Human Cancer Biology

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Relapsed Classic E-Cadherin (*CDH1*)–Mutated Invasive Lobular Breast Cancer Shows a High Frequency of *HER2* (*ERBB2*) Gene Mutations

Jeffrey S. Ross[1,2], Kai Wang[2], Christine E. Sheehan[1], Ann B. Boguniewicz[1], Geoff Otto[2], Sean R. Downing[2], James Sun[2], Jie He[2], John A. Curran[2], Siraj Ali[2], Roman Yelensky[2], Doron Lipson[2], Gary Palmer[2], Vincent A. Miller[2], and Philip J. Stephens[2]

Abstract

Purpose: We queried whether comprehensive genomic profiling using a next-generation sequencing–based assay could identify novel and unanticipated targets of therapy for patients with relapsed invasive lobular carcinoma (ILC).

Experimental Design: DNA sequencing (Illumina HiSeq 2000) was conducted for 3,320 exons of 182 cancer-related genes and 37 introns of 14 genes frequently rearranged in cancer on indexed, adaptor-ligated, hybridization-captured libraries using DNA isolated from formalin-fixed paraffin-embedded sections from 22 histologically verified ILC.

Results: A total of 75 genomic alterations were identified with an average of 3.4 alterations per tumor (range, 1–6), of which 35 were actionable for an average of 1.59 actionable alterations per patient (range, 0–3). Nineteen of 22 (86%) of the ILC samples harbored at least one actionable alteration. Six (27%) cases featured alterations in *ERRB2* including 4 (18%) with *ERBB2* mutation, 1 (5%) with an *ERBB2* gene fusion, and 1 (5%) with an *ERBB2* copy number gain (amplification). The enrichment of *ERBB2* mutations/fusion in *CDH1*-mutated ILC (5 of 22, 23%) compared with the 5 *ERBB2* mutations in a series of 286 non-*CDH1*-mutated breast cancers from which the ILC cases were obtained (5 of 286, 2%) was significant ($P = 0.0006$).

Conclusions: Comprehensive genomic profiling of relapsed *CDH1*-mutated ILC revealed actionable genomic alterations in 86% of cases, featured a high incidence of *ERBB2* alterations, and can reveal actionable alterations that can inform treatment decisions for patients with ILC. *Clin Cancer Res; 19(10); 2668–76. ©2013 AACR.*

Introduction

Substantial progress has been made over the past 3 decades in our understanding of the epidemiology, clinical course, and basic biology of breast cancer and the integration of routine and molecular biomarkers into patient management. Modern techniques designed to detect the disease at an earlier stage combined with new methods of determining risk assessment and more optimized combined modality treatment that have enhanced our ability to manage, and in some cases, achieve a cure for the disease. The morphologic classification of invasive carcinomas of the breast has had a significant role in managing individual patients but has not been a major driver of therapy development and clinical trials. In addition, the application of standard slide-based biomarker status for the estrogen receptor (ER), progesterone receptor (PR), and *HER2* gene copy number (FISH) and/or protein expression [immunohistochemistry (IHC)] currently play a more significant role in clinical trial design than routine histologic subtyping (1). This is also true when the mRNA expression profile-based molecular portraits classification of invasive breast cancer including the luminal A, luminal B, normal, HER2-positive, and basal-like (typically "triple negative") nomenclature has been used to classify the tumors (2). In drug development, strategies have approached invasive ductal and invasive lobular breast carcinomas as essentially the same disease (3–6).

Most published studies have found that the overall prognosis for invasive lobular carcinoma (ILC) of the breast is similar to that of invasive ductal carcinoma (IDC; refs. 7–9).

Authors' Affiliations: [1]Department of Pathology and Laboratory Medicine, Albany Medical College, Albany, New York; and [2]Foundation Medicine, Inc., Cambridge, Massachusetts

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Corresponding Author: Jeffrey S. Ross, Department of Pathology, Albany Medical College, Mail Code 81, 47 New Scotland Avenue, Albany, NY 12208. Phone: 518-262-5461; Fax: 518-262-8092; E-mail: rossj@mail.amc.edu

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Translational Relevance

This study describes the application of a novel comprehensive next-generation sequencing–based diagnostic test on active clinical cases of relapsed lobular breast cancer and how the results of the analysis can drive the selection of treatment for these patients by discovering unanticipated therapeutic targets. Use of this approach could facilitate accrual to clinical trials of small molecules and antibodies in patients with breast cancer with *ERBB2* mutations.

However, most of these outcome studies have excluded the less common, but more aggressive pleomorphic variant of ILC in the clinical outcome data analysis (10), which could overestimate the prognosis of patients with ILC. In addition, although ER-positive ILC of the breast generally has a favorable prognosis, relapsed lobular breast cancer originally treated only with hormonal therapy may, on occasion, follow an aggressive clinical course (7–9). All of these observations favor the study of ILC as a separate entity from the more frequent invasive ductal breast carcinoma.

Recent studies suggest that lobular breast cancer, which makes up 10% of all invasive breast cancers and approximately 20,000 new cases in the United States each year, may be well characterized by a distinct genotype featuring a mutation in *CDH1* that encodes the E-cadherin protein product, in contrast with the far more frequent ductal breast cancer, which typically has an unaltered *CDH1* (11–15). In this study, restricted to 22 *CDH1*-mutated ILC from patients with relapsed disease, we queried whether a next-generation sequencing (NGS)–based assay could identify novel and unanticipated targets of therapy for these patients with relapsed, metastatic, and therapy-resistant disease.

