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Lobular invasive carcinoma of the breast is a molecular entity distinct from luminal invasive ductal carcinoma

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ARTICLE INFO

Article history:
Received 29 January 2010
Received in revised form 1 April 2010
Accepted 7 May 2010
Available online 21 June 2010

Keywords:
Breast cancer
Invasive lobular carcinoma
Luminal carcinomas
Array-CGH
Gene expression profiling

ABSTRACT

In order to get insight into the molecular alterations of invasive lobular carcinoma (ILC), comparative genomic hybridisation array and transcriptomic analyses of a series of 62 oestrogens-positive (ER) invasive tumours [21 ILC and 41 invasive ductal carcinomas (IDC)] were performed. ILC and IDC shared highly recurrent regions of gains (1q12–q44^{+} in more than 60% of the cases, 16pter–p11.2^{+} in 45% and 63% of ILC and IDC, respectively) and losses (16q11.2–q24.2^{-} in 84% of ILC and 67.5% of IDC and 17pter–p12^{-} in 50% of ILC and IDC). However, ILC genomic signature was characterised by significantly more frequent losses of 13q21.33–q31.3 region (46.5%) and 22q11.23–q12.1 region (50%) whereas IDC showed significantly more frequent losses of 11q23.1–q23.2 region (in 44% of IDC). Nine different regions of high level amplifications were found in 38% of ILC (8/21 cases). Localised on chromosome 11 (11q13.2 region), the most frequent region of amplification encompassing the *CCND1* and *FGF3* genes was observed in five different ILC. Unsupervised hierarchical clustering of transcriptomic data showed that ILC and IDC clustered apart. Genes involved in cell adhesion, cell communication and trafficking, extra cellular matrix-interaction pathways or cell mobility contributed to this clustering. Despite these differences, the overall clinical outcome of ILC was identical to that of IDC. This molecular study highlights that lobular and oestrogens-positive ductal invasive carcinomas share common genomic alterations but that ILC present some specific molecular alterations. These molecular specificities should help with the identification of new therapeutic targets for ILC patients.

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doi:10.1016/j.ejca.2010.05.013

1. Introduction

Invasive lobular carcinomas (ILCs) of the breast represent 15% of invasive breast carcinomas.[1,2] ILCs are characterised by a proliferation of non-cohesive cells with a lower histological grade, a higher rate of hormone receptors positivity[1,2] and a lower rate of ERBB2 overexpression[3] than invasive ductal carcinomas (IDCs). Moreover, ILCs present a higher rate of multiple metastases[4] with a distinct and uncommon pattern of visceral metastatic involvement[2,5] and a high incidence of controlateral disease[6] when compared with IDC. Despite the biological differences between ILC and IDC, the overall outcome of ILC is not different from that of IDC and is accurately determined by histological grade.[2,3,7] In addition, ILCs are less responsive to chemotherapy in relation to their biological characteristics (i.e. low proliferation with high oestrogens and progesterone receptors expression)[8–11] and are rarely candidate for anti-HER2-targeted therapy.[3] Therefore, there is a real need for new therapeutic options in this disease.

The molecular mechanisms underlying the differences between ILC and IDC have not entirely been elucidated. Nonetheless, it has been demonstrated that the inactivation of the E-cadherin/catenin adhesion complex and related molecules, observed at the earliest stage of the lobular carcinogenesis,[12] could account for the non-cohesive pattern of the epithelial tumour-cells. Some previous ILC CGH and array-CGH analysis[13,14] have reported recurrent chromosomal aberrations, such as chromosomes 1q and 8q gains, losses of chromosomes 16q, 17q and 22. Meanwhile *HER2* amplification is reported to be infrequent in ILC,[15] and *CCND1* amplification is frequently found in ILC.[16,17] More recently, it has been proposed that *FGFR1* could be the chromosome 8p12 driver amplicon, observed in 15% of the 13 analysed ILC cases.[14]

It has recently been shown that the lobular and low grade ductal carcinoma *in situ* lesions, the well-known precursors of ILC and IDC, respectively, are frequently associated. This observation raised the hypothesis of a common initiating precursor cell for these entities.[18] However it remains unclear which molecular alterations, in addition to E-cadherin inactivation, might be involved in the distinct development of oestrogens receptor-positive (ER^+) IDC and ILC.

In that context, we perform a comprehensive comparison of 21 ILCs and 41 ER-positive (i.e. luminal) IDC in order to provide a detailed assessment of the biological phenotype and clinical behaviour of ILC.

We showed that ILC and IDC harboured common highly recurrent regions of gain and loss but with distinct genomic regions specifically associated with each group. Furthermore, a specific ILC transcriptomic signature was identified that provided a biological insight into the ILC specificities that could play a role in their metastatic pattern.

2. Material and methods

2.1. Tumour material

Twenty one classical ILCs and 41 luminal IDCs of the breast were retrospectively selected. These IDCs were defined as luminal according to their oestrogen receptor nuclear expression by immunohistochemistry and their transcriptomic characteristics (data not shown, available upon request). The selection was based on the availability of both a paraffin-embedded and a frozen sample among all the available frozen breast cancers samples, at the time of the study design and for which clinical follow-up was available. As gene expression and genomic profiles are different according to tumour grade, IDC and ILC samples were matched on grade. Surgery was the first step of treatment for all selected cases. All cases were reviewed according to the World Health Organization (WHO) classification criteria by two experienced breast pathologists (XSG and AVS). Experiments were performed in agreement with the Bioethic Law No. 2004-800 and the Ethic Charter from the National Institute of Cancer (INCa).

