Clinical Studies

Cancer Research

Intrinsic Subtypes and Gene Expression Profiles in Primary and Metastatic Breast Cancer

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Abstract

Biological changes that occur during metastatic progression of breast cancer are still incompletely characterized. In this study, we compared intrinsic molecular subtypes and gene expression in 123 paired primary and metastatic tissues from breast cancer patients. Intrinsic subtype was identified using a PAM50 classifier and χ^2 tests determined the differences in variable distribution. The rate of subtype conversion was 0% in basal-like tumors, 23.1% in HER2-enriched (HER2-E) tumors, 30.0% in luminal B tumors, and 55.3% in luminal A tumors. In 40.2% of cases, luminal A tumors converted to luminal B tumors, whereas in 14.3% of cases luminal A and B tumors converted to HER2-E tumors. We identified 47 genes that were expressed differentially in metastatic versus primary disease. Metastatic tumors were enriched for proliferation-related and migration-related genes and diminished for luminal-related genes. Expression of proliferation-related genes were better at predicting overall survival in metastatic disease (OSmet) when analyzed in metastatic tissue rather than primary tissue. In contrast, a basal-like gene expression signature was better at predicting OSmet in primary disease compared with metastatic tissue. We observed correlations between time to tumor relapse and the magnitude of changes of proliferation, luminal B, or HER2-E signatures in metastatic versus primary disease. Although the intrinsic subtype was largely maintained during metastatic progression, luminal/HER2-negative tumors acquired a luminal B or HER2-E profile during metastatic progression, likely reflecting tumor evolution or acquisition of estrogen independence. Overall, our analysis revealed the value of stratifying gene expression by both cancer subtype and tissue type, providing clinicians more refined tools to evaluate prognosis and treatment. *Cancer Res; 77(9); 2213–21. ©2017 AACR.*

Introduction

Despite new systemic treatment advances, metastatic breast cancer is still an incurable disease and a major cause of cancer-related death (1). Median overall survivals of patients with triple-negative, hormone receptor (HR)-positive/HER2-negative and HER2-positive diseases are approximately 12, approximately 20, and approximately 56 months, respectively (2–5). Thus, there is a need to better understand the biology behind the progression of tumor cells toward metastasis. To date, evidence suggests that both intrinsic properties of breast cancer cells and host organ microenvironment participate actively to this matter (6).

In general, detectable distant breast cancer metastases occur years, or even decades, after primary tumor diagnosis. These secondary lesions are believed to originate from disseminated tumor cells that underwent a period of dormancy (7). Tumor dormancy is the result of equal rates of cell proliferation and cell death (8). However, the molecular factors that promote the formation of detectable metastasis from disseminated tumor cells are largely unknown. To try to approach it, studies have started to identify the molecular differences between primary tumor and their matched metastatic lesions (9). At the DNA level, although significant differences have been observed, the vast majority (80%–85%) of molecular alterations are similar in both settings.

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Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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doi: 10.1158/0008-5472.CAN-16-2717

For example, the discordance of *HER2* gene amplification by FISH in primary versus metastatic tissue is 3%–10% (10). Similarly, at the protein level, estrogen and progesterone receptors by IHC are discordant in 13%–28% of the cases (11). Overall, these results suggest that minor but important molecular changes occur during metastatic progression such as *ESR1* mutations (12).

In terms of global gene expression, 4 main molecular subtypes [luminal A, luminal B, HER2-enriched (HER2-E) and basal-like], and a normal breast-like group, have been identified and intensively studied for the last 15 years in early breast cancer (13–16). Known as the "intrinsic subtypes of breast cancer," these groups of tumors have revealed critical differences in incidence (17, 18), survival (19–21), and response to treatment (22, 23). Importantly, the information provided by the intrinsic subtypes complements and expands the information provided by classical clinical parameters (e.g., age, node status, tumor size, histologic grade) and pathologic markers [estrogen receptor (ER), progesterone receptor (PR), and HER2; refs. 24, 25], all of which are routinely used today in the clinic to stratify patients for prognostic predictions and to select treatments.