Materials and Methods

We reviewed the records of a series of 308 invasive breast cancers submitted for NGS-based diagnostic testing at Foundation Medicine, Inc. and identified 22 *CDH1*-mutated ILCs all of which had relapsed after primary surgical and one or more systemic treatment approaches. NGS was conducted on hybridization-captured, adaptor ligation–based libraries using DNA extracted from 4 formalin-fixed paraffin-embedded sections cut at 10 μm. The pathologic diagnosis of each case was confirmed on routine hematoxylin and eosin (H&E)–stained slides and all samples forwarded for DNA extraction contained a minimum of 20% tumor cells. DNA sequencing was conducted for 3,320 exons of 182 cancer-related genes and 37 introns of 14 genes frequently rearranged in cancer (1.14 million total base pairs) on indexed, adaptor-ligated, hybridization-captured libraries (Agilent SureSelect Custom Kit) and fully sequenced using 49-bp paired reads on the Illumina HiSeq 2000 to at an average depth of 877× and evaluated for genomic alterations including base substitutions, insertions, deletions, copy number alterations (CNA; amplifications and homozygous deletions), and select gene fusions/rearrangements as previously described (16). To maximize mutation-detection accuracy (sensitivity and specificity) in impure clinical specimens, the test was optimized and validated to detect base substitutions at a 5% or more mutant allele frequency (MAF) and indels with a 10% or more MAF with 99% or more accuracy. To validate base substitution detection, 2 pools of 10 normal cell lines were used each containing a total of 2,057 known base substitutions representing a broad range of allele frequencies. We compared the alterations detected with those expected from base substitutions present in individual cell line constituents as previously described (17–20). To validate indel detection, 28 tumor cell lines were used containing a total of 47 known somatic indel alterations in 22 genes to generate 41 pools of 2 to 10 cell lines each creating a test set of 227 indels spanning a wide range of allele frequencies and indel lengths (17–20). To validate CNA detection, 7 tumor cell lines bearing 19 focal gene amplifications (6–15 copies, 15 genes) and 9 homozygous gene deletions (6 genes) were pooled with their matched normal cell lines (thereby maintaining consistent genotypes) in 5 ratios ranging from low to high tumor content (20%–75%), creating a test set of 210 CNAs (17–20).

CNAs had a validated accuracy of more than 95%. Actionable genomic alterations were defined as those identifying anticancer drugs in the market or available in registered clinical trials, the mechanism of action of which was predicted to be relevant based on the genomic alteration identified. Local site permissions to use clinical samples were used for this study.

Results

The mean age of the female patients in this study was 56 years (range, 44–74). Tissue samples obtained from 22 cases of ILC originate from 22 unique patients. Either the primary tumor or a recurrent/metastatic tumor sample was available for each patient and histologically graded as follows: 1 (5%) grade 1, 16 (73%) grade 2, and 5 (23%) grade 3 tumors. Six ILC (27%) were stage III and 16 (73%) were stage IV at the time of sequencing (Table 1). Of the cases for which hormone receptor and HER2 slide-based test results were available, 15 of 19 (88%) were ER-positive; 11 of 18 (61%) were PR-positive; and 1 of 19 (5%) was HER2-positive by either IHC or FISH. Sequencing was conducted on the primary ILC in $n = 9$ (41%) cases and biopsies of metastatic sites in $n = 13$ (59%) cases including lymph node metastases in $n = 3$ (14%) cases, liver metastases in $n = 3$ (14%) cases, pleural fluid cell blocks and bone metastases in $n = 2$ (9%) cases each, and brain metastases in $n = 1$ (5%) cases.

Among the 22 ILCs, we identified 75 genomic alterations with an average of 3.4 alterations per tumor (range, 1–6), of which 35 were considered actionable for an average of 1.59 actionable alterations per patient (range, 0–3;

Table 1. Clinical features, standard biomarker status, and genomic alterations in 22 cases of CDH1-mutated invasive lobular breast cancer

Case	Age	Coverage depth	Specimen tested by NGS	Tumor grade	Tumor stage	ER	PR	HER2	Primary tumor E-cad IHC	Genomic alterations	Actionable genomic alterations	*AKT1*	*ARID1A*	*BRCA2*	*CCND1*	*CDH1*	*ERBB2*	*ERBB4*	*ESR1*	*FGFR1*	*KRAS*	*MAP2K4*	*MEN1*	*MYC*	*MCL1*	*NF1*	*PIK3CA*	*RB1*	*RICTOR*	*RUNX1*	*SMAD4*	*TP53*	*VHL*
1	65	842	Pleural fluid CB	II	IV	Pos	Pos	Neg (IHC)	Neg	2	0					Y37fs* 1																E286K	
2	68	897	Primary tumor	II	IV	Pos	Pos	Neg (FISH)	Neg	2	1					T823fs* 23											H1047R						
3	65	1,250	Skin recurrence	II	IV* inflammatory	Neg	Neg	Neg (IHC)	NA	4	1	E17K				Splice site - deletion												E693*				D259Y	
4	66	885	Liver metastasis	III	IV	Pos	Pos	Neg (IHC)	NA	6	2					I594fs* 19				Amplified				Amplified			E545K					R280K	
																																E285K	
5	74	1,085	Primary tumor	II	III	Pos	Pos	Neg (IHC)	Neg	3	2				Amplified	E167*											H1047R						
6	64	1,293	Lymph node metastasis	II	IV	Pos	Pos	Neg (IHC)	NA	2	1					L355fs* 1											N1044K						
7	63	1,135	Liver metastasis	III	IV	Neg	Neg	Neg (FISH)	NA	6	1					A348fs* 8	L755S		Amplified					Amplified						T270fs* - 213+		G245V	
8	68	301	Lymph node metastasis	II	IV	Unk	Unk	Neg (IHC)	NA	3	2					Splice site - 532-1G>T		S1246N			G13C												
9	NA	454	Primary tumor	II	IV	Pos	Pos	Neg (IHC)	NA	3	2					A719fs* 29	A775_G776ins - YVMA, R1153*																
10	NA	764	Primary tumor	II	III	Pos	Pos	Neg (IHC)	Neg	2	1					E763*											H1047L						
11	63	1,426	Lymph node metastasis	II	III	Pos	Pos	Neg (IHC)	Neg	3	2				Amplified	I650fs* 13				Amplified													
12	55	1,464	Pleural fluid CB	III	IV	Neg	Neg	Neg (IHC)	NA	4	1	E17K				D337_L343del+splice -												E693*				D259Y	
13	54	1,098	Brain metastasis	III	IV	Pos	Neg	IHC2+/FISH equivocal (ratio 1.9)	NA	4	2				Amplified	P309fs* 29				Amplified												P81S	
14	54	647	Primary tumor	I	III	Pos	Pos	Neg	Neg	4	3				Amplified	K653*					G12R						H419_-F421 >T						
15	58	1,182	Liver metastasis	II	IV	Pos	Pos	Neg	NA	4	2		Homozygous deletion	Homozygous deletion		T40fs* 19										Homozygous deletion							
16	54	547	Primary tumor	II	III	Pos	Neg	Neg	NA	3	2					E388*									Amplified		E542R						
17	65	929	Primary tumor	III	III	Unk	Unk	Unk	NA	4	2					L343R (novel)	P780_Y781ins - GSP					Splice Site 513+2T>C 513+2T>C			Amplified			Amplified					
18	62	211	Bone metastasis	II	IV	Pos	Pos	Neg	NA	4	3				Amplified	Q23*	*ERBB2–GRB7* - fusion										H1047R						
19	61	188	Bone metastasis	II	IV	Unk	Unk	Unk	NA	1	0					Q611fs*10																	
20	68	405	Cecal metastasis	II	IV	Pos	Neg	Neg (IHC)	Neg	1	0					L731fs* 39																	
21	44	1,475	Primary tumor	III	IV	Neg	Neg	Pos (IHC)	NA	5	3				Amplified	A408V	Amplified								Amplified							R175H	
22	59	821	Primary tumor	II	IV	Pos	Neg	Neg (FISH)	Neg	5	2					K314fs*4	S310F V842I						Q171*								Q83*		

NOTE: , actionable alteration.

