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Integrated molecular analysis of Tamoxifen-resistant invasive lobular breast cancer cells identifies MAPK and GRM/mGluR signaling as therapeutic vulnerabilities

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ABSTRACT

Invasive lobular breast cancer (ILC) is an understudied malignancy with distinct clinical, pathological, and molecular features that distinguish it from the more common invasive ductal carcinoma (IDC). Mounting evidence suggests that estrogen receptor-alpha positive (ER+) ILC has a poor response to Tamoxifen (TAM), but the mechanistic drivers of this are undefined. In the current work, we comprehensively characterize the SUM44/LCCTam ILC cell model system through integrated analysis of gene expression, copy number, and mutation, with the goal of identifying actionable alterations relevant to clinical ILC that can be co-targeted along with ER to improve treatment outcomes. We show that TAM has several distinct effects on the transcriptome of LCCTam cells, that this resistant cell model has acquired copy number alterations and mutations that impinge on MAPK and metabotropic glutamate receptor (GRM/mGluR) signaling networks, and that pharmacological inhibition of either improves or restores the growth-inhibitory actions of endocrine therapy.

Contents

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1. Introduction

Invasive lobular breast cancer (ILC) is a special histologic subtype of breast cancer that accounts for 10–15% of annually diagnosed cases – an identical percentage to triple negative breast cancer. ILC has unique clinical features that distinguish it from the more common invasive ductal breast cancer (IDC) (Barroso-Sousa and Metzger-Filho, 2016; Christgen et al., 2016; Sledge et al., 2016) – it forms a long, thin mass that is often missed by screening mammograms, and metastatic ILC frequently spreads to distinct sites (e.g. peritoneum, gastrointestinal tract, orbital cavity) that differ from IDC. This unique biology of ILC impacts disease prognosis. ILC has a greater risk of late recurrence and death (>10 years post-diagnosis) than IDC (Pestalozzi et al., 2008; Rakha et al., 2008). Grade 2 (moderately differentiated) ILC has an equally poor breast cancer-specific survival to grade 3 (poorly differentiated) IDC (Engstrøm et al., 2015). The most common genetic lesion in ILC is mutation of *CDH1* leading to loss of E-cadherin expression, which is thought to underlie ILC's unusual metastatic pattern as well as its tendency to be multifocal and affect the contralateral breast. Recent studies have performed genomic, transcriptomic, and proteomic characterization of ILC clinical specimens to identify additional events that are enriched in ILC vs. IDC; these include higher rates of *PTEN* loss, *FOXA1* mutation, and AKT phosphorylation (Ciriello et al., 2015; Desmedt et al., 2016; Michaut et al., 2016).

Molecular profiling of breast cancer classifies most ILC as Luminal A – estrogen receptor positive (ER+), and slow-growing – for which 10 years of treatment with the antiestrogen Tamoxifen (TAM) or an aromatase inhibitor (AI) is recommended (Azim et al., 2016; Rugo et al., 2016). Within ILC, there are 2–3 additional molecular subtypes (Ciriello et al., 2015; Michaut et al., 2016). Among ER + or Luminal A breast tumors, lobular histology is still independently and significantly associated with worse long-term survival outcome than ductal histology (Pestalozzi et al., 2008; Rakha et al., 2008), and multiple studies show that ER + ILC has a significantly worse response to TAM than the non-steroidal AIs letrozole or anastrozole (Knauer et al., 2015; Metzger Filho et al., 2015). ILC patients also have inferior overall survival on the steroidal AI exemestane vs. anastrozole, while in IDC they have equivalent efficacy (Strasser-Weippl et al., 2016).

ILC is an understudied malignancy and thus the reasons for its relatively poor response to TAM are not well defined. We previously established the first cellular model of TAM-resistant ILC (Riggins et al., 2008); SUM44 cells are the parental, TAM sensitive cell line while LCCTam cells are TAM-resistant. These initial studies identified an orphan member of the nuclear receptor superfamily, estrogen-related receptor gamma (ERRγ, *ESRRG*), as a key mediator of the TAM-resistant phenotype. In the current work, we comprehensively characterize this ILC model system through integrated analysis of genome-wide gene expression, copy number, and whole exome sequencing (WES), with the goal of identifying actionable alterations relevant to clinical ILC that can be targeted to improve therapeutic outcomes. We find that 4-hydroxytamoxifen (4HT) has a distinct effect on the transcriptome of LCCTam cells, that this resistant cell model has acquired copy number alterations and specific gene mutations that impinge on MAPK and metabotropic glutamate receptor (GRM/mGluR) signaling networks, and that pharmacological inhibition of either improves or restores the growth-inhibitory actions of endocrine therapy.

2. Materials and methods

2.1. General study design

We have previously reported the establishment and initial validation of a TAM-resistant ILC cell line (LCCTam) established from the parental SUM44 (Riggins et al., 2008). Here, we demonstrate the utility of this model system for studying TAM-resistant ILC by comparing SUM44 with publicly available breast cancer patient datasets. Subsequent comprehensive molecular analysis of LCCTam cells was performed in comparison to SUM44 cells utilizing gene expression array, Whole Exome Sequencing (WES), and Array Comparative Genomic Hybridization (aCGH). Selected molecular targets from this analysis were validated and functional analyses were performed using drugs of interest that resensitize TAM-resistant cells to endocrine therapy as measured by growth inhibition.

2.2. TCGA and METABRIC datasets, and tumor-derived ILC signature (TIS)

cBioPortal (Cerami et al., 2012; Gao et al., 2013) was used to query processed gene expression microarray, whole-exome, and RNA sequencing data for Luminal A IDC and Luminal A ILC tumors from The Cancer Genome Atlas (TCGA, (Ciriello et al., 2015)) and the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC, (Curtis et al., 2012; Pereira et al., 2016)). To establish the tumor-derived ILC signature (TIS, Fig. 1A), RNA-seq data for Luminal A ILC (n = 106) and IDC (n = 201) were downloaded from the TCGA Portal (http://cbio.mskcc.org/cancergenomics/tcga/brca_tcga/). The differentially expressed genes between these two histologic subtypes were identified using the 'ComparativeMarkerSelection' module from GenePattern (Reich et al., 2006), and plotted by alignment of their tested t-score after permutation. The top 100 genes including both directions (2 × 50) were defined as a 'tumor-derived ILC gene signature' (TIS) for the comparison of signature profiles among different cell models, using a t-score method as described previously (Creighton et al., 2010).

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