Development of a panel of breast cancer patient-derived xenograft models (PDX) with estrogen independence and/or acquired resistance to endocrine treatment

Abstract #5281

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Introduction

Breast cancer (BC) is the most common cancer and the leading cause of cancer related death among women in the world [1]. At least 70% of breast cancers are classified as estrogen receptor positive (ER+) and/or progesterone receptor positive (PR+), and HER2 negative tumors commonly called luminal breast cancers [2]. Interfering with the ER pathway with antiestrogens (e.g., tamoxifen or fulvestrant) or estrogen deprivation (e.g., aromatase inhibitors or ovariectomy), decreases mortality from ER+ breast cancer. However, development of hormonal therapy resistance (HTR) in patients remains a major clinical issue [3]. The main mechanisms of resistance to these therapies are lack of ER expression, deregulation of ER-associated transcription factors, coactivators, activation of receptor tyrosine kinase signaling, and aberrant expression of cell-cycle regulators [4]. A huge research effort has over the years deciphered key biological mechanisms of HTR. Unfortunately, results obtained in biology-based clinical studies showed only very small and short-term clinical benefits, underlining the need for more in-depth molecular understanding of HTR and adequately predictive preclinical investigations. Consequently, there is a need for new experimental models that better replicate the diversity of human tumor biology in a preclinical setting. Utilization of patient-derived xenograft (PDX) models in preclinical breast cancer research has been recognized as a more realistic solution to recapitulate human tumor biology and predict patient drug response [5] by directly comparing drug responses in patients and their corresponding xenografts. To extend such observations to a greater number of human cancers, OncoDesign and Eisai have collaboratively developed an extensive collection of breast cancer PDXs. Starting with luminal hormone dependent PDX models, we generated PDX sublines with acquired resistance to fulvestrant or the ability to grow in the complete absence of estrogen (ovariectomy or without estrogen supplementation). Each generated subline was then analyzed by IHC (for ER/PR expression), whole exome sequencing, RNA sequencing and DNA methylation analyses and compared to the parental tumor.

Similar to what is observed in the clinic, 60% of our breast PDX panel is classified as estrogen receptor (ER) positive. Immunohistochemical analyses performed on patient's tumors and xenografts showed striking similarities in the tumor morphology as well as in the expression level of ER, PR, and HER2. Response to hormone therapy showed different sensitivities, thus exhibiting heterogeneity similar to what is observed in the clinic. RNA sequencing and DNA methylation of PDX sublines with acquired resistance to hormone therapies showed specific deregulation of ER-mediated gene expression and DNA hypermethylation. These models offer a clinically relevant tool to evaluate specific anticancer therapies in the context of endocrine resistance, as well as to use as mechanistic models to investigate both the acquisition and mitigation of such resistance.

References

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Results

Breast Cancer PDX Model

Designation	ER / PR /Her2 Status	Patient and Cancer Details	Patient Prior Therapies
OD-BRE-0438	ER+/PR+/HER2−	51-year-old female patient with luminal B invasive lobular breast carcinoma	No

Schematic figure of breast cancer PDX amplification and utilization to test drug efficacy

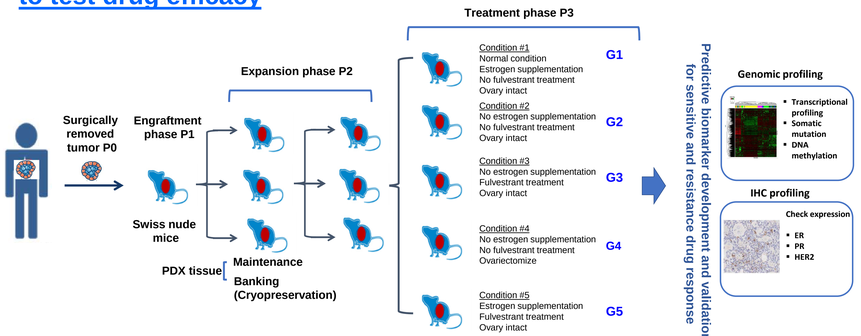

Figure 1: Schematic presentation of PDX establishment and utilization. The first step was the establishment of the PDX from breast cancer patients' tumors by engraftment of tumors samples in Swiss Nude mice. The second phase consisted of tumor expansion by serial re-engraftment. All established PDX tissue could be flash frozen and subsequently successfully engrafted, ensuring the persistence of the living biobank. The third step was for *in vivo* efficacy of antitumor activity of endocrine therapy to establish hormone independent models. Additionally, this last step was used for biomarker (ER, PR and HER2) and multi-omic profiling (RNA sequencing, whole exome sequencing, methylome) analysis.