Abbreviations: CB, cell block; NA, not available.

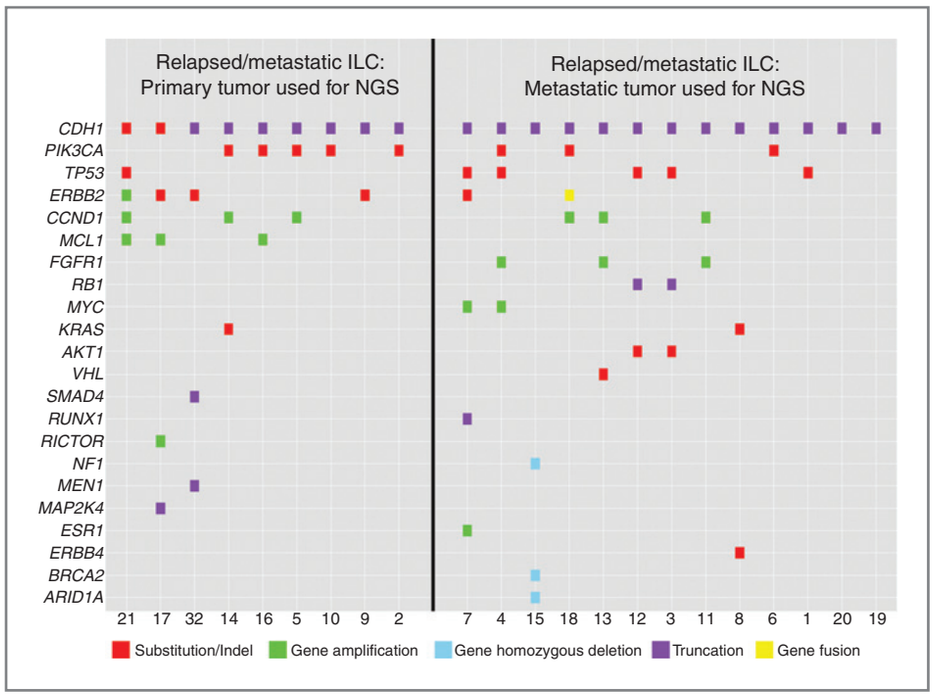

Figure 1. Relapsed/metastatic ILC: primary tumor genomic profiles. The figure is divided into 2 groups: (i) relapsed/metastatic ILC where the patient's primary tumor was sequenced, and (ii) relapsed/metastatic ILC where a metastatic lesion biopsy was sequenced.

Fig. 1; Table 1). Nineteen of 22 (86%) of the ILC harbored at least 1 actionable alteration potentially associated with clinical benefit of targeted therapies.

As a required study entry criterion, all 22 (100%) of the ILC featured a mutation in *CDH1*. There was a wide variety of *CDH1* base substitutions and splice site mutations identified with no repeated pattern of sequence alteration in any of the 22 *CDH1*-mutated ILC cases in this study (Table 1).

Frequency of genomic alterations in ERBB2

Six (27%) of the *CDH1*-mutated ILC cases featured alterations in *ERRB2* including 4 (18%) with *ERBB2* mutations, 1 (5%) with an *ERBB2–GRB7* gene fusion, and 1 (5%) with an *ERBB2* copy number gain (amplification). There was a trend for high-grade tumors to harbor *ERBB2* alterations with 3 (60%) of 5 grade 3 tumors having either *ERBB2* mutations or amplification. Of the 9 primary tumors evaluated, 4 (44%) had an *ERBB2* alteration (3 cases with mutations and 1 case with amplification) compared with 2 (15%) of 13 metastatic cases (1 case with an *ERBB2* mutation and 1 case with an *ERBB2* fusion). The 4 cases with *ERBB2* mutations included study case number 7, a grade 3 tumor with a L755S *ERBB2* mutation in a liver metastasis sample from an ER^-/PR^-/HER2 $FISH^-$ 63-year-old patient (Fig. 2A). This tumor also featured an amplification of *ESR1* as well as amplification in *MYC* and mutations in *RUNX1* and *TP53*. Case study number 9, a primary tumor sequence from an ER^-/PR^-/HER2 IHC^- ILC harbored 2 *ERBB2* mutations: a A775_G776insYVMA mutation and a R1153* truncation and no additional genomic alterations (Fig. 2B). Case study number 17 showed a P780_Y781insGSP *ERBB2* mutation in the primary tumor from a 65-year-old patient. In addition to the *ERBB2* mutation, this tumor also featured a splice site mutation in the *MAP2K4* gene and amplification of *MCL1* and *RICTOR*, respectively (Fig. 2C). In case number 22, a woman with relapsed metastatic ILC, her primary tumor was $ER^+/PR^-/HER2^-$ (FISH). When this primary tumor was sequenced, 2 discrete *ERBB2* mutations were found (S310F and V842I) as well as mutations in the *MEN1* and *SMAD4* genes (Fig. 2D). In case study number 18, a $ER^+/PR^+/HER2^-$ bone metastasis from a 62-year-old patient, a novel *ERBB2–GRB7* fusion was identified (Figs. 2E and 3). This tumor also had an amplification of the *CCND1* gene and a H1047R *PIK3CA* mutation. There was insufficient archived extracted DNA or tumor tissue remaining on this sample for further DNA and RNA-based characterization of this gene fusion. Finally, in case study number 21, a *CDH1*-mutated stage IV high-grade ER^-/PR^- pleomorphic breast cancer with papillary features, an *ERBB2* gene copy number gain was detected by NGS, which correlated with the positive HER2 overexpression on immunohistochemical staining and gene amplification detected by FISH testing of the same specimen. This tumor also had amplifications in the *CCND1* and *MCL1* genes and a *TP53* mutation. Only this *ERBB2* amplification case also had a positive HER2 slide-based test result (both IHC and FISH) and the 5 ILC cases with either a mutation or fusion of *ERBB2* were uniformly HER2-negative by IHC and/or FISH testing.

