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Gene expression profiling of lobular carcinoma *in situ* reveals candidate precursor genes for invasion

Victor P. Andrade[a,b,1], Mary Morrogh[a,1], Li-Xuan Qin[c,1], Narciso Olvera[d], Dilip Giri[e], Shirin Muhsen[a], Rita A. Sakr[a], Michail Schizas[d], Charlotte K.Y. Ng[e], Crispinita D. Arroyo[d], Edi Brogi[e], Agnes Viale[f], Monica Morrow[a], Jorge S. Reis-Filho[e], Tari A. King[a,*]

[a]Breast Service, Department of Surgery, Memorial Sloan Kettering Cancer Center, 300 E. 66th St., New York, NY, 10065, USA
[b]Department of Pathology, A.C. Camargo Cancer Center, Sao Paulo, Brazil
[c]Department of Epidemiology and Biostatistics, Memorial Sloan Kettering Cancer Center, New York, NY, 10065, USA
[d]Sloan-Kettering Institute, Memorial Sloan Kettering Cancer Center, New York, NY, 10065, USA
[e]Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, NY, 10065, USA
[f]Genomics Core, Memorial Sloan Kettering Cancer Center, New York, NY, 10065, USA

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ABSTRACT

Purpose: Lobular carcinoma *in situ* (LCIS) is both a risk indicator and non-obligate precursor of invasive lobular carcinoma (ILC). We sought to characterize the transcriptomic features of LCIS and ILC, with a focus on the identification of intrinsic molecular subtypes of LCIS and the changes involved in the progression from normal breast epithelium to LCIS and ILC.

Methods: Fresh-frozen classic LCIS, classic ILC, and normal breast epithelium (N) from women undergoing prophylactic or therapeutic mastectomy were prospectively collected, laser-capture microdissected, and subjected to gene expression profiling using Affymetrix HG-U133A 2.0 microarrays.

Results: Unsupervised hierarchical clustering of 40 LCIS samples identified 2 clusters of LCIS distinguished by 6431 probe sets ($p < 0.001$). Genes identifying the clusters included proliferation genes and other genes related to cancer canonical pathways such as TGF beta signaling, p53 signaling, actin cytoskeleton, apoptosis and Wnt-Signaling pathway. A supervised analysis to identify differentially expressed genes ($p < 0.001$) between normal epithelium, LCIS, and ILC, using 23 patient-matched triplets of N, LCIS, and ILC, identified 169 candidate precursor genes, which likely play a role in LCIS progression, including PIK3R1, GOLM1, and GPR137B. These potential precursor genes map significantly more frequently to 1q and 16q, regions frequently targeted by gene copy number alterations in LCIS and ILC.

Abbreviations: LCIS, lobular carcinoma *in situ*; ILC, invasive lobular carcinoma; ER, estrogen receptor; MSKCC, Memorial Sloan Kettering Cancer Center; H&E, hematoxylin and eosin; LCM, laser capture microdissection; DAVID, Database for Annotation, Visualization and Integrated Discovery; FFPE, formalin-fixed paraffin-embedded; TCGA, The Cancer Genome Atlas.

* *Corresponding author*. Tel.: +1 646 888 5352; fax: +1 646 888 4921.
E-mail address: kingt@mskcc.org (T.A. King).

[1] V. P. Andrade, M. Morrogh, and L. X. Qin contributed equally to this article.

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Conclusion: Here we demonstrate that classic LCIS is a heterogeneous disease at the transcriptomic level and identify potential precursor genes in lobular carcinogenesis. Understanding the molecular heterogeneity of LCIS and the potential role of these potential precursor genes may help personalize the therapy of patients with LCIS.

1. Introduction

Classical lobular carcinoma *in situ* (LCIS) is a non-invasive neoplastic lesion of the breast characterized by distention of the lobules and terminal duct-lobular units by a proliferation of atypical but monomorphic dishesive cells that almost invariably express estrogen receptor (ER) and lack HER2 expression (Lakhani et al., 2012). LCIS is most often an incidental finding in benign breast biopsies, reported in 0.5–4.0% of cases and confers one of the greatest risks for the subsequent development of invasive breast cancer (lifetime risk, 20–25%) (Rosen et al., 1978). Although LCIS is typically perceived as a marker of increased breast cancer risk, emerging evidence of genotypic similarities between co-existing LCIS and invasive lobular breast cancer (ILC) suggests that a subset of LCIS may carry a higher risk for progression to invasive disease, exhibiting true precursor potential. In fact, molecular analyses have demonstrated losses on chromosomes 16q and 17p in both LCIS and ILC (Buerger et al., 1999; Lu et al., 1998; Nishizaki et al., 1997), identical truncating mutations in the *E*-cadherin gene and LOH of wild-type *E*-cadherin allele have been found in LCIS and adjacent ILCs (Vos et al., 1997), and recent studies from our group and others have demonstrated that at least some LCIS are clonally related to co-existing ILCs (Andrade et al., 2012; Aulmann et al., 2008; Hwang et al., 2004; Mastracci et al., 2006).