Recently, we evaluated the prognostic value of the intrinsic subtypes in 821 samples from a phase III clinical trial, where postmenopausal patients with metastatic HR^+ breast cancer were treated with first-line letrozole ± lapatinib (26). The vast majority of samples (∼80%) were from the primary tumor years before the patient relapsed. Interestingly, we observed that intrinsic subtype provided the largest amount of prognostic information in this setting beyond HER2 status, treatment, visceral disease, and other clinical–pathological variables. Overall, these results suggested that intrinsic subtype does not change substantially when recurrence occurs.

Material and Methods

Study population

This retrospective study included nonconsecutive female patients over the age of 18 years with a histologic diagnosis of metastatic breast cancer detected at the time of diagnosis, at first relapse or after successive disease progressions. Tissues were collected from five independent sources: GEICAM/2009-03 ConvertHER trial (11), Hospital Clínico Universitario de Valencia, Vall d'Hebrón Institute of Oncology, University-AO Papardo and Hospital Clinic of Barcelona. To be included, samples were required to have a formalin-fixed paraffin-embedded (FFPE) tissue sample from primary and metastatic tumor. Biopsies were performed by core biopsy or surgical process, according to the routine clinical practice of the hospitals. For each sample, receptor status (ER, PR, and HER2) were analysed at the local laboratory.

Gene expression analysis

All primary and metastatic tissues were analysed using the same methodology. A section of FFPE breast tissue was first examined with a hematoxylin and eosin staining to confirm the diagnosis and determine the tumour surface area. For RNA purification, three 10-µm FFPE slides were cut for each tumor, and macrodissection was performed, when needed, to avoid normal breast contamination. A minimum of approximately 100 ng of total RNA was used to measure the expression of 105 breast cancer-related genes and 5 housekeeping genes (*ACTB*, *MRPL19*, *PSMC4*, *RPLP0*, and *SF3A1*) using the *nCounter* platform (Nanostring Technologies; ref. 27). Data was log base2–transformed and normalized using the housekeeping genes. Raw data have been deposited in the Gene Expression Omnibus under the accession number GSE92977.

Gene list

The list of 105 breast cancer–related genes includes genes from the following three signatures: PAM50 intrinsic subtype predictor ($n = 50$; ref. 28), claudin-low subtype predictor ($n = 43$; ref. 29), VEGF/Hypoxia signature ($n = 13$; ref. 30). In addition, we included 8 individual genes that have been found to play an important role in breast cancer [i.e., *CD24* (29), *CRYAB* (31), *ERBB4* (32), *PIK3CA* (13), *PTEN* (13), *RAD17* (33), *RAD50* (33), and *RB1* (13)]. The complete list of genes can be found in Supplementary Table S1.

Intrinsic subtype

All tumors were assigned to an intrinsic molecular subtype of breast cancer (luminal A, luminal B, HER2-E, and basal-like) and the normal-like group using the previously reported PAM50 subtype predictor (28).

Claudin-low intrinsic subtype

We applied the previously reported 9-Cell line claudin-low predictor (29). A sample was identified as claudin-low independently of the PAM50 subtype call, as previously reported.

Gene signatures

The expression of 10 independent signatures was evaluated as a continuous variable. The PAM50 predictor calculates, for each sample, the correlation coefficient to each of the 5 PAM50 centroids (luminal A, luminal B, basal-like, HER2-enriched, and normal-like). Each centroid was considered a single signature. In addition, the PAM50 predictor outputs a risk of recurrence (ROR) score at 10 years. The ROR score based on subtype (ROR-S) and subtype and proliferation (ROR-P) were developed in a microarray-based cohort of node-negative, untreated early breast cancer (34). In addition, we evaluated the following three signatures: proliferation score, which is the mean expression of 11 proliferation-related genes (21), VEGF/Hypoxia signature (30), which is the mean expression of 13 hypoxia-related genes, and claudin-low signature (as a continuous variable; ref. 29).