2.2. DNA and RNA extractions

All tumour samples contained more than 50% of cancer cells, as assessed by haematoxylin and eosin staining of histological sections adjacent to the samples used for the nucleic acids extraction. In this work, to avoid potential artefacts, which may due to DNA or RNA amplification of microdissected samples, we privileged the investigation of samples large enough to extract good quality nucleic acids without need for pre-amplification steps. DNAs and RNAs were extracted from frozen tumour samples by the caesium chloride protocol. The concentration and integrity/purity of each RNA sample were measured using RNA 6000 LabChip kit with the Agilent 2100 bioanalyzer (Agilent Technologies, Palo Alto, CA).

2.3. Immunohistochemistry

Immunostaining was performed on 4 μm tissue sections prepared from a representative sample of the tumour. After rehydration and antigenic retrieval in citrate buffer (10 mM, pH 6.1), the tissue sections were stained for oestrogen receptor (ER, clone 6F11, Novocastra, 1/200), progesterone receptor (PR, clone 1A6, Novocastra, 1/200) and E-cadherin (E-cadh, clone HECD1, Zymed Laboratories Inc., 1/50). Revelation of staining was performed using the Vectastain Elite ABC peroxidase mouse IgG kit (Vector Burlingame, CA) and diaminobenzidine (Dako A/S, Glostrup, Denmark) as chromogen. Positive and negative controls were included in each slide run. Cases were considered positive for ER and PR according to standardised guidelines using $\geqslant$10% of positive nuclei per carcinomatous duct. Cases were considered negative for E-cadherin when no tumour cell presented any strong membrane staining in the presence of accurate external/internal positive controls (surrounding normal glands).

2.4. Array-CGH

The BAC-array (one 250 kb clone per megabase on average) together with experimental procedures used for hybridisation and washing was as previously published.[19] All BACs and PACs were verified by end sequencing before spotting.

2.5. Analysis of array-CGH data

Data analysis was performed following the procedures previously published by our group. A region of gain or loss was considered as recurrent when it was observed in at least 40% of tumours. The comparison of altered regions between two groups was based on an odd ratio test. The hypothesis of no difference between the two groups was rejected when the Benjamini–Hochberg False Discovery Rate (FDR), calculated over the 3264 BAC loci analysed, was less than or equal to 0.05. A region was considered to be significantly different between the two groups when more than half of the loci of the region presented adjusted *p*-values less than 0.05. Amplicons were defined as BAC loci exhibiting a Cy5/Cy3 ratio greater than or equal to 2.

BACs known to harbour copy number variation in public databases (Data-base of Genomic variants, http://projects.tcag.ca/variation/cgi-bin/gbrowse) were excluded from the analysis.

2.6. Survival analysis

Statistical analyses were carried out using MedCalc software. The cumulative overall survival was calculated using the Kaplan–Meier method, and the log-rank test was used to analyse differences in the survival times. A *p*-value $\leqslant 0.05$ was considered significant.

2.7. Gene expression profile analysis

The DNA microarrays used in this study were the Human Genome U133A, containing almost 23,000 probe sets and B (Affymetrix, Santa Clara, CA). Microarray data were analysed as previously published.[20] Gene expression analysis for ILC and IDC was performed on the same Affymetrix platform but using two different scanners. In order to detect a possible scanner bias on gene expression levels, we performed an unsupervised clustering of ILC and IDC using the common 100 probesets present on both the U133A and U133B and corresponding to control genes whose expression was not associated to the tumour groups. The Fisher test, performed to evaluate the enrichment of top level groups by scanner type, was not significant ($p = 0.7$) demonstrating that the unsupervised clustering did not detect a scanner-dependent classification. We therefore concluded that the branches in the unsupervised clustering with ILC and IDC corresponded to true biological differences in gene expression levels.

Genomic/transcriptomic correlations were performed for each probeset in the IDC and the ILC groups following previously published procedures.[20] The correlation with Cy5/Cy3 ratios of the corresponding BAC clone was calculated using Pearson correlation tests. Then, we determined which genomic/transcriptomic correlations were observed in both lobular and ductal carcinomas and those specific for each group.

2.8. Quantitative real-time reverse transcription-PCR

Reverse transcription was performed with 1 μg of total RNA and 50 units of MultiScribe™ reverse transcriptase (High-Capacity cDNA Reverse Transcription kit, Applied Biosystems).

The analysed genes are listed in Fig. 3 and have been chosen among the group of downregulated genes in the unsupervised clustering and according to the importance of their biological putative role in carcinogenesis.

Assays-on-Demand for assessing expression level of the *CCND1* and other genes as well as the control TATA-binding protein (*TBP*) genes were obtained from Applied Biosystems. PCR was carried out in an ABI PRISM 7500 real-time thermal cycler using the Taqman master mix (Applied Biosystems).