Despite its characteristic shared pathological and molecular features with ILC, LCIS is best classified as a non-obligate precursor of breast cancer, as only a subset of these lesions will progress to invasive disease. This heterogeneity in clinical behavior represents a distinct clinical challenge and led us to posit that LCIS would comprise a heterogeneous collection of lesions at the molecular level, and that by elucidating this heterogeneity, we may be able to identify potential markers of progression in lobular carcinogenesis.

The first application of microarray expression profiling in invasive breast cancer reshaped breast cancer classification and now provides the basic framework for outlining the molecular heterogeneity of all breast cancer subtypes (reviewed in Sorlie et al., 2001). The application of this technology to the study of LCIS has been limited by its clinically occult nature and the lack of available fresh frozen tissue. Our research team has overcome these limitations (Morrogh et al., 2007) and performed microarray-based gene expression profiling from fresh frozen patient-matched samples of normal breast epithelium, LCIS, and ILC. These data offered us an opportunity to carry out a hypothesis-generating analysis investigating whether LCIS would constitute a heterogeneous disease at the transcriptomic level and define the gene expression changes in the progression from LCIS to ILC.

2. Materials and methods

Patients with a documented history of LCIS, presenting for prophylactic or therapeutic mastectomy, were identified preoperatively and enrolled in a Memorial Sloan Kettering Cancer Center (MSKCC) institutional review board-approved protocol for the collection of fresh frozen tissue and genomic analyses (IRB 01-135). Following standard clinical sampling, mastectomy specimens were subject to random sampling, and up to 10 fresh frozen blocks per quadrant were harvested and stored at −80 °C for subsequent analysis. For the purposes of this study, 5 μm hematoxylin and eosin (H&E) frozen sections were reviewed by two study pathologists (DG, EB) to identify blocks with LCIS, with or without invasive cancer. Histologic criteria of classic LCIS, classic ILC, and IDC were those described by the 2012 World Health Organization Tumors of the Breast (Lakhani et al., 2012). Only lesions that fulfilled the histologic criteria for classic LCIS were included in this study. Cases classified as pleomorphic LCIS were excluded.

From 2005 to 2009, tissue collection and screening of mastectomy specimens from 140 patients resulted in the identification of fresh frozen LCIS lesions suitable for laser capture microdissection (LCM) from 88 (63%) patients. Selected blocks were further sectioned, and 10 μm slides were prepared for LCM as previously described (Morrogh et al., 2007). LCM of normal epithelium, LCIS, and invasive tumor cells (where available) was performed using the PALM laser catapult system (Carl Zeiss, Germany), followed by extraction of RNA with standard methodologies (*mir*VANA miRNA Isolation Kit; Ambion, Foster City, CA). A total of 224 samples from 88 patients were microdissected and evaluated for RNA concentration, purity, and quality using the NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE) and the Agilent 2100 Bioanalyzer (Agilent Technologies, Colorado Springs, CO).

Samples with at least 250 ng, $A_{260\ nm/280\ nm}$ ratios >1.8, and RNA integrity number above 5 were labeled and hybridized in 2 batches to HG-U133A 2.0 oligonucleotide arrays (Affymetrix, Santa Clara, CA) according to the MSKCC Genomics Core Facility protocols (Sanchez-Carbayo et al., 2003). The first array batch (Cohort No. 1) contained 60 samples from 23 patients accrued from April 2003 to April 2006; 3 patients with LCIS only (prophylactic mastectomy specimens) and 20 patients with LCIS and ILC (n = 18) or IDC (n = 2). The second array batch (Cohort No. 2) contained 115 samples from 40 patients accrued from December 2006 to May 2009; 5 patients with LCIS only (prophylactic mastectomy specimens), 3 patients with LCIS and DCIS, and 32 patients with LCIS and either ILC (n = 20) or IDC (n = 12). For this analysis, we did not use data from DCIS or IDC samples. (The arrays were scanned with the GeneChip System confocal scanner Agilent