Statistical analysis

χ^2 tests were performed to determine the differences in the distribution of variables. To identify genes whose expression was significantly different between paired primary and metastatic samples, we used a paired two-class significance of microarrays (SAM) with a false discovery rate (FDR) <5% (35). Biologic analysis of gene lists was performed with DAVID annotation tool (http://david.abcc.ncifcrf.gov/; ref. 36). Time to tumor recurrence (TTR) was defined as the period of time from surgery to the date of the first distant relapse. Overall survival from metastatic disease (OSmet) was defined as the period of time of metastatic disease to death or last follow-up. Estimates of survival were from the Kaplan–Meier curves and tests of differences by the log-rank test. Univariate Cox models were used to test the independent prognostic significance of each variable. All statistical computations were carried out in R v2.15.1 (http://cran.r-project.org). All statistical tests were two sided, and the statistical significance level was set to less than 0.05.

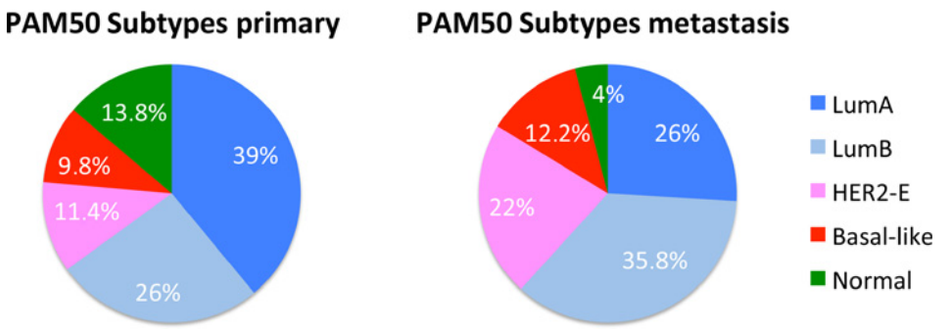

Figure 1.
Distribution of intrinsic subtype in primary versus metastatic disease.

Results

Clinical–pathologic characteristics

A total of 123 patients were included (Supplementary Table S2). The median age at breast cancer diagnosis was 52.5 years (range, 28–90). In primary disease, the immunohistochemical analyses showed 73.17% ($n = 90$) of patients had HR-positive (HR+), 15.45% ($n = 19$) HER2-positive (HER2+), and 9.76% ($n = 12$) triple-negative disease. In metastatic disease, 69.92% ($n = 86$) of patients had HR+, 19.51% ($n = 24$) HER2+, and 9.76% ($n = 12$) triple-negative disease. No significant differences ($P > 0.502$) were observed in the distribution of the three IHC groups in primary versus metastatic disease. Fourteen patients (11.38%) presented with *de novo* metastatic disease. Median follow-up and OSmet were 76.5 and 84 months, respectively (Supplementary Fig. S1).

Type of metastatic tissues

The organs of origin of the metastatic biopsies analysed in this study were skin ($n = 35$; 28.4%), lymph nodes ($n = 24$; 19.5%), liver ($n = 20$; 16.3%), bone ($n = 16$; 13%), lung ($n = 7$; 5.7%), ovarian and peritoneum ($n = 7$; $n = 5.7$%), pleural ($n = 6$; 4.9%) and others ($n = 8$; 6.5%), including brain, pericardial fluid, and colon metastases (Supplementary Fig. S2).

Subtype distribution

The distribution of the PAM50 intrinsic subtype classification in primary tumor versus metastatic disease was 39% versus 26% for luminal A ($P = 0.029$), 26% versus 35.8% for luminal B ($P = 0.097$), 11.4% versus 22% for HER2-E ($P = 0.026$) and 9.8% versus 12.2% for basal-like tumors ($P = 0.540$; Fig. 1). Individually, subtype concordance was high for basal-like (100%), HER2-E (76.9%), and luminal B (70.0%) tumors (Table 1). Regarding luminal A primary tumors, 44.7% remained luminal A in the metastasis, switching to luminal B in 40.4% and HER2-E in 14.9% of the cases. Overall, primary luminal tumors (A and B combined) changed to a HER2-E in 14.28%, despite 81% of them being clinically HER2 negative. Cohen kappa coefficient was 0.38 [95% confidence interval (CI), 0.27–0.5, $P < 0.001$]. These results were not affected when the claudin-low classification was investigated as no claudin-low tumor was identified in this series. Finally, we observed that liver and lung metastases showed the highest and lowest subtype conversion rate (75% and 14%), respectively. However, these results by site of metastasis need further validation due to the small sample sizes (Supplementary Table S3).