3. Results

3.1. Patient and tumour characteristics (Table 1)

The clinico-pathological characteristics and the most recurrent genomic alterations (16q22.1 loss and *CCND1* amplification) of the 21 ILCs and 41 IDC cases are described in Table 1. Besides E-cadherin expression, none of the clinico-pathological parameters was significantly different between the two groups. For 7 IDC, E-cadherin staining was not assessed because tumour tissue was not available anymore on the representative paraffin-embedded block.

3.2. Genomic analysis (Tables 1–3 and Fig. 1)

We investigated genomic profiles of ILC and compared them to that of the IDC. In the ILC group, all but 1 sample exhibited altered profiles, indicating that the amount of stromal or other normal cells in the samples was not sufficient to mask copy number changes.

We found 9 different amplicons in 8 ILCs (38%, 8/21 cases) and 12 amplicons in 11 IDC (27%, 11/41 cases). One amplification, located on chromosome on 11q13.2–q13.4 region encompassing *Cyclin D1* and *FGF3* genes, was observed in both groups (24%, 5/21 ILC cases; 12%, 5/41 IDC cases; Fisher test $p = ns$). One ILC harboured an amplification of the 8p12 *FGFR1* region (5%, 1/21 cases). In IDC, three other recurrent regions of amplifications, found in at least 2 tumours, are located on chromosomes 8p12–p11.21, 11q13.4–q14.1 and 13q14.12–q14.2 (Table 2).

The most frequent gains observed in both ILC and IDC were on chromosomes 1q12–q44 (72.4% of ILC and 60% of IDC) and 16pter–p11.2 (45% of ILC and 62.7% of IDC) whereas the most frequent losses were on chromosomes 16q11.2–q24.2 (observed in 84.4% of ILC and 67.5% of IDC) and 17pter–p12 (50.1% of ILC and 48.8% of IDC).

Genomic alterations that significantly distinguished ILC from IDC were losses of 13q21.33–q31.3 region, observed in 46.5% of ILC and 22.2% of IDC and losses of 22q11.23–q12.1 region (50% of ILC and 27.1% of IDC). Losses of 11q23.1–q23.2 region distinguished IDC from ILC and were observed in 33.5% of ILC and 44.4% of IDC (*p*-value $\leqslant 0.05$) (Fig. 1 and Table 3).

The majority of ILC cases (14/21 cases, 67%) presented a complex genomic pattern encompassing 16q loss (86%, 18/21 cases) associated with 1q gain and others alterations in 15 cases out of 18 (83%).

More than 71% of ILC (15/21 cases) presented more than five chromosomal alterations (control IDC: 80%, 33/41 cases, $p = ns$). Moreover, ILC presented significantly fewer whole

