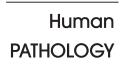


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Original contribution

Genetic up-regulation and overexpression of PLEKHA7 differentiates invasive lobular carcinomas from invasive ductal carcinomas[☆]

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Summary Molecular differentiation between invasive lobular carcinomas (ILCs) and invasive ductal carcinomas (IDCs) of the breast has not been well defined. We investigated gene expression differences between ILCs and IDCs and their correlation with variations in invasiveness and tumor growth. Total RNA was isolated from 30 frozen tumor samples: 10 from ILCs and 20 from IDCs. Gene expression was investigated using the Affymetrix GeneChip Human Gene 1.0 ST Array (Affymetrix, Santa Clara, CA). Data and validation were performed by reverse transcriptase polymerase chain reaction and immunohistochemistry. Gene expression differences between ILCs and IDCs were found in 140 genes. Overall, ILCs showed up-regulation of genes related with cell migration, lipid and fatty acid metabolism, and some transcription factors and showed down-regulation of cell adhesion, actin cytoskeleton, cell proliferation, and energetic metabolism of the tumor cells. Our reverse transcriptase polymerase chain reaction results showed that PLEKHA and TMSB10 expression discriminated ILCs from luminal A IDCs, whereas PLEKHA7, TMSB10, PRDX4, and SERPINB5 discriminated ILCs from luminal B IDCs. At the protein level, Plekha7 was overexpressed in ILCs but not in normal tissue or low-grade IDCs. Moreover, Plekha7 overexpression had an inverse relation with E-cadherin expression. The gene expression profile in ILCs and IDCs differs in several signaling pathways. Our findings suggest that overexpression of PLEKHA7 is common in ILCs and could be a molecular marker to differentiate ILCs from IDCs.

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1. Introduction

Breast carcinomas are usually classified as either invasive ductal carcinomas (IDCs) or invasive lobular carcinomas (ILCs) [1-3], despite the fact that 20 pathologic variants of breast cancer have already been described [4]. ILCs have a higher tendency to metastasize in gastrointestinal and genital tracts, meninges, and serosal cavities, whereas IDCs tend to metastasize to bone, lung, and liver [4,5]. Morphologically, IDCs usually form glandular structures or a solid nest. Lobular tumors, in contrast, are formed of discohesive epithelial cells that are sometimes organized in small clusters in association with loss of E-cadherin expression [6] and slower growth rate. Functionally, 90% of ILCs are hormone receptor (HR) positive, either estrogen receptor (ER) and/or progesterone receptor (PR), and they rarely overexpress HER2 (<1%). In contrast, 70% of IDCs express hormone receptors (HR) and HER2 overexpression is detected in 20% of these tumors [4]. Taken together, these differences suggest that ILCs and IDCs develop and progress following different signaling pathways.

The use of gene expression profiling in human breast cancer has allowed a new molecular classification of these neoplasms and has facilitated the development of new prognostic and therapeutic approaches [7]. The initial gene expression profiling studies in breast cancer included mostly IDCs and only began to specifically analyze other histologic subtypes in recent years [8-14]. However, differences between IDCs and ILCs based on their gene expression profile have not yet been fully identified [12-14].

In this study, we compared the gene expression profile between ILCs and IDCs to gain insight into the biologic differences in the invasion and growth mechanisms of these 2 types of breast tumors.

2. Materials and methods

2.1. Patients and tumor samples

This study followed the principles of the Helsinki Declaration and was approved by the ethics committee at the Institut de Recerca, Hospital de la Santa Creu i Sant Pau. All patients gave informed consent for use of tumor tissue for clinical research. A total of 10 ILCs and 20 IDCs were selected retrospectively from the tumor bank and archives at the Department of Pathology at Hospital de la Santa Creu i Sant Pau, Barcelona, Spain. Patients were staged according to the World Health Organization system, and tumors were graded using the conventional grading system. Clinicopathologic data were revised, and the main characteristics are summarized in Table 1. Gene expression profiling analysis, quantitative reverse transcriptase polymerase chain reaction, and immunohistochemistry were studied using the same tumors.

		ILC $(n = 10)$	IDC $(n = 20)$
Histologic grade	Ι	3 (30%)	3 (15%)
	II	6 (60%)	8 (40%)
	III	1 (10%)	9 (45%)
Mitosis (mm ² /10)	<7	9 (90%)	9 (45%)
	7-13	0	5 (25%)
	>13	1 (10%)	6 (30%)
Nuclear atypia	1	2 (20%)	1 (5%)
	2	6 (60%)	5 (25%)
	3	2 (20%)	14 (70%)
Ki-67	<20	10 (100%)	13 (65%)
	≥20	0	7 (35%)
Her-2/neu	Positive	1 (10%)	13 (65%)
	Negative	9 (90%)	7 (35%)
E-cadherin	Present	3 (30%)	20
	Loss	7 (70%)	0
IHC subtype	HR+/Her 2-	9 (90%)	10 (50%)
	HR+/Her2+	1 (10%)	7 (35%)
	HR-/Her2+	0	2 (10%)
	HR-/Her2-	0	1 (5%)

2.2. RNA isolation

Total RNA was obtained from all tumors and from 8 normal breast tissue samples (from control subjects). RNA was isolated from selected areas of OCT (Sakura, Alphen aan dern Rijn, The Netherlands) embedded frozen tissue using Trizol reagent (Invitrogen, Carlsbad, CA) and purified using the RNeasy mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The quality and concentration of RNA were assessed with a Nanodrop 2000 c spectrophotometer (Thermo Scientific, Wilmington, DE). RNA integrity was analyzed with the RNA 6000 Nano LAbChip Kit (Agilent Technologies, Boeblingen, Germany) following the manufacturer's protocol in an Agilent 2100 Bioanalyzer (Agilent Technologies, Boeblingen, Germany).

2.3. Gene-expression profile analysis

Gene-expression profile analysis was performed using the Affymetrix GeneChipHuman Gene 1.0 ST Array (Affymetrix, Santa Clara, CA). The procedure was performed following the manufacturer's instructions. The amplification and labeling processes were controlled using the GeneChip Eukaryotic Poly-A RNA Control Kit (Affymetrix) in which exogenous positive controls were spiked into the total RNA before complementary (DNA) synthesis. In all cases, 25 μ g of biotinylated cRNA preparation was fragmented and placed in hybridization cocktail containing biotinylated hybridization Controls, Affymetrix). Samples were hybridized onto the GeneChipHuman Gene 1.0 ST Array (Affymetrix) in a Hybridization Oven 640 (Affymetrix). Microarray-

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