Expression changes of individual signatures

We evaluated the expression changes of each individual signature between primary and their metastatic samples. Luminal A and normal-like signatures were found significantly less expressed in metastatic tumors than in primary tumor (Fig. 2). In contrast, luminal B, HER2-E, and proliferation signatures were found more expressed in metastatic tumors than in primary tumors. Finally, the expression of basal-like, VEGF/hypoxia and claudin-low signatures was similar between primary and metastatic disease (Fig. 2).

Expression changes of individual genes

Among 105 breast cancer–related genes, 16 and 31 genes were found up- and downregulated in metastatic tissues compared with primary tissues (FDR < 5%; Table 2). The upregulated gene list was enriched for genes involved in survival and migration (e.g., *FGFR4*), cell cycle (e.g., *CDC6* and *CCNB1*), and DNA repair (e.g., *TYMS*). The downregulated gene list was enriched for genes involved in response to hormone stimulus (e.g., *BCL2* and *PGR*; Fig. 2), differentiation (e.g., *GATA3*) and chromatin regulation (e.g., *CXXC5*).

A similar analysis was performed within each of the subtypes identified in primary disease. Concordant with the subtype changes, 25, 8, 7, and 0 genes were found differentially expressed in luminal A, luminal B, HER2-E, and basal-like primary disease, respectively, compared with metastatic disease (Supplementary Table S4).

Association with overall survival

We evaluated the ability of the 10 signatures to predict OSmet in primary (Fig. 3A) versus metastatic (Fig. 3B) disease. Interestingly, no signature consistently predicted OSmet in both primary

Table 1. Subtype concordance between primary and metastatic disease

	Metastatic disease				
Primary disease	**Basal-like**	**HER2-E**	**Luminal A**	**Luminal B**	**Genes differentially expressed (FDR<5%)**
Basal-like	12 (100%)	0	0	0	0
HER2-E	2 (15.38%)	10 (76.92%)	1 (7.7%)	0	7
Luminal A	0	7 (14.9%)	21 (44.68%)	19 (40.42%)	25
Luminal B	0	4 (13.33%)	5 (16.67%)	21 (70%)	8

Abbreviation: FDR, false discovery rate.