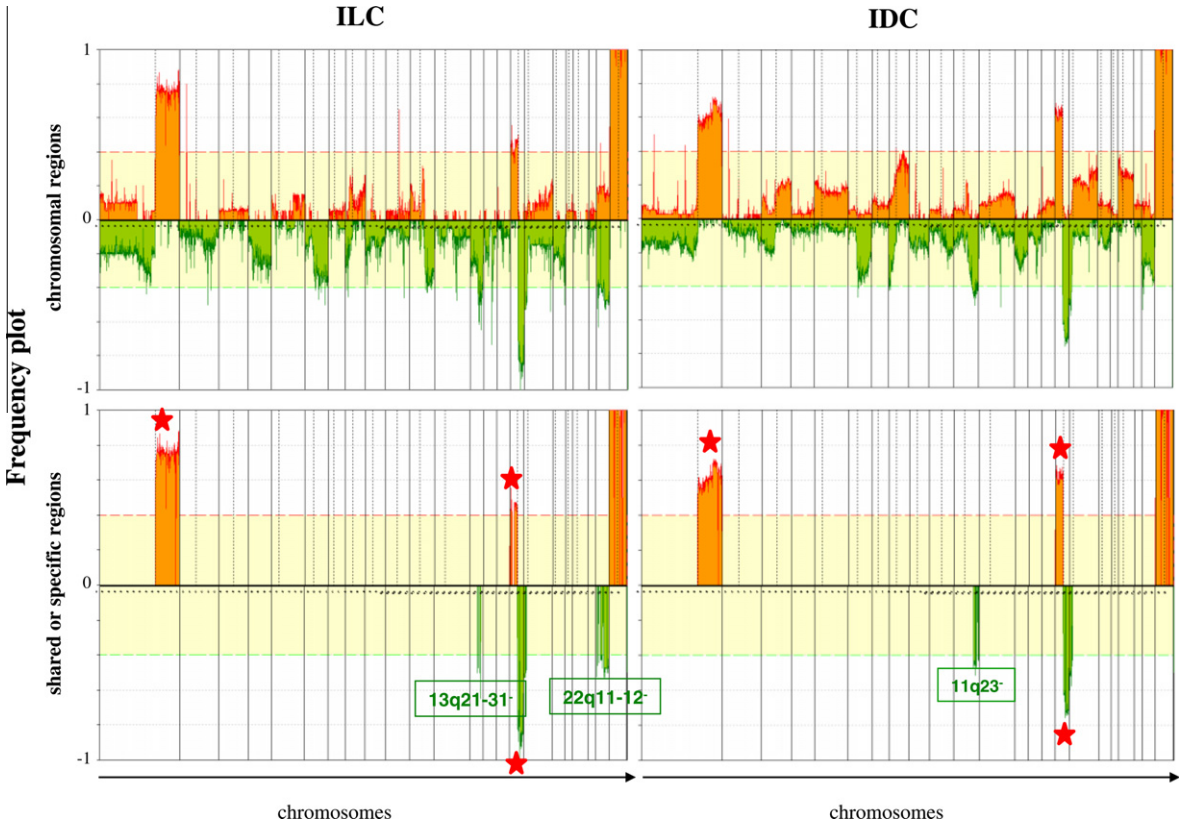

Fig. 1 – Frequency plot of gains and losses of ILC and IDC (top panels) and of regions shared (indicated by a star) by the ILC and the IDC or specific from ILC or IDC (indicated by their genomic localisation in green) (lower panels). The two top panels show the frequency of gains and losses in 21 ILC (upper left panel) and in 41 ER$^+$ IDC (upper right panel). Gains are indicated by orange bars ranging from 0 to 1 and losses, by green bars ranging from 0 to –1, from chromosome 1pter on the left to chromosome Xq on the right. Vertical lines indicate chromosome boundaries, and vertical hemi-dashed lines indicate centromere locations. The lower panels display shared or specific regions observed in two groups.

chromosomal arm gains than control IDC (47 and 150 gains observed in ILC and in IDC, respectively, $p = 0.0013$).

3.3. *Gene expression analysis (Figs. 2 and 3)*

We then investigated transcriptomic differences between ILC and ER$^+$ IDC. A subset of 57 samples (16 ILC and 41 IDC) for which good quality RNAs were available, was analysed on Affymetrix U133A (Fig. 2). A total of 445 probesets exhibited an interquartile range (IQR) greater than or equal to 2. These genes discriminated two major clusters of tumours. Cluster 1 was composed of IDC (35/41 cases). Cluster 2 encompassed two sub-branches (2-1 and 2-2, Fig. 2) according to their histological type. Cluster 2-1 was composed of 16/16 ILC and cluster 2-2 of 6/41 IDC cases.

As expected in ILC, *CDH1* gene was downregulated. Genes of the *IGFR1* pathway, some genes involved in chromatine maintenance such as *SMARCC2* and *UBN1* were also downregulated. Other genes participating in cell adhesion (*ADAM12* and *LOXL2*), cell communication and trafficking (*CLIC4*, *LYST* and *AP3D1*), receptor or extra cellular matrix-interaction pathways (*MMP11*, *COL11A1*, *COL10A1*, *PLAU* and *RHOBTB3*), cell shape and motility (*TWF1* and *ACTR2*) also contributed to this clustering.

The most of these genes under-expressions observed with the Affymetrix analysis were further confirmed by RT-qPCR (Fig. 3).

To assess if differences existed within ILC according to grade, we performed an unsupervised clustering with the 445 probesets with an IQR $\geqslant 2$ among the 16 ILC and did not show any clear group according to grade (Supplementary Fig. 1).

3.4. *Genomic and transcriptomic correlations (Supplementary Table 1 and Fig. 4)*

In order to determine the impact of genomic changes on gene expression levels, we combined the analyses of transcriptomic (Affymetrix signals) and genomic data (array-CGH ratios) for the 16 ILCs and the 41 IDCs analysed with both approaches. We observed that the expression level of 78 genes was significantly correlated with the ratio of their corresponding BAC locus in the ILC group (Supplementary Table 1). Correlations corresponding to amplifications were located on chromosomes 8p12, from 36.8 to 38.4 Mb