Frequency of genomic alterations in primary tumor samples versus metastatic tumor biopsies

Although all ILCs in this study ultimately relapsed, 9 of the ILCs sequenced in this study were archived specimens

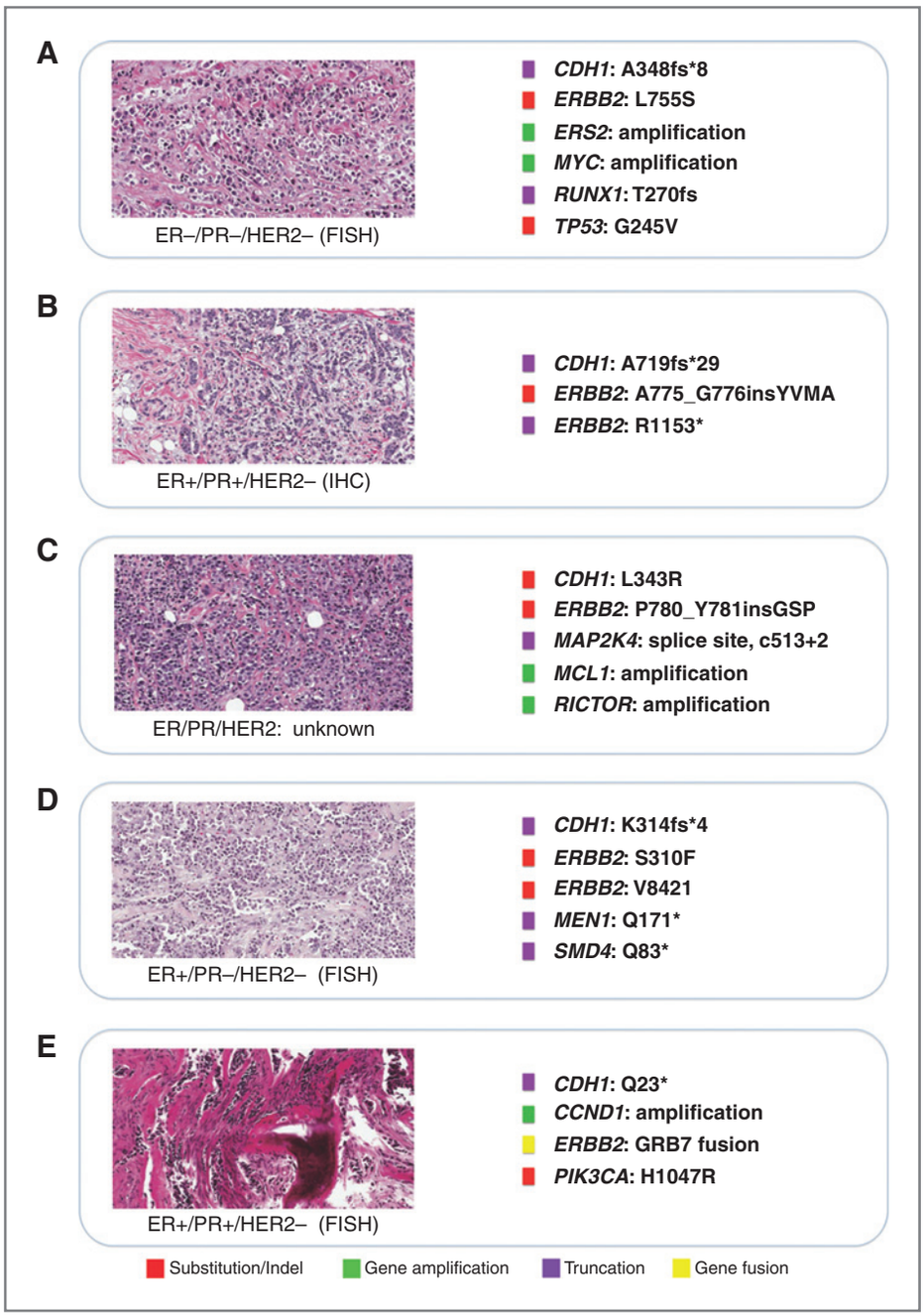

Figure 2. *ERBB2* mutations in invasive lobular breast cancer detected by NGS. Actionable alterations detected on 5 *ERBB2* mutation patients were listed next to H&E images. A, core needle biopsy of a grade 3 invasive ILC metastatic to the liver in a 63-year-old woman with a triple-negative (ER^-/PR^-/HER2 $FISH^-$) tumor. The tumor featured a L755S *ERBB2* mutation. In addition to the *CDH1* mutation, this ER IHC-negative tumor also had amplifications of the *ESR1* and *MYC* genes, and *TP53* and *RUNX1* mutations. B, this grade 2 primary ILC was ER^+/PR^+/HER2 IHC^- and featured only a *CDH1* mutation in addition to 2 distinct *ERBB2* mutations: A775_G776insYVMA and R1153*. C, this grade 3 ILC showed a P780_Y781insGSP *ERBB2* mutation in the primary tumor from a 65-year-old patient. In addition to the *ERBB2* mutation, this tumor also featured a splice site mutation in the *MAP2K4* gene and amplification of *MCL1* and *RICTOR*, respectively. D, this grade 2 relapsed metastatic ILC featured a primary tumor that was ER^+/PR^-/$HER2^-$ (FISH). When this primary tumor was sequenced, 2 discrete *ERBB2* mutations were found (S310F and V842I) as well as mutations in the *MEN1* and *SMAD4* genes. E, this $ER^+/PR^+/HER2^-$ bone metastasis from a 62-year-old patient showed a novel *ERBB2–GRB7* fusion. This tumor also had an amplification of the *CCND1* gene and a H1047R *PIK3CA* mutation.

of the primary disease. The incidence of genomic alterations in the primary tumors and metastatic site biopsies were virtually identical. When the original primary tumor was used for sequence analysis in this cohort of advanced and metastatic ILC, the 9 tumors yielded 31 total genomic alterations and 3.44 alterations per tumor (Fig. 1). In comparison, the 13 ILC cases where a metastatic site provided the sample for the NGS assay yielded 43 total alterations and 3.31 alterations per tumor. Interestingly, the incidence of actionable alterations was higher in the primary tumor sample group (2.0 actionable alterations per tumor) than in the metastatic site biopsy group (1.23 actionable alterations per tumor). However, given the relatively small numbers of cases in these 2 groups, this observation is preliminary and requires validation on a larger series of cases.