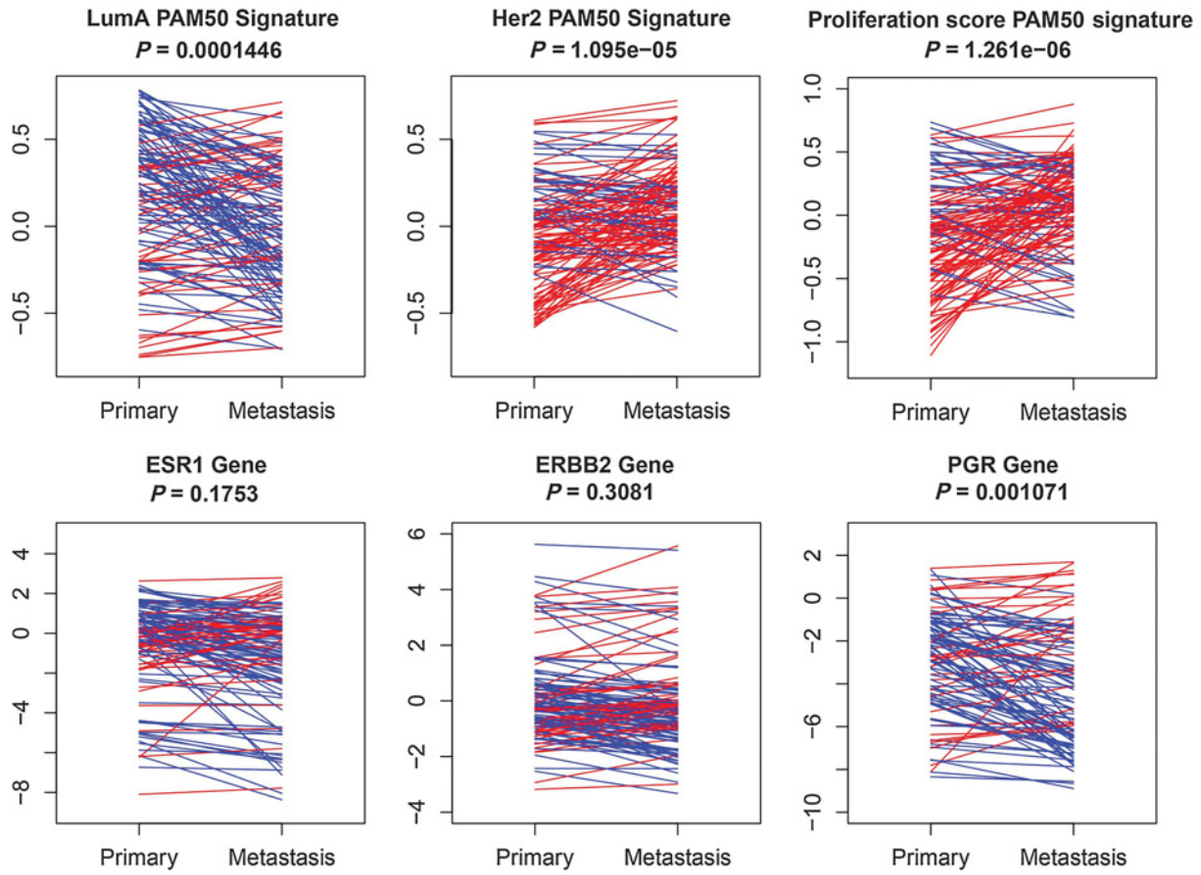

Figure 2.
Gene and signature expression changes between primary and metastasis. *P* value was obtained after performing a paired *t* test.

and metastatic disease. In primary disease, basal-like signature was found associated with worse outcome (HR = 1.50, P = 0.007), while the VEGF/Hypoxia signature was associated with a better outcome (HR = 0.65, P = 0.016). In metastatic disease, proliferation was found associated with worse outcome (HR = 1.40, P = 0.047).

These results suggested that OSmet might be better predicted by measuring either the primary tumor or the metastatic tumor depending on the biological process (e.g., proliferation) being evaluated. To further explore this, we evaluated the ability of each individual gene to predict OSmet in primary versus metastatic disease. Among 105 genes, 14 and 10 genes were found associated with OSmet in primary and metastatic disease, respectively. Interestingly, only one gene (*GATA3*) consistently predicted favorable outcome in both settings (Fig. 4). In primary disease, high expression of 13 of the 14 genes was found associated with better outcome. These 13 genes (e.g., *PGR*, *ESR1*, and *FOXA1*) were mostly tracking luminal-related biological processes. On the contrary, high expression of 8 of the 10 genes in metastatic disease was found associated with worse outcome. These 8 genes (e.g. *MYC*, *CCNE1*, and *CCNB1*) were mostly tracking cell cycle/proliferation-related biological processes.

Finally, we explored the ability of each gene signature to predict OSmet in patients with tumors with no subtype conversion (n = 59) versus patients with tumors without subtype conversion (n = 49). The results revealed that in patients with no subtype conversion, the associations of signatures with OSmet were very similar when the primary or the metastatic tumors were evaluated. In patients with subtype conversion, the associations of signatures with OSmet were generally different when the primary or the metastatic tumors were evaluated. Among them, the HER2-E signature was found significantly associated with poor outcome (HR = 1.86, P = 0.046) when evaluated in metastatic tumors but not when evaluated in primary disease (Supplementary Fig. S3).