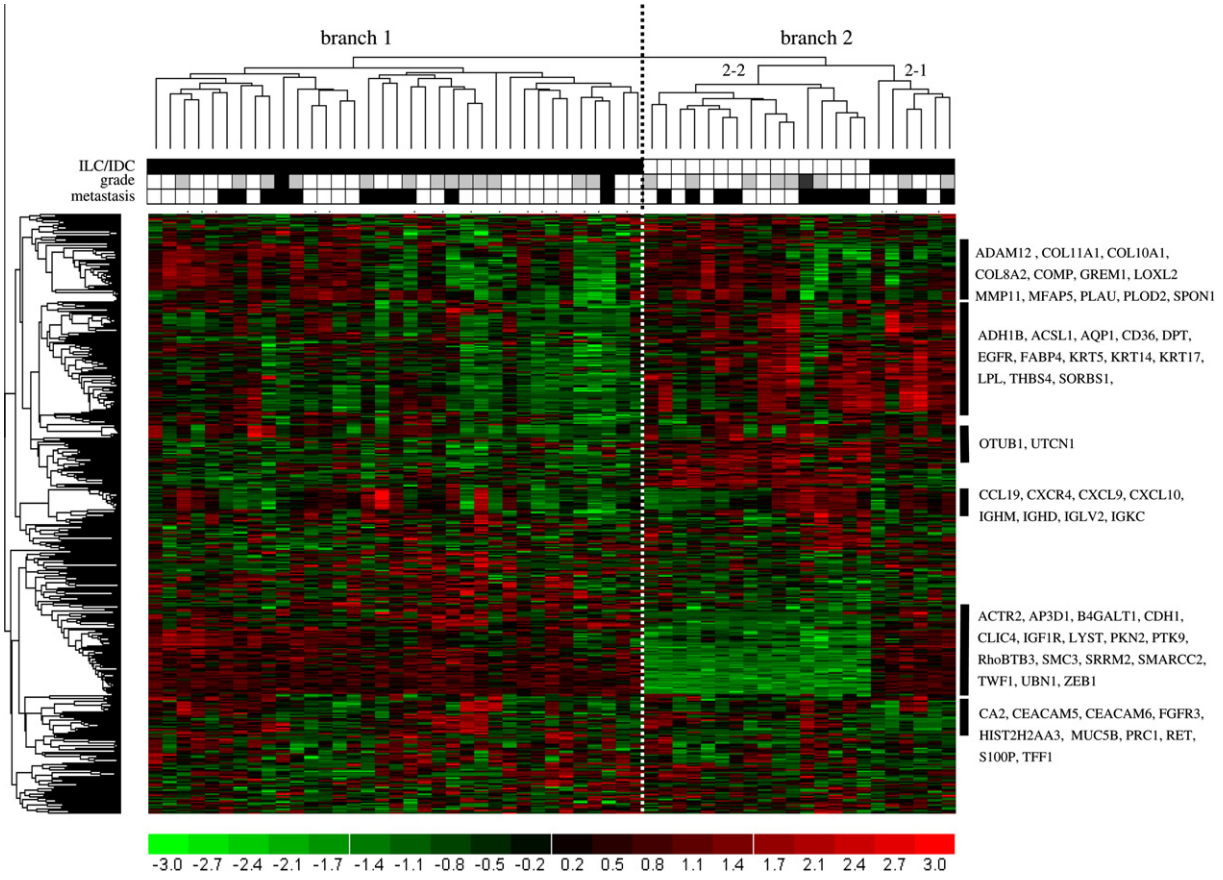

Fig. 2 – Unsupervised hierarchical clustering of 16 ILC and 41 ER$^+$ IDC tumours. We have used intrinsically variable gene expression of 445 probesets (interquartile range $\geqslant$2). Each column represents a different tumour, and each row represents one of 445 genes used for this unsupervised clustering. Histological status (ILC: white; IDC: black), grade (grade I: white; grade II: grey; grade III: black) and the presence of metastasis/other cancers (black) are indicated.

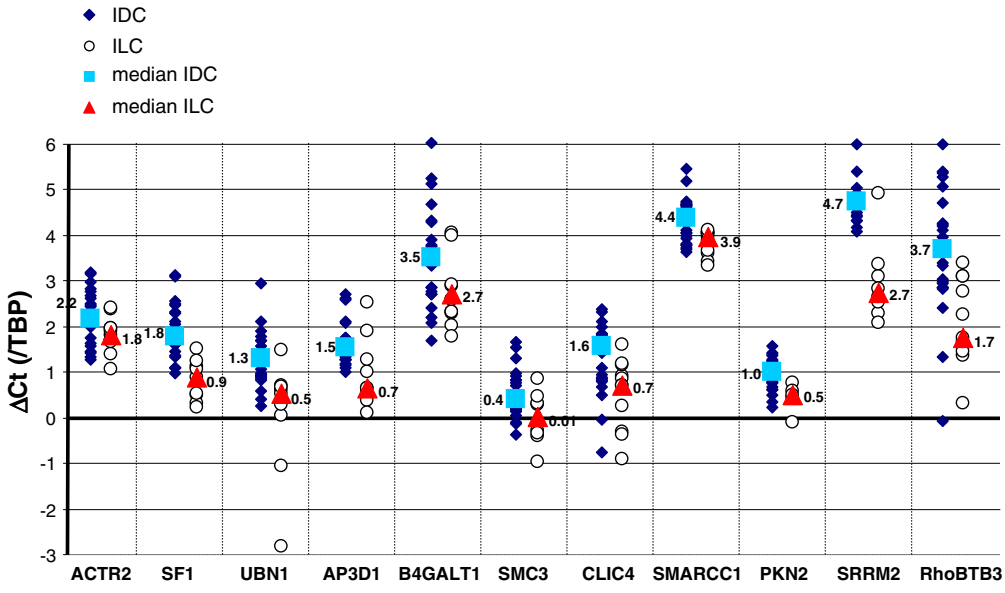

Fig. 3 – Quantitative PCR analysis of under-expressed genes compared to the *TATA-binding protein (TBP)* gene. Results are indicated as ΔCt values. The values for IDC are represented by a dark blue full diamond, for ILC by a dark blue empty circle. The median values for each group are indicated by a clear blue square (IDC) and a red full triangle for ILC.

Table 1 – Clinico-pathological characteristics and summary of the main genomic alterations found in the 21 ILC and 41 IDC cases.