In vivo tumor growth of OD-BRE-0438 xenografts and response to endocrine therapies

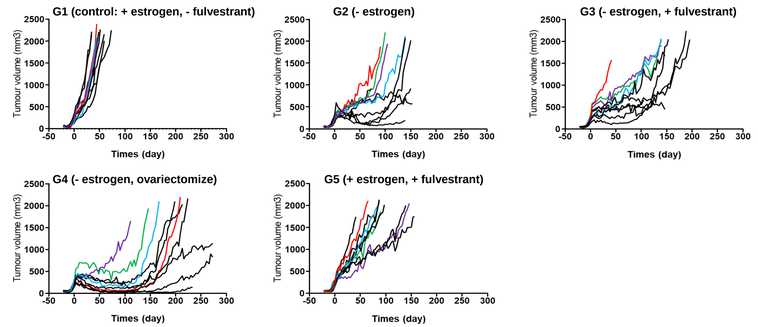

Figure 2: *In vivo* effect of estrogen deprivation (ovariectomy or without estrogen supplementation) and/or fulvestrant treatment on OD-BRE-0438 PDX models. Intact or ovariectomized Swiss Nude mice bearing OD-BRE-438 tumors were randomized into 5 groups and treated +/- estrogen supplementation and +/- fulvestrant as shown. Two groups of animals received fulvestrant treatment at 2.5 mg/mouse. The data show individual tumor volumes for each animal. Individual tumors with colors were chosen for biomarkers analyses.

Patient tumor phenotype is reproduced in xenografts

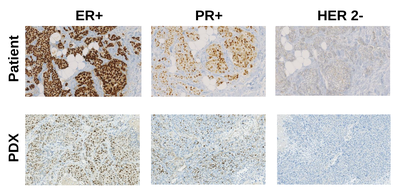

Figure 3: Phenotypic stability between the original patient tumor and its corresponding xenograft. Immunohistochemistry analyses of ER, PR and HER2 markers in parental breast tumor from patient and corresponding derived xenografts in Swiss Nude mice.

The hormone receptor expression are affected by endocrine therapy

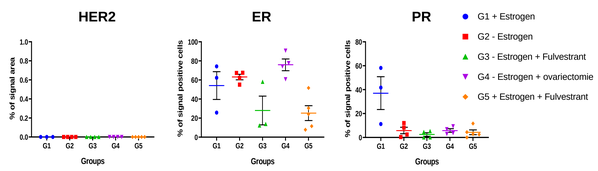

Figure 4: Immunohistochemistry analysis of ER, PR and HER2 from PDX tumor tissues generated from the 5 different treatment groups. The graph represents the individual value for each tumor and the mean +/- SEM for each group for ER, PR and HER 2. Tumors treated with endocrine therapy (fulvestrant) or estrogen deprivation (ovariectomy and/or without estrogen supplementation) induced change in hormone receptor expression compared to the original tumor from group 1.

Independence and/or acquired resistance to endocrine treatment of xenografts is associated with tumor specific molecular changes

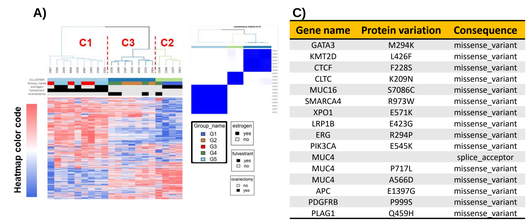

Figure 5: Characterization of molecular features analysis of the tissue tumor generated from the 5 groups treated or not with fulvestrant.
A) Differential gene expression of PDX tumors generated from the 5 different groups (G1, G2, G3, G4, G5) treated or not with fulvestrant compared to the control PDX (G1). The categorized subtype information is illustrated as a heat map with hierarchical clustering into 3 clusters (C1, C2, C3) according to gene expression patterns. **B)** Heat map representing up-regulated and down-regulated pathways associated with genomic alterations in individual PDX tumors from groups in clusters 1 and 3 compared to G1. **C)** Table shows driver mutations and related protein variations. **D)** Pan-genomic profiles show very similar patterns of copy number between all PDX from different groups; these included 3 amplifications frequently gained in breast cancer: KAT6A (chromosome 8), PHF12 and TUBD1 (both on chromosome 17), and focal homozygous deletion of TP53 on chromosome 17 (first 5 exons). **E)** Supervised analysis-differential methylation in gene-based features: the number of differentially methylated features by category (TSS/gene body) is indicated in the graph comparing groups from C1 and C3 versus G1. As for CGI-based features, we observe hypermethylation in C1 and to a lesser extent C3. Also shown are Venn diagrams representing overlapping genes hypermethylated between C1 and C3 in the TSS and gene body.

Comments and Conclusions

- ✓ Our breast cancer PDX (ER+/PR+/HER2−) models maintain high phenotype similarities with the original patients' tumors.
- ✓ We generated a biobank of PDX models out to several passages, and cryopreserved those tissues in liquid nitrogen.
- ✓ We established OD-BRE-438 subline-based PDX preclinical tumor models which show resistance to hormone therapy treatment (fulvestrant) in presence or absence of estradiol supplementation.
- ✓ Characterization of molecular features revealed differences between genomic profiles of PDX models treated with fulvestrant versus untreated PDX, as compared to the original parental PDX tumor.
- ✓ The PDX tumor model treated with fulvestrant displays differential gene expression indicative of activation of many pathways, thus providing relevant models to test targeted agents and new treatment combinations.