Tumor-specific molecular changes in luminal B breast cancer patient-derived xenografts (PDXs) with acquired resistance to endocrine treatment

Abstract #1399

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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 A 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 molecular tumor changes associated with acquisition of resistance 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 A hormone dependent breast cancer 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), thus corresponding to acquisition of resistance following hormonotherapy in the clinical setting. To understand phenotypic changes associated with resistance acquisition, each generated PDX 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 showed that all derived resistant PDXs had complete loss of PR expression regardless of selection method, whereas ER expression was only decreased in resistant PDXs that had been selected by fulvestrant treatment. Response to hormone therapy showed different sensitivities, thus exhibiting heterogeneity similar to what is observed in the clinic. Genetic analyses showed that resistant PDX tumors fell into two different transcriptomic signatures, depending on whether resistance was driven by estrogen deprivation or fulvestrant administration. Compared to the naïve parental tumor, the cluster corresponding to fulvestrant-selected PDX showed up-regulated and down-regulated pathways associated with genomic alterations related to endocrine therapy pathways. Interestingly, the other cluster in which PDX were selected without fulvestrant showed only a partial genomic alterations. Furthermore, whole exome sequencing of the PDX revealed similar driver genes mutations existed in both derived resistant and sensitive parental PDX. Finally, we confirmed pharmacological resistance to fulvestrant in the resistant PDX. New experiments investigating drug combinations in the context of endocrine resistance are ongoing.

References

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Results

Breast patient derived xenograft 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

Patient tumor phenotype is reproduced in its patient derived xenograft

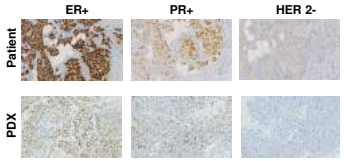

Figure 2: Phenotypic stability between the original patient tumor and its corresponding xenograft. Immunohistochemistry analyses of ER, PR and HER2 markers in parental breast tumor from patient and corresponding derived xenografts in Swiss Nude mice.

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

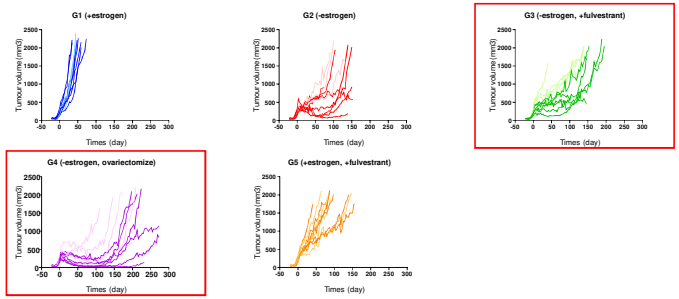

Figure 1: *In vivo* effect of estrogen deprivation (ovariectomy or without estrogen supplementation) and/or fulvestrant treatment on OD-BRE-0438 PDX models. Intact or ovariectomized Swiss Nude mice bearing OD-BRE-438 tumors were randomized into 5 groups and treated +/- estrogen supplementation and +/- fulvestrant as shown. Two groups of animals received fulvestrant treatment at 2.5 mg/mouse. The data show individual tumor volumes for each animal, with Day 0 defined as the day of initiation of the resistance-inducing protocols. Individual tumors with light colors were chosen for biomarkers analyses.

Hormone receptor expression is affected by endocrine therapy

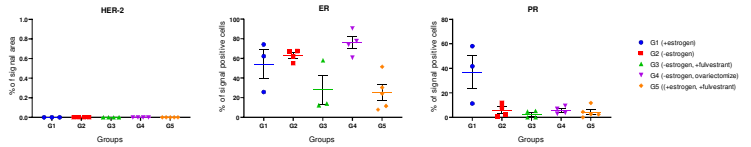

Figure 3: Immunohistochemistry analysis of ER, PR and HER2 from PDX tumor tissues generated from the 5 different treatment groups. The graph represents the individual value for each tumor and the mean ± SEM for each group for HER2, ER, and PR. Tumors treated with endocrine therapy (fulvestrant; G3, G5) or estrogen deprivation (without estrogen supplementation and/or ovariectomy; G2, G3, G4) showed changes in hormone receptor expression levels compared to the untreated tumor from group 1 (G1).

Estrogen independence and/or acquired resistance to endocrine treatment is associated with tumor-specific molecular changes

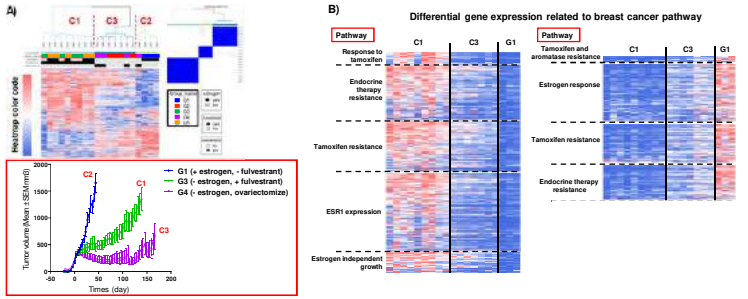

Figure 4: Characterization of molecular features of tumors generated from the 5 groups treated or not with fulvestrant.
A) Differential gene expression of PDX tumors generated from the 5 different groups (G1, G2, G3, G4, G5) treated or not with fulvestrant compared to the control PDX (G1). The categorized 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.

Characterization of estrogen independence fulvestrant resistance in PDX derived from G3 and G4

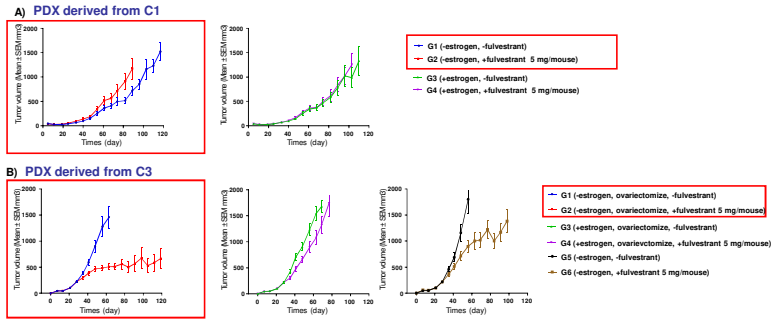

Figure 5: Characterization of PDX estrogen independence issued from G3 and G4 to test their sensitivity of resistance to fulvestrant treatment. A). Swiss Nude Mice bearing OD-BRE-438 PDX received fulvestrant at 2.5 mg/mouse (original growth condition of PDX derived from G3; see Figure 1) and were then randomized into 4 groups and treated ± estrogen and ± fulvestrant 5 mg/mouse (maximum tolerated dose). The graph in the red box represents the growth condition of the tumor issued from G3.
B). Intact or ovariectomized Swiss nude mice were randomized into 6 groups and treated ± estrogen and ± fulvestrant 5 mg/mouse (maximum tolerated dose). The graph in the red box represents the growth condition of the tumor issued from G4.

Comments and Conclusions

- ✓ We established OD-BRE-438 subline-based PDX preclinical tumor models which show resistance to hormone therapy treatment (fulvestrant) in presence or absence of estrogen supplementation.
- ✓ Characterization of molecular features revealed differences between transcriptomic 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.
- ✓ We confirmed the resistance of PDX to fulvestrant treatment generated from group 3 which already shows resistance to hormone therapy treatment.
- ✓ New experiments are ongoing using this PDX model resistance to fulvestrant with combinations therapy.