Parameters	ILC ($n = 21$) n (%)	IDC ($n = 41$) n (%)	p-Value
Age (median; years)	59	56	ns[b]
Grade			
I	12 (57)	24 (58)	ns[c]
II	8 (38)	15 (36.5)	
III	1 (5)	2 (5)	
Node status			
–	14 (70)	25 (61)	ns[c]
+			
1–3	2 (10)	0	
>3	4 (20)	16 (39)	
ND	1	–	
ER			
+	21 (100)	41 (100)	ns[c]
–	0 (0)	0	
PR			
+	17 (81)	41 (100)	ns[c]
–	4 (19)	0	
E-cadherin			
+	1 (5)	33 (97)	4.7×10^{-14}
–	20 (95)	1 (3)	
ND	–	7	
Genomic data			
16q22.1 loss	18 (86)	30 (73)	ns[b]
CCND1 amplifier[a]	5 (24)	5 (12)	ns[b]

Cases were considered positive for ER and PR using ⩾10% of positive nuclei per carcinomatous duct. Cases were considered positive for E-cadherin when a membrane staining was observed in >10% of tumour-cells with an internal positive control (normal glands).

ER: oestrogen receptor; PR: progesterone receptor; E-cadh: E-cadherin; ND: not determined.

[a] Cy5/Cy3 ratio greater or equal than 2.

[b] Wilcoxon test.

[c] Fisher exact test.

(*WHSC1L1*), and 11q13.1–q14.1, from 65.3 to 78.4 Mb (*CCND1*, which overexpression was further confirmed by RT-qPCR, Fig. 4).

The main correlations due to low copy number gains or losses were observed on chromosomes 1q21.2–q43 (in the region of gain located from 149.2 to 234.7 Mb), 16p13.3 (gain, from 3.3 to 3.6 Mb), 16q12.1–q22.1 (loss, from 46 to 67.1 Mb) and 17p13.3–p13.2 (loss, from 0.8 to 5.8 Mb). Some other genes located in the region of gain 11q13, below the *CCND1* amplicon, were found to be correlated to their expression status, including genes such as *FADD* and *PAK1*.

In the IDC group (Supplementary Table 1), the level of expression of 173 genes was significantly correlated with the ratio of their corresponding BAC locus, either in regions of amplifications on the chromosomes 6q25.3, from 155.6 to 155.7 Mb (encompassing the *TFB1M* gene), 11q13–q14 (encompassing the *CCND1*, *PAK1* and *ALG8* genes) or in regions of gains (chromosomes 1q, 8q) and losses on chromosomes 8p encompassing *MTUS1* and *CNOT7* and on chromosome 16q. We next compared the correlations that were common to ILC and IDC and found eight genes located on chromosomes 1, 8 and 11 in common regions of gains and amplifications.

3.5. *Association with outcome (Fig. 5)*

Despite genomic and transcriptomic differences between ILC and IDC, patients with ILC tumours harboured at 10 years, the same overall survival than patients with luminal IDC tumours (76% for lobular versus 73% for ductal, $p = ns$; median follow-up of 142 [95–171] and 131 [18–167] months for ILC and IDC, respectively) and the same recurrence-free survival (49% for lobular versus 65% for ductal, $p = ns$; median follow-up of 120 [95–171] and 131 [55–167] months for ILC and IDC, respectively).

4. Discussion

In this analysis, we show that ILCs are a distinct molecular entity from luminal ductal carcinomas.

The majority of ILC and IDC from this series presented complex genomic profiles with copy-number alterations with no simple profile (i.e. tumours harbouring 1q gains associated with 16q losses as unique alterations) and rare recurrent amplification. Notably, highly recurrent regions of gains and losses were shared by two groups such as 1q and 16p gains, 16q and 17p losses. These alterations have been frequently observed in previous studies comparing ductal and ILC[14,21] and give biological credit to the hypothesis of a common precursor initiating cancer cell, a hypothesis based on morphological data showing the co-existence of ductal and lobular *in situ* lesions in more than 60% of the cases.[18] The different regions of losses and gains observed between ILC and IDC not only refined the genomic analysis reported previously[13,14,21,22] but also showed new regions of interest in ILC. Indeed, ILCs were characterised by 13q21.33–q31.3 and 22q11.23–q12.1 losses that were significantly less often observed in IDC. Interestingly, some genes located in the lost regions, in the 13q region *KLF5* that promotes cell proliferation and in the 22q region *ADORA 2A* and *CYTSA*, two genes involved in cell cycle or cytokinesis may contribute, when lost, to the low proliferation rate that characterises ILC.

In the present series, only one case harboured an amplification of the 8p12 region (1/21, 5%). This observation contrasts with the 46% (6/13 cases) rate of amplification observed in a previous published series.[14] In our series, the genes that demonstrated an overexpression within this region of amplification were rather *WHSC1L1* than *FGFR1* although it is rather impossible to draw any definite conclusion with only one case harbouring the region of amplification. Nevertheless, this observation is in agreement with our recently published data regarding the putative 8p12 amplicon driver in ductal carcinomas.[23] The role of *FGFR1* in ILC should be further confirmed in other series.

The most recurrent amplification observed in this study, in the two groups, encompassed the *CCND1* region on the 11q13.2 chromosome. It has recently been shown that *CCND1* amplification was observed in 29% of a series of 93 ER-positive

Table 2 – List of amplifications found in the ILC and IDC groups.

