived the experiment design and wrote the manuscript. N.D. and A.M. analysed the sequencing data. K.H. prepared the samples for whole genome sequencing. M.L, J.W, A.G. provided data for mutational validations in PDXs.

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Figure 1: Summary of single nucleotide variations (SNVs) in six breast cancer cell lines, including two replicates for MCF-7. (A) Classification of SNVs into different categories: overlap with 1000 genome project; overlap with dbSNP but not in 1000 genome project; cell-line specific events; (B) Distribution of the SNVs in MCF-7 in respect to location of protein coding genes. (C) unsupervised hierarchical clustering of MCF7 in this study together with 27 MCF7 strains in Ben-David et al, based on their missense mutation obtained from supplementary information from (Ben-David et al) (D) Percentage of overlapping missense mutations between our findings and those identified in multiple strains of MCF7s in Ben-David et al. (E) Venn diagram shows the overlap of missense mutations in Ben-David et al and this study.

Figure 2: (A) Genomics landscape of copy number and structural variants in six breast cancer cell lines. Circos plots of each individual cell lines are included. Copy number events are summarized in the inner circle with red and blue color indicates copy number gains and blue respectively, two inner circles are represented two replicates of MCF7 samples (one 30X and one 60X). Arcs connecting two loci of difference chromosomes indicate inter-chromosomal structural variations. (B) Venn diagram shows the overlap of CNV genes between COSMIC, Ben-David et al and this study. (C) Representative genome browser view of copy number alterations covering a common CNV gene, CDKN2A. Tracks from top to bottom: depth of coverage in an NA12878 control (control), all reads in the sample (all reads), or reads with mapping quality ≥ 20 (MQ ≥ 20), the average mapping quality of aligned reads from the sample (MQ, if no reads align MQ=0), coverage standard deviation from 500 controls (Coverage SD, indicating common CNV), overlapping segmental duplications published by Bailey JA et al. 2002 (SEG-DUP, used as control for

germline CNVs), discordant pairs (DP), split reads (SR), variants from the Database of Genomic Variants (DGV), and RefSeq genes (Genes). (D) Representative genome browser view plot of a novel CNV gene LINC00290 in this study.

Figure 3: Summary of SNVs in breast cancer patient-derived xenograft models: (A) classifications of SNVs into three categories: overlap with 1000 genome project; overlap with dbSNP but not in 1000 genome project; PDX-specific variants. (B) Distribution of the SNVs in HCI002 model in respect to location of protein coding genes. (C) Representative genome browser view of copy number alteration covering *PTEN* in HCI004 model. Tracks from top to bottom: depth of coverage in HCI004 germline (control) and HCI004 PDX, and the average mapping quality of aligned reads from the sample (MQ, if no reads align MQ=0), coverage standard deviation from 500 controls (Coverage SD, indicating common CNV), overlapping segmental duplications published by Bailey JA et al. 2002 (SEG-DUP, used as control for germline CNVs), discordant pairs (DP), split reads (SR), variants from the Database of Genomic Variants (DGV), and RefSeq genes (Genes).

Figure 4: Genes with enriched genomic alterations in PDXs (A,B) Genes with frequent copy number alterations in PDXs samples showing higher copy number variations in a metastatic breast cancer cohort than a primary breast cancer cohort (cbiportal). Each row indicates a gene and each column indicates a breast cancer sample. Copy number gains are in red and deletions are in blue. CSMD1, for example, showing a much high frequency of copy number deletion in the metastatic cohort. (C) Kaplan-Meier survival analysis of METABRIC samples

445 stratified by CSMD1 expression status. Top 25% samples with high CSMD1 is in red,
446 showing a better survival outcome compared to those with low CSMD1 expression.
447

Figure 1

Figure 2

A

B

C

D

Figure 3

A

B

C

Figure 4

A. Metastatic Breast Cancer Project (cbiportal)

B. METABRIC (cbiportal)

C

Survival of CSMD1 in M1 ALL (p = 0.00466)

Supplementary Information

Deep whole genome sequencing identifies recurrent genomic alterations in breast cancer cell lines and patient derived xenograft models

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Number of citations for each of the cell lines obtained from PubMed.

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Correlation analysis of genotyping calls from this study to compare with SNP calls from Heiser et al and CCLE.

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484 *average mapping quality of aligned reads from the sample (MQ , if no reads align*
485 *$MQ=0$), coverage standard deviation from 500 controls (Coverage SD, indicating*
486 *common CNV), overlapping segmental duplications published by Bailey JA et al.*
487 *2002 (SEG-DUP, used as control for germline CNVs), discordant pairs (DP), split*
488 *reads (SR), variants from the Database of Genomic Variants (DGV), and RefSeq*
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Figure S1

Figure S2

Figure S3

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FOXA1- 8 copies, focal amplification

GATA3, broad gain, 4 copies

ESR1, partial gain

Supplementary Files

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- [SuppTable14.xlsx](#)
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