Additional genomic alterations identified in the cohort included mutations in *PIK3CA* (8 cases; 36%), *TP53* (6 cases; 27%), *RB1* (2 cases; 9%), *KRAS* (2 cases; 9%), *AKT1* (2 cases 9%), and *ERBB4* (1 case; 5%). The *PIK3CA* mutation frequency was higher in the 9 cases where the primary tumors were sequenced (56%) than in the metastatic site

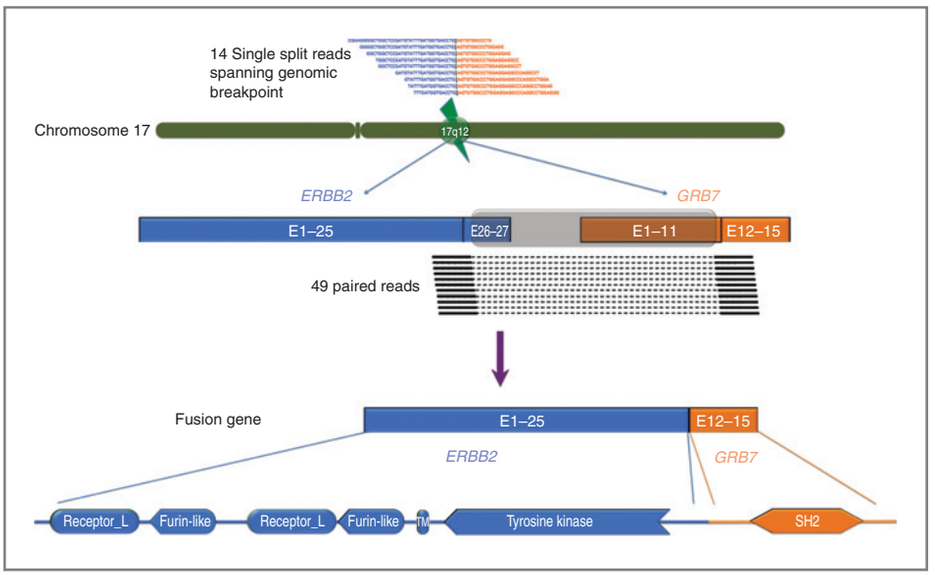

Figure 3. Schematic illustration of the *ERBB2–GRB7* gene fusion. The schematic is created from a $ER^+/PR^+/HER2^-$ ILC bone metastasis showing a novel *ERBB2–GRB7* fusion. This tumor also had an amplification of the *CCND1* gene and a H1047R *PIK3CA* mutation. The figure shows an 18-kb segment on chromosome 17, which is deleted fusing *ERBB2* and *GRB7*. ERBB2 is represented in blue as 5′ partner and GRB7 is represented in orange as 3′ partner. Forty-nine high-quality paired-end reads span the chimeric structure of *ERBB2–GRB7* fusion; a subset is shown as dashed black lines. Fourteen reads span the exact genomic breakpoint, mapping it unambiguously to the single-nucleotide resolution. E1–25, exons 1 to 25; E12–15, exons 12 to 15; exons 26–27 of *ERBB2* and exons 1–11 of *GRB7* in gray box are within the deleted segment. Receptor L, receptor L domain; Furin-like, Furin-like cysteine rich region; TM, transmembrane; Tyrosine kinase, tyrosine kinase domain; and SH2, Src homology 2 domain (44, 45).

biopsy samples (23%), whereas the *TP53* mutation frequency was higher in the metastatic lesions (31%) than for the cases where the primary tumor samples (11%) was used for genomic analysis. Mutations in *PIK3CA* and *TP53* have been reported in 41% and 50% of ILC, respectively [Catalogue of Somatic Mutations in Cancer (COSMIC); Oct 2012]. Whether the observed differences between the 2 groups are significant requires validation on a larger series of cases. DNA copy number gains were seen in *CCND1* (6 cases; 27%), *FGFR1* (3 cases; 14%), *MYC* (2 cases; 9%), and *ESR1* (1 case; 5%).

Finally, in order to assess clonality, the mutant allele frequencies of the 43 reported mutations in the 4 most commonly mutated genes in the study, *CDH1*, *TP53*, *PIK3CA*, and *ERBB2*, were assessed. Considering tumor suppressors (*CDH1*, *TP53*) and oncogenes (*PIK3CA*, *ERBB2*) separately, we observed mean mutant allele frequencies of 39.7% and 18.5%, respectively (Supplementary Table S2). Taken in the context of an average estimated tumoral purity of approximately 40% (range, 10%–95%) in the study, these mutant allele frequencies are consistent, on average, with a clonal homozygous model in the case of tumor suppressor mutations and the clonal heterozygous model in the case of oncogene mutations.

Discussion

To define a more genetically homogenous population of ILC cases, patient entry into this study required the presence of a *CDH1* mutation (7, 21). Using this approach, we found that a striking 19 of 22 (86%) ILC cases had one or more actionable alterations including changes in *PIK3CA*, *CCND1*, *ERBB2*, *FGFR1*, *MCL1*, *KRAS*, *NF1*, *AKT1*, and *BRCA2*. The incidence of *CDH1* mutations in ILC varies significantly in public databases and literature from a low of 31% (COSMIC; July 2012) to 62% (22). Most investigators consider a mutation in *CDH1* to represent a genomic alteration characteristic of the lobular subtype of breast cancer (7, 21). Other mechanisms that lead to loss of E-cadherin function include methylation of *CDH1* (15). The *CDH1* mutations in the 22 ILC cases included in this study were all unique and involved point mutations in 20 (91%) cases and splice site mutations/deletions in 2 (9%) cases. In triple-negative IDC of the breast, loss of E-cadherin expression has been linked to adverse clinical outcome (23). Finally, neither *CDH1* mutation nor loss of E-cadherin function by other mechanisms, are considered targets for anticancer therapy at this time (24). Six (27%) of the *CDH1*-mutated ILC cases featured alterations in *ERRB2* including 4 cases with mutations (L755S in 1 case, A775_G776 and insYVMA in 1 case, R1153* in 1 case, P780_Y781insGSP in 1 case, and S310F and V842l in 1 case), 1 case with an *ERBB2–GRB7* fusion and 1 case with *ERBB2* amplification. For ILC, *HER2* gene amplification and protein overexpression are generally considered to be rare events typically below a 5% incidence in most published studies (25–27). *ERBB2* mutations are rare in breast cancer and were not listed in a recently published major DNA sequence analysis study of more than 100 cases that also included whole-genome analysis in 22 cases (13). In