Magnitude of gene expression changes versus TTR

To evaluate if the gene expression changes observed in metastatic tissues are a reflection of tumor evolution over time, we plotted the magnitude of change of the expression of each signature versus TTR (Fig. 5). The results revealed a positive correlation between TTR and HER2-E (corr = 0.324, P < 0.001), luminal B (corr = 0.27, P = 0.004), Proliferation score (corr = 0.291, P = 0.002), and ROR-P (corr = 0.295, P = 0.001). In contrast, normal-like and luminal A signatures showed a negative correlation with TTR (corr = −0.285, P = 0.002; corr = −0.219, P = 0.019, respectively).

Gene-by-gene analysis revealed a positive correlation between TTR and the magnitude of change of genes implicated in cell proliferation (*CEP55*: corr = 0.244, P = 0.024), mitogenesis, and differentiation biological process (*FGFR4*: corr = 0.211, P =

Table 2. List of up- and downregulated genes differentially expressed between metastatic versus primary disease across all samples (FDR<5%)

Gene name	Gene symbol	Score (d)	Fold change	FDR (%)
Fibroblast growth factor receptor 4	FGFR4	3.38	1.74	0
Cell division cycle 6	CDC6	2.14	1.29	1.90
Maternal embryonic leucine zipper kinase	MELK	1.99	1.27	1.90
Pituitary tumor-transforming	PTTG1	1.80	1.21	1.90
Cell division cycle 20	CDC20	1.79	1.23	1.90
Thymidylates synthetase	TYMS	1.75	1.22	1.90
Centrosomal protein	CEP55	1.73	1.24	1.90
Cyclin B1	CCNB1	1.71	1.19	1.90
Phosphoglycerate dehydrogenase	PHGDH	1.69	1.23	1.90
Keratin 8, Type II	KRT8	1.68	1.17	1.90
Ribonucleotide reductase M2	RRM2	1.67	1.22	1.90
Epithelial cell adhesion molecule	EPCAM	1.50	1.15	3.60
BCL2-Associated athanogene	BAG1	1.42	1.14	3.60
Ubiquitin-conjugating enzyme E2T	UBE2T	1.38	1.17	4.82
V-Myb myeloblastosis viral oncogene homolog-like 2	MYBL2	1.36	1.21	4.82
Serine Peptidase Inhibitor, Kunitz Type, 2	SPINT2	1.33	1.13	4.82
Transmembrane protein 158	TMEM158	−6.29	−1.34	0
Keratin 17, Type I	KRT17	−5.55	−1.22	0
Matrix metallopeptidase 11	MMP11	−5.39	−1.32	0
Secreted frizzled-related protein 1	SFRP1	−4.26	−1.39	0
Zinc finger E-box binding homeobox 1	ZEB1	−4.19	−1.57	0
Progesterone receptor	PGR	−3.72	−1.40	0
Crystallin, alpha B	CRYAB	−2.90	−1.59	0
Melanoma inhibitor activity	MIA	−2.82	−1.55	0
V-Myc myelocytomatosis viral oncogene homolog	MYC	−2.69	−1.67	0
N-Acetyltransferase 1	NAT1	−2.31	−1.62	0
Microtubule-associated protein tau	MAPT	−2.25	−1.64	0
Estrogen receptor	ESR1	−2.07	−1.65	0
Retinoid-inducible nuclear factor	CXXC5	−2.07	−1.80	0
Fibrilin 1	FBN1	−1.99	−1.72	0
AXL receptor tyrosine kinase	AXL	−1.88	−1.78	0
Forkhead box A1	FOXA1	−1.87	−1.72	0
Solute carrier family 16	SLC16A3	−1.82	−1.78	0
Lipoma HMGIC fusion partner	LHFP	−1.74	−1.78	0
Solute carrier family 39 member 6	SLC39A6	−1.73	−1.75	0
Caveolin 1	CAV1	−1.59	−1.78	0
GATA binding protein 3	GATA3	−1.54	−1.79	0
Erb-B2 receptor tyrosine kinase 2	ERBB2	−1.52	−1.82	0
B-Cell CLL/Lymphoma 2	BCL2	−1.41	−1.80	1.35
DNA damage inducible transcript 4	DDIT4	−1.17	−1.85	3.60
Claudin 7	CLDN7	−1.10	−1.87	3.60
Cadherin 3	CDH3	−1.10	−1.82	3.60
Adrenomedullin	ADM	−1.07	−1.83	4.82
Phosphatase and tensin homolog	PTEN	−1.06	−1.91	4.82
Forkhead Box C1	FOXC1	−1.03	−1.82	4.82
MET proto-oncogene, receptor tyrosine kinase	MET	−1.02	−1.83	4.82
Vimentin	VIM	−0.99	−1.88	4.82

