Multiplatform molecular profiling of invasive lobular breast cancer

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Abstract #123

Background: Invasive lobular breast cancer (ILC) is the second most common subtype of invasive breast cancer accounting for 10% of breast cancer diagnosis. ILC has particular histological and clinical characteristics and a distinct response to therapy. Characterizing the molecular alterations in ILC may lead to an improved understanding of its biology and provide new therapeutic options. The purpose of this study is to describe the molecular profile of ILC and compare it to the one of invasive ductal cancer (IDC).

Methods: Three-hundred and thirty-nine pure ILC specimens profiled from January 2012 – November 2015 were evaluated (Caris Life Sciences, Phoenix, AZ). Multiplatform profiling consisted of gene sequencing (next generation sequencing [NGS]), gene amplification (CISH or FISH), and protein expression (immunohistochemistry [IHC]). Molecular characteristics of estrogen receptor (ER) positive and human epidermal growth receptor factor 2 (HER2) negative pure ILC (n= 236) and IDC (n=286) were compared.

Results: 198 (58.4%) pure ILC specimens were from the primary site, two (0.6%) were breast recurrences, and 139 (41.0%) were lymph node or distant metastases. By IHC, ER expression was present in 87.7% (277/316), progesterone receptor in 59.6% (198/313), HER2 in 3.5% (11/313), androgen receptor in 87% (262/301), PD-L1 in 8.1% (12/148) and PTEN in 63.3% (198/313). Amplifications were detected in *MYC* (7.7%, 2/26), *EGFR* (8.3%, 2/24), *ERBB2* (4.5%, 13/290) and *TOP2A* (1.3%, 3/236). Mutations were detected in *AKT1* (4.7%, 9/191), *ATM* (3.7%, 7/190), *BRCA1* (4.2%, 4/96), BRCA2 (9.5%, 9/95), *ERBB2* (7.5%, 14/186), *PIK3CA* (54.5%, 103/189), *PTEN* (7.9%, 15/189), and *TP53* (13.4%, 25/186). A comparison of ER-positive/HER2-negative invasive lobular and ductal carcinomas revealed significant differences in AR expression (89.7% vs. 79.6%, p = 0.0022), *ERBB2* (8.2% vs. 2.1%, p = 0.0079), and *TP53* (10.3% vs. 31.8%, 0.0001).

Conclusion: Multiplatform testing of this large series of ILC reveals recurrent alterations and a distinct molecular profile when compared to IDC. These support the definition of ILC as biologically distinct entity. High AR expression and high rates of dysregulation along the PIK3CA/AKT/mTOR pathway are consistent with recent reports in the literature.

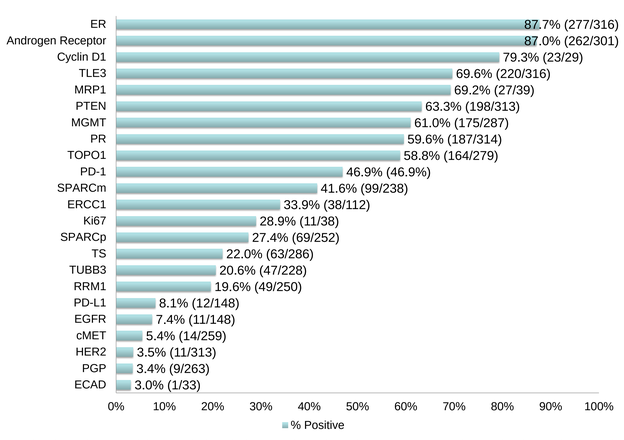
Methods

Three-hundred and thirty-nine pure ILC specimens profiled from January 2012 – November 2015 were evaluated at a CLIA-certified, centralized laboratory (Caris Life Sciences, Phoenix, AZ). Diagnosis of every specimen was confirmed by a staff pathologist. Multiplatform profiling consisted of gene sequencing (next generation sequencing [NGS] using Illumina MiSeq or NextSeq), gene amplification (chromogenic [CISH] or fluorescence [FISH] *in situ* hybridization), and protein expression (immunohistochemistry [IHC]). After calculating the overall distributions, a comparison study was done on hormone receptor (ER/PR) positive and negative ILC. Molecular characteristics of estrogen receptor (ER) positive and human epidermal growth receptor factor 2 (HER2) negative pure ILC (n= 236) and IDC (n=286) were also compared.