the COSMIC database, 14 (1.52%) of 919 listed breast cancers have an *ERBB2* mutation that alters the Her2 protein (COSMIC; Jan 2013). Similarly, in a currently unpublished prior assessment of a database of 308 clinical breast cancer samples of all types sequenced at Foundation Medicine, Inc. from which the 5 ILC with *ERBB2* mutations that are also included in the current study were identified, only 10 (3.25%) total invasive breast cancers (5 ductal and 5 *CDH1* mutated lobular) featured *ERBB2* mutations. When the 5 of 22 (23%) *CDH1*-mutated ILC with *ERBB2* mutation/fusion are compared with the 5 of 286 (2%) of the *CDH1* WT (nonmutated) invasive breast cancers with *ERBB2* mutations, this difference is statistically significant ($P = 0.0006$). When the *ERBB2–GRB7* fusion case is excluded from analysis and only *ERBB2*-mutated cases are included, the enrichment of ERBB2 alterations in *CDH1*-mutated ILC remains significant ($P = 0.003$). Thus, the filtering of breast cancers by combining lobular histologic phenotype with *CDH1* mutation significantly enriches for the presence of *ERBB2* mutations. Given that the 5 *ERBB2*-mutated *CDH1* WT tumors did not display a lobular histologic phenotype, these data indicate that it is unlikely that subsets of ILC in which E-cadherin is inactivated by other mechanisms such as methylation are also enriched for *ERBB2* mutations.

Interestingly, *ERBB2* mutations have been reported more frequently in lung cancer than in breast cancer reaching as high as 10% in the adenocarcinoma subtype (28, 29). The mutational spectrum of *ERBB2* in breast cancer identified in this study is distinct from the mutations described for lung cancer, which has also recently been reported by others (30). The L755S mutation identified in case 7 of this series of ILC is located in the kinase domain of Erbb2, and has been shown to be an activating mutation, capable of inducing oncogenic transformation in cell culture (31, 32). Mutations in exon 20 of *ERBB2*, such as A775_G776insYVMA found in case 9 of this study, have been associated with Erbb2 activation and sensitivity to Erbb2 inhibitors and dual inhibitors of Erbb2 and Egfr (29, 33–36). Preclinical studies have shown that the L755S *ERBB2* mutation is resistant to the reversible dual Egfr/Erbb2 inhibitor lapatinib but may be sensitive to irreversible dual Egfr/Erbb2 inhibitors, such as afatinib, which are under investigation in clinical trials (37). The P780_Y781insGSP mutation is located at exon 20 of *ERBB2* and has also been linked to Erbb2 activation and sensitivity to Erbb2 inhibitors and dual inhibitors of Erbb2 and Egfr (33–36). The monoclonal antibodies trastuzumab and pertuzumab have been approved for use in *ERBB2*-amplified breast cancer, but their efficacy in the context of the L755S mutation is unknown.

The *ERBB2–GRB7* putative fusion seen in study case 18 has not been previously reported. The fusion retains the kinase domain of *ERBB2* (uniprot.org), which suggests that it could result in *ERBB2* activation. The 17q12-21 amplicon, which includes both *ERBB2* and *GRB7* is frequently amplified in breast cancer and preclinical studies suggest that it may be a recombination hotspot (38). An expression screening study has reported that *GRB7* can function as an *ERBB2*-dependent oncogene (39). Interestingly, 2 previous studies focused on breast cancer rearrangements did not report this fusion although the number of CDH1-mutated lobular cancers in these studies may have been limited (40, 41). *GRB7* encodes an adaptor protein that interacts with *ERBB2* and has been shown in a preclinical study to enhance its transformative capacity and increase *ERBB2* phosphorylation in fibroblasts (41). In that the sample that yielded the *ERBB2–GRB7* fusion was a small bone metastasis biopsy, there was insufficient remaining material available to further characterize the alteration at the RNA level. However, this fusion protein has not been functionally characterized, and its effect is therefore unknown and the relevance to the efficacy of clinical use of anti-HER2–targeted therapies is unclear.

The concept of targeting of *ERBB2* mutations for anti-HER2 therapies has recently been expanded to breast cancer (30). Investigators identified 25 patient samples with *ERBB2* mutations from a series of 8 independent breast cancer-sequencing projects. Using *in vivo* kinase assays, analysis of protein structure and animal models, the investigators were able to identify 7 activating *ERBB2* mutations, G309A, D769H, D769Y, V777L, P780ins, V842I, and R896C that were sensitive to the kinase inhibitor neratinib (30). The P780_Y781insGSP mutation of *ERBB2* in study case 17 is included in this list of activating *ERBB2* alterations. Neither the A775_G776insYVMA, R1153*mutation identified in case 9 nor the novel *ERBB2–GRB7* fusion from study case 18 were reported by these investigators. The L755S mutation found in case 7 of the current report was considered not to be an activating mutation by these investigators but conferred resistance to the drug lapatinib in their experimental systems. The authors concluded that *ERBB2* (*HER2*) somatic mutation was capable of activating the gene in breast cancer and concluded that *ERBB2* somatic mutations are potential drug targets for breast cancer treatment (30).

Several questions are raised by this study. Are all lobular breast cancers, or just *CDH1*-mutated ILCs enriched for *ERBB2* mutations? If this relationship holds true, other *CDH1*-mutated cancers such as diffuse gastric carcinoma may also have a disproportionate enrichment of *ERBB2* mutation. Moreover, the biologic mechanism for this apparent enrichment of *ERBB2* alterations in *CDH1* mutated ILCs is not known at this time. Interestingly, however, both the loss of cell adhesion associated with *CDH1* mutation and the well-documented enhancement of cell motility attributed to *ERBB2* activation (42, 43) could conceivably synergize to drive invasion and metastasis in these patients with difficult to treat, relapsed ILC. Finally, is *ERBB2* mutation simply associated with relapsed breast cancer, rather than specifically being enriched in relapsed ILC? At this time, is not known whether a similar enrichment of *ERBB2* alterations in *CDH1*-mutated ILC would be seen in cases that were successfully treated in the primary setting and, unlike the ILC in the current study, did not relapse or metastasize. This may explain whether *ERBB2* mutation is

typically present at the early stages of cancer for the patient or is an additionally acquired mutation as the cancer evolves, perhaps under selection pressure. Understanding the chronologic nature of this mutation could also explain distinct spectrums of *ERBB2* mutation between lung and breast cancer.