Chr	Start (Mb)	End (Mb)	Cytoband	Genes	Max ratio	ILC ($n = 21$)	IDC ($n = 41$)
5	162.8	164.8	5q34		2.2	0	1
6	154.2	157.7	6q25.2–6q25.3	*TFB1M*	2.5	0	1
8	36.8	40	8p12–8p11.21	*WHSC1L1*, *PPAPDC1B*, *FGFR1*	3.9	1	2
10	76.5	85.2	10q22.2–q23.1		3.9	1	0
11	58.8	61.5	11q12.1–q12.3		2.5	0	1
11	68.6	71.2	11q13.2–q13.4	*CCND1*, *FGF3*	4.5	5	5
11	71.5	75.5	11q13.4–q13.5	*OSBP*, *STX3*, *MRPL16*, *TCN1*, *MS4A6A*, *MS4A12*, *CCDC86*, *PRPF19*, *TMEM109*, *TMEM132A*, *VPS37C*, *DDB1*, *DAK*, *C11orf10*, *FEN1*, *FADS1*, *FADS2*, *FADS3*	2.7	1	0
11	74.6	80	11q13.4–q14.1	*SLCO2B1*, *ARRB1*, *RPS3*, *WNT11*, *LRRC32*, *OMP*, *MYO7A*, *RSF1*, *C11orf67*, *NDUFC2*, *ALG8*, *GAB2*	4.2	1	4
13	44.8	46.2	13q14.12–q14.2	*NUFIP1*, *GTF2F2*, *TPT1*, *SLC25A30*, *CPB2*, *LCP1*, *C13orf18*, *LRCH1*	2.4	0	2
14	74.9	75	14q24.3	*BATF*	2.9	0	1
15	94.9	100	15q26.2–26.3	*IGF1R*, *DMN*, *MEF2A*, *CHSY1*, *SNRPA1*, *PCSK6*, *TM2D3*	4.6	0	1
20	22.8	29.7	20p11.21–q11.21	*THBD*, *CD93*, *NXT1*, *CST3*, *CST4*, *C20orf39*, *ENTPD6*, *PYGB*, *GINS1*, *ZNF337*	2.6	0	1
20	47.3	48.6	20q13.13		4.3	1	0
20	49	51.6	20q13.13–q13.2	*ATP9*	3	1	1
20	54.5	57.4	20q13.31–13.32		2.4	1	0
X	150.9	152.8	Xq28	*MAGEA6*, *MAGEA3*, *NSDHL*, *BGN*, *PLXNB3*, *SRPK3*, *IDH3G*	2.7	1	1

Table 3 – List of representative regions found in ILC and control IDC groups.

Chr	Cytoband	Alteration	Start (Mb)	End (Mb)	ILC (%)	IDC (%)	Signif.
Shared regions							
1	q12–q44	Gain	128.20	244.25	72.4	60.0	
16	pter–p11.2	Gain	0.00	33.54	45.0	62.7	
16	q11.2–q24.2	Loss	45.06	87.35	84.4	67.5	
17	pter–p12	Loss	0.00	15.85	50.1	48.8	
Regions found more specifically in ILC							
13	q21.33–q31.3	Loss	71.30	90.92	46.5	22.2	*
22	q11.23–q12.1	Loss	23.00	24.37	50.0	27.1	*
Region found more specifically in IDC							
11	q23.1–q23.2	Loss	112.07	132.48	33.5	44.4	*

tumours.[24] Here we show that it is also recurrent among ILC and that *CCND1* amplification drives its overexpression. This recurrent amplification may open perspectives of targeted therapies using *EglN2* hydroxylase inhibitors, as it has been recently shown that EglN2 hydroxylase could control breast carcinogenesis through *CCND1* interactions.[25]

Gene expression unsupervised analysis in our study clearly demonstrated that ILC and IDC clustered apart. One cluster was entirely composed of IDC and the second formed by two sub-clusters, one being composed by all ILC cases. Previous transcriptomic analyses comparing ILC with IDC[26–29] have found genes differentially expressed between the two entities and, as expected, the *E-cadherin* gene was found under-expressed in all studies despite technical or sample selection differences such as grade of the tumour samples, or platforms and statistical analysis used. Specific downregulation of *E-cadherin* gene expression with small interference RNA experiments in an E-cadherin-positive cell-line would be helpful to assess the consequences of *E-cadherin* mutation/downregulation in gene expression modification and its oncogenic properties. Interestingly enough, even the E-cadherin-positive ILC case did not cluster with the IDC. This observation suggests that beside E-cadherin downregulation in ILC, the specific biology of these carcinomas relies on a larger spectrum of molecular alterations.