Results

Background on Cohort			
Age		Specimen Location	
Median Age	59	Primary	59.0% (200)
Age Range	29 - 87	Metastatic	41.0% (139)

Figure 1 – Demographics of ILC cohort. All ILC patients analyzed were female. Shown above is information on age and specimen location from outside ordering physician. Of those with metastatic disease, 20.9% (29/139) were from lymph nodes. Staging information and treatment information was not available.



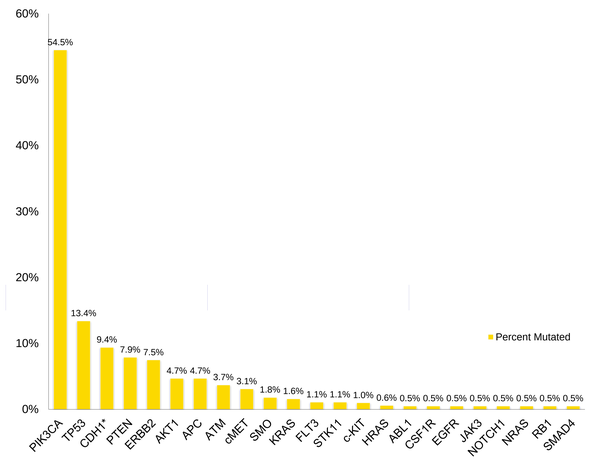
Thresholds for determining protein (positive) expression or lack of expression and antibody utilized were determined based on literature review.

Figure 2 – Immunohistochemistry distribution in pure lobular breast carcinoma. Several potentially theranostic biomarkers were tested. Variability in the number of tests performed (i.e. denominator) is secondary to physician request.

Biomarker	Number Amplified	Total Number	Percent Amplified
MET	0	199	0.0%
MYC	2	26	7.7%
EGFR	2	24	8.3%
ERBB2 (HER2)	13	290	4.5%
TOP2A	3	236	1.3%

Table 1 – Gene amplification using In situ hybridization (ISH) using FISH or CISH.

Results (cont.)



* *CDH1* is being retested using NextSeq NGS testing.

Figure 3 – Sequencing (by NGS) distribution in pure lobular breast carcinoma using targeted NGS platform. High rates of *PI3KCA, PTEN*, and *AKT1* are observed in this cohort. *ERBB2* mutations were observed in patients who had no amplification by IHC or FISH. Various other genetic aberrations were detected, albeit at low amounts but consistent with what is known in advanced breast cancer.

Biomarker	Platform	Hormone Receptor Positive	Hormone Receptor Negative	P-value
AR	IHC	89.8%	68.4%	0.001
TLE3	IHC	73.4%	43.2%	0.0004
TS	IHC	19.7%	36.8%	0.0328
TP53	NGS	9.9%	36.0%	0.0018

Table 2 – Comparison of hormone receptor positive versus negative ILC. With hormone receptor (HR) positive status defined as expression in ER and/or PR (defined as 1+ staining intensity in 1% or more cells), a comparison was performed between the two ILC subtypes. AR and TLE3 were higher in HR-positive ILC while TS expression and *TP53* gene mutations were significantly higher in HR-negative ILC.

Results (cont.)

Biomarker	Platform	ILC	IDC	p-value
AR	IHC	89.7%	79.6%	0.0022
*CDH1**	NGS	10.1%	0.0%	0.0001
cMET	IHC	6.7%	0.4%	0.0001
TS	IHC	19.2%	36.2%	0.0001
TUBB3	IHC	21.5%	35.5%	0.0019
ERBB2	NGS	8.2%	2.1%	0.0079
TP53	NGS	10.3%	31.8%	0.0001

Table 3 – Comparison of ER-positive/HER2-negative invasive lobular versus invasive ductal carcinoma cohort. Differences were noted between pure histologic subtypes of lobular (n=236) and ductal carcinoma (n=286). Although not significant, a trend was found in PD-L1 expression between ILC and IDC (10.6% versus 5.0%, respectively, p= 0.0609). **CDH1* is currently being retested using NextSeq NGS testing.

Conclusion

Comparisons between ILC and IDC show differential expression patterns and distinct disease entities.

Multiplatform testing reveals multiple potential targets in invasive lobular breast carcinoma. More comprehensive sequencing assays like NextSeq NGS are needed to detect CDH1 mutations in lobular breast carcinoma.

High rates of AR expression and dysregulation of the PI3KCA/AKT/mTOR pathway may be potential targets in future clinical trials. *ERBB2* mutations may be an additional target for treatment in patients without HER2 overexpression or amplification using FDA-approved testing.

Future molecular studies should continue to clarify the biology of ILC and identify potential targets for therapy.

References

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