Clinical trials focused on *ERBB2* mutations and fusions in breast cancer using both on the market antibody therapeutics and tyrosine kinase inhibitors as well as new agents in late stages of development seem highly warranted. In conclusion, deep sequencing of genomic DNA using an optimized clinical grade diagnostic assay can provide a broad cancer-related gene survey at a depth of coverage that provides sensitive detection for all classes of cancer-related genomic alterations, and when applied to patients with ILC can reveal actionable alterations that have the potential to inform treatment decisions in the majority of patients. Wider use of this testing strategy could accelerate accrual to therapeutic trials of agents targeting these (and other mutations) and allow more efficient identification of more active single agents or combinations.

Disclosure of Potential Conflicts of Interest

J.S. Ross, K. Wang, G. Otto, S.R. Downing, J. Sun, J. He, J.A. Curran, S. Ali, R. Yelensky, D. Lipson, G. Palmer, V.A. Miller, and P.J. Stephens are employees and stock owners of Foundation Medicine, Inc. V.A. Miller is a consultant/advisory board member of Boehringer Ingelheim. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: J.S. Ross, R. Yelensky
Development of methodology: J.S. Ross, G. Otto, S.R. Downing, R. Yelensky
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J.S. Ross, C.E. Sheehan, A.B. Boguniewicz, S.R. Downing, J.A. Curran
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J.S. Ross, K. Wang, A.B. Boguniewicz, S.R. Downing, J. Sun, J. He, J.A. Curran, R. Yelensky, D. Lipson, V.A. Miller, P.J. Stephens
Writing, review, and/or revision of the manuscript: J.S. Ross, K. Wang, C. E. Sheehan, A.B. Boguniewicz, S.R. Downing, S. Ali, R. Yelensky, G. Palmer, V.A. Miller
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): J.S. Ross, C.E. Sheehan, P.J. Stephens

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References

1. Ross JS. Multigene classifiers, prognostic factors, and predictors of breast cancer clinical outcome. Adv Anat Pathol 2009;16:204–15.
2. Perou CM, Sørlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, et al. Molecular portraits of human breast tumours. Nature 2000; 406:747–52.
3. Tavassoli FA. Lobular neoplasia: evolution of its significance and morphologic spectrum. Int J Surg Pathol 2010;18:174S–7S.
4. Venkitaraman R. Lobular neoplasia of the breast. Breast J 2010; 16:519–28.
5. Fortunato L, Mascaro A, Poccia I, Andrich R, Amini M, Costarelli L, et al. Lobular breast cancer: same survival and local control compared with ductal cancer, but should both be treated the same way? analysis of an institutional database over a 10-year period. Ann Surg Oncol 2012; 19:1107–14.
6. Jung SY, Jeong J, Shin SH, Kwon Y, Kim EA, Ko KL, et al. The invasive lobular carcinoma as a prototype luminal A breast cancer: a retrospective cohort study. BMC Cancer 2010;10:664.
7. O'Malley FP. Lobular neoplasia: morphology, biological potential and management in core biopsies. Mod Pathol 2010;23(Suppl 2):S14–25.
8. Rakha EA, Ellis IO. Lobular breast carcinoma and its variants. Semin Diagn Pathol 2010;27:49–61.
9. Iorfida M, Maiorano E, Orvieto E, Maisonneuve P, Bottiglieri L, Rotmensz N, et al. Invasive lobular breast cancer: subtypes and outcome. Breast Cancer Res Treat 2012;133:713–23.
10. Jacobs M, Fan F, Tawfik O. Clinicopathologic and biomarker analysis of invasive pleomorphic lobular carcinoma as compared with invasive classic lobular carcinoma: an experience in our institution and review of the literature. Ann Diagn Pathol 2012;16:185–9.
11. Weigelt B, Geyer FC, Natrajan R, Lopez-Garcia MA, Ahmad AS, Savage K, et al. The molecular underpinning of lobular histological growth pattern: a genome-wide transcriptomic analysis of invasive lobular carcinomas and grade- and molecular subtype-matched invasive ductal carcinomas of no special type. J Pathol 2010; 220:45–57.
12. O'Toole SA, Selinger CI, Millar EK, Lum T, Beith JM. Molecular assays in breast cancer pathology. Pathology 2011;43:116–27.
13. Banerji S, Cibulskis K, Rangel-Escareno C, Brown KK, Carter SL, Frederick AM, et al. Sequence analysis of mutations and translocations across breast cancer subtypes. Nature 2012;486: 405–9.
14. Shah SP, Roth A, Goya R, Oloumi A, Ha G, Zhao Y, et al. The clonal and mutational evolution spectrum of primary triple-negative breast cancers. Nature 2012;486:395–9.
15. Morrogh M, Andrade VP, Giri D, Sakr RA, Paik W, Qin LX, et al. Cadherin–catenin complex dissociation in lobular neoplasia of the breast. Breast Cancer Res Treat 2012;132:641–52.
16. Lipson D, Capelletti M, Yelensky R, Otto G, Parker A, Jarosz M, et al. Identification of new ALK and RET gene fusions from colorectal and lung cancer biopsies. Nat Med 2012;18:382–4.
17. Wang K, Yelensky R, Dogan S, Borsu L, Frampton GM, Lipson D, et al. An evaluation of next-generation sequencing for diagnostic use in cancer care [abstract]. 2012 Meeting of Association of Molecular Pathology. J Mol Diagn 2012;14:637–748.
18. Beltran H, Yelensky R, Frampton GM, Park K, Downing SR, Macdonald TY, et al. Targeted next-generation sequencing of advanced prostate cancer identifies potential therapeutic targets and disease heterogeneity. Eur Urol 2013;63:920–6.
19. Frampton GM, Downing S, Brennan K, Donahue A, White E, Garcia L, et al.. Validation of a comprehensive NGS-based cancer genomic assay for clinical use. In: Proceedings of the AACC Annual Meeting 2012; 2012 Jul 15–19; Los Angeles, CA. Presentation nr C-19.
20. Lipson D, He J, Yelensky R, Miller V, Sheehan C, Brennan K, et al. Next-generation sequencing of FFPE breast cancers demonstrates high concordance with FISH in calling HER2 amplifications and commonly identifies additional clinically relevant genomic alterations. Cancer Res 2012;72(Suppl 24):PD02-07.
21. Yoder BJ, Wilkinson EJ, Massoll NA. Molecular and morphologic distinctions between infiltrating ductal and lobular carcinoma of the breast. Breast J 2007;13:172–9.
22. Bertucci F, Orsetti B, Nègre V, Finetti P, Rougé C, Ahomadegbe JC, et al. Lobular and ductal carcinomas of the breast have distinct genomic and expression profiles. Oncogene 2008;27:5359–72.