0.044). In contrast, a negative correlation was observed with genes that participate in cell-to-cell adhesion (*CLDN4*: corr = −0.207, $P = 0.027$; *F11R*: corr = −0.237, $P = 0.01$), regulation of DNA damage repair (*RAD17*: corr: −0.226, $P = 0.017$), tumor suppression (*GRHL2*: corr = −0.186, $P = 0.05$), mammary gland development (*PGR*: corr = −0.203, $P = 0.045$), and that attenuate cell migration (*ESRP1*: corr = −0.252, $P = 0.006$).

Discussion

Here, we explored RNA-based expression differences between paired primary and metastatic breast tumors and made the following observations: (i) intrinsic molecular subtype is largely maintained during metastatic recurrence, except for luminal A disease, which converted to luminal B and HER2-enriched in 55% of the cases; (ii) metastatic tissues show higher expression of proliferative and lower expression of luminal-related genes compared to primary tumors, except for basal-like disease, which seems to be very stable from a RNA-based perspective; (iii) different biological processes can predict overall survival from recurrence when evaluated in primary versus metastatic disease; (iv) an intriguing relationship seems to exist between the time taken to develop detectable metastases and the aggressiveness of the tumor, indicating that a tumor might evolve towards a more aggressive phenotype as time evolves.

Previous studies have evaluated the rates of change of the three classical pathologic biomarkers (i.e., ER, PR, and HER2) between primary and metastatic tumors (37, 38). Overall, the rates of ER, PR, and HER2 conversion were 13%, 28%, and 3%–10%, respectively (11). Among the three genes, we also observed *PGR* as the top downregulated gene in metastatic compared with primary tissues. Nonetheless, the three classical biomarkers are largely

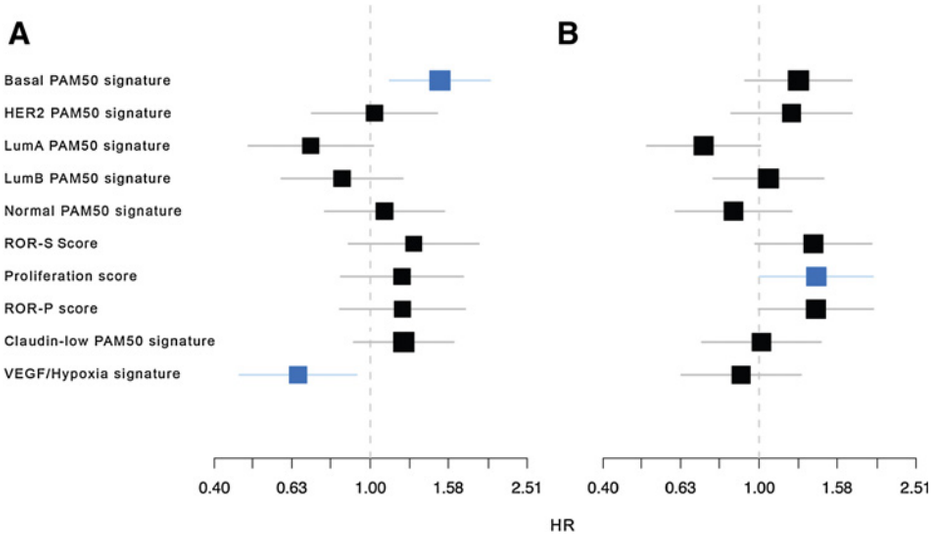

Figure 3.
Association of 10 signatures with OSmet when evaluated in primary (**A**) and metastatic (**B**) disease. Each signature was evaluated as a continuous variable and was standardized to have a mean of 0 and a SD of 1. The size