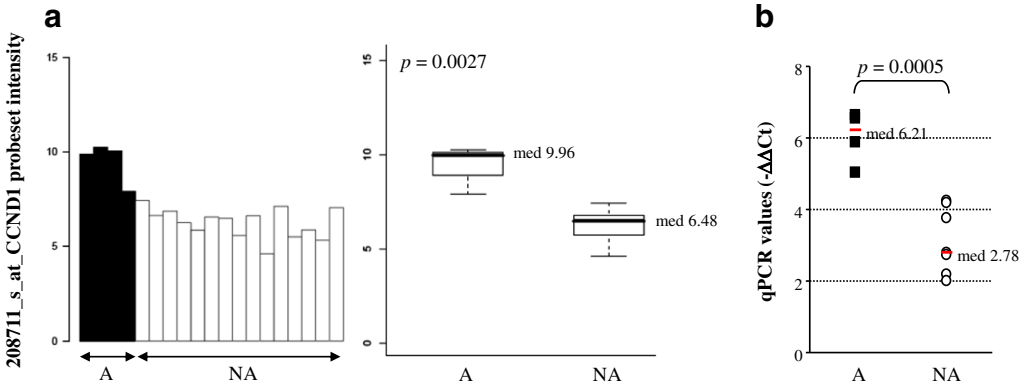

Fig. 4 – Correlation of amplification and overexpression of *CCND1* in ILC tumours. Bar-plot and box-plot (a) of Affymetrix log 2 ratios of the 208711_s_at probeset (*CCND1* gene) in amplified (A) and non-amplified (NA) ILC. Quantitative PCR analysis (b) of *CCND1* gene compared to the *TATA-binding protein* (*TBP*) gene. The results are indicated as −ΔΔCt values. This analysis was performed on a subset of 19 tumours (4 *CCND1*-amplified and 15 *CCND1*-non-amplified ILC). Median signal (med) is indicated.

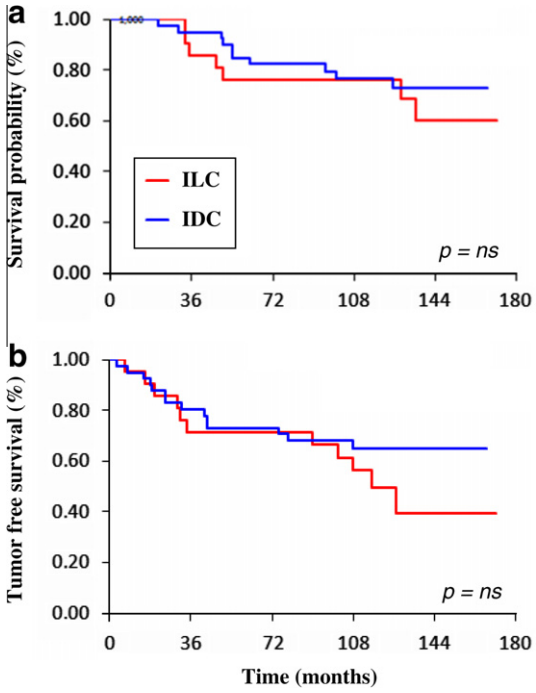

Fig. 5 – Survival curves for ILC and IDC. Upper panel (a) represents the overall survival. Lower panel (b) represents the disease-free survival curve.

In our analysis, genes belonging to the *IGF1R* pathway are also downregulated. The Gene Ontology analysis showed that genes differentially expressed between ILC and IDC are involved in cell adhesion such as *ADAM 12*, cell communication and trafficking (*LYST*, *CLIC4*, *AP3D1*), extra cellular matrix-interaction pathways or cell mobility (Rho GTPases, *ACTR2*). Some genes involved in chromatine maintenance or RNA metabolism (*SMARCC2*, *SMC3*, *UBN1*, *SRRM2*) were also downregulated in ILC and confirmed by RT-qPCR. A recent study highlighted also that actin cytoskeleton remodelling genes involved in cell motility and cell adhesion genes were also modified in ILC compared to IDC.[29] Altogether, these transcriptomic data could in part explain the ILC wide metastatic spreading through the modification of the expression of genes involved in extracellular matrix interactions, cell motility[4] and the ILC low proliferation rate and genes involved in chromatin maintenance and RNA metabolism.

As previously shown by Weigelt et al.,[29] we did not observe any clustering within the ILC tumours according to grade, demonstrating that ILC tumours, whatever may be their grade, share common transcriptomic profiles.

Genomic/transcriptomic correlations highlighted candidate genes in regions of gains and amplification. *CCND1* and *FADD* were the candidate genes in the 11q13 regions of amplification, and *PAK1* in the region of gain on the same chromosome arm. *FADD* gene encodes for a protein that mediates apoptotic signals. PAK1 protein regulates cell motility and morphology. This protein family links RhoGTPases to cytoskeleton re-organisation and nuclear signalling. Though, when *FADD* and *PAK1* are over-expressed through amplification/gains, these two gene products could participate to the low proliferation level of ILC via the apoptosis activation and the specific motility of ILC cells.

In conclusion, we demonstrated that ILC represents a genomic and a transcriptomic distinct entity from luminal (ER^+ grade I and II) IDC and therefore ILC should not be considered as luminal carcinoma. To identify lobular tumours as a specific entity should help to identify specific therapeutic strategies in the future.

Competing interests

The authors declare that they have no competing financial interests.

Author's contributions

All authors have read and approved the final manuscript.

Conflict of interest statement

The authors have no conflict of interest to declare.

Acknowledgements

We thank Ingrid Lebigot and her team from the Institut Curie's tumour bank.

This work was supported by grants from the INSERM, the Institut Curie (Programme Incitatif et Coopératif). Construction of the BAC-array was supported by grants from the 'Carte d'Identité des Tumeurs' (CIT) program of the Ligue Nationale Contre le Cancer. Affymetrix analyses were supported by grants from AstraZeneca. Dr. Anne Vincent-Salomon was supported by an 'Interface INSERM' grant. Dr. Manuel J Rodrigues was supported by a Fondation Pour la Recherche Médicale grant.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejca.2010.05.013.

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