23. Xie ZM, Li LS, Laquet C, Penault-Llorca F, Uhrhammer N, Xie XM, et al. Germline mutations of the E-cadherin gene in families with inherited invasive lobular breast carcinoma but not diffuse gastric cancer. Cancer 2011;117:3112–7.
24. Tang D, Xu S, Zhang Q, Wang J, Lu K, Song Y, et al. The expression and clinical significance of the androgen receptor and E-cadherin in triple negative breast cancer. Med Oncol 2012;29:526–33.
25. Ross JS, Slodkowska EA, Symmans WF, Pusztai L, Ravdin PM, Hortobagyi GN. The HER-2 receptor and breast cancer: ten years of targeted anti-HER-2 therapy and personalized medicine. Oncologist 2009;14:320–68.
26. Monhollen L, Morrison C, Ademuyiwa FO, Chandrasekhar R, Khoury T. Pleomorphic lobular carcinoma: a distinctive clinical and molecular breast cancer type. Histopathology 2012;61:365–77.
27. Yu J, Dabbs DJ, Shuai Y, Niemeier LA, Bhargava R. Classical-type invasive lobular carcinoma with HER2 overexpression: clinical, histologic, and hormone receptor characteristics. Am J Clin Pathol 2011; 136:88–97.
28. Arcila ME, Chaft JE, Nafa K, Roy-Chowdhuri S, Lau C, Zaidinski M, et al. Prevalence, clinicopathologic associations, and molecular spectrum of ERBB2 (HER2) tyrosine kinase mutations in lung adenocarcinomas. Clin Cancer Res 2012;18:4910–8.
29. Stephens P, Hunter C, Bignell G, Edkins S, Davies H, Teague J, et al. Lung cancer: intragenic ERBB2 kinase mutations in tumours. Nature 2004;431:525–6.
30. Bose R, Kavuri SM, Searleman AC, Shen W, Shen D, Koboldt DC, et al. Activating HER2 mutations in HER2 gene amplification negative breast cancer. Cancer Discov 2013;3:224–37.
31. Lee JW, Soung YH, Seo SH, Kim SY, Park CH, Wang YP, et al. Somatic mutations of ERBB2 kinase domain in gastric, colorectal, and breast carcinomas. Clin Cancer Res 2006;12:57–61.
32. Trowe T, Boukouvala S, Calkins K, Cutler RE Jr, Fong R, Funke R, et al. EXEL-7647 inhibits mutant forms of ErbB2 associated with lapatinib resistance and neoplastic transformation. Clin Cancer Res 2008;14: 2465–75.
33. Wang SE, Narasanna A, Perez-Torres M, Xiang B, Wu FY, Yang S, et al. HER2 kinase domain mutation results in constitutive phosphorylation and activation of HER2 and EGFR and resistance to EGFR tyrosine kinase inhibitors. Cancer Cell 2006;10:25–38.
34. Cappuzzo F, Bemis L, Varella-Garcia M. HER2 mutation and response to trastuzumab therapy in non–small-cell lung cancer. N Engl J Med 2006;354:2619–21.
35. Minami Y, Shimamura T, Shah K, LaFramboise T, Glatt KA, Liniker E, et al. The major lung cancer-derived mutants of ERBB2 are oncogenic and are associated with sensitivity to the irreversible EGFR/ERBB2 inhibitor HKI-272. Oncogene 2007;26:5023–7.
36. Tomizawa K, Suda K, Onozato R, Kosaka T, Endoh H, Sekido Y, et al. Prognostic and predictive implications of HER2/ERBB2/neu gene mutations in lung cancers. Lung Cancer 2011;74:139–44.
37. Kancha RK, von Bubnoff N, Bartosch N, Peschel C, Engh RA, Duyster J. Differential sensitivity of ERBB2 kinase domain mutations towards lapatinib. PLoS ONE 2011;6:e26760.
38. Katoh M, Katoh M. Evolutionary recombination hotspot around GSDML-GSDM locus is closely linked to the oncogenomic recombination hotspot around the PPP1R1B-ERBB2-GRB7 amplicon. Int J Oncol 2004;24:757–63.
39. Saito M, Kato Y, Ito E, Ishikawa K, Doi A, Kumazawa K, et al. Expression screening of 17q12-21 amplicon reveals GRB7 as an ERBB2-dependent oncogene. FEBS Lett 2012;586:1708–14.
40. Stephens PJ, McBride DJ, Lin ML, Varela I, Pleasance ED, Simpson JT, et al. Complex landscapes of somatic rearrangement in human breast cancer genomes. Nature 2009;462:1005–10.
41. Kumar-Sinha C, Ignatoski KW, Lippman ME, Ethier SP, Chinnaiyan AM. Transcriptome analysis of HER2 reveals a molecular connection to fatty acid synthesis. Cancer Res 2003;63:132–9.
42. Wolf-Yadlin A, Kumar N, Zhang Y, Hautaniemi S, Zaman M, Kim HD, et al. Effects of HER2 overexpression on cell signaling networks governing proliferation and migration. Mol Syst Biol 2006;2:54–8.
43. Ueda Y, Wang S, Dumont N, Yi JY, Koh Y, Arteaga CL. Overexpression of HER2 (erbB2) in human breast epithelial cells unmasks transforming growth factor beta-induced cell motility. J Biol Chem 2004;279:24505–13.
44. Finn RD, Mistry J, Tate J, Coggill P, Heger A, Pollington JE, et al. The Pfam protein families database. Nucleic Acids Res 2010;38: D211–22.
45. Hunter S, Jones P, Mitchell A, Apweiler R, Attwood TK, Bateman A, et al. InterPro in 2011: new developments in the family and domain prediction database. Nucleic Acids Res 2012;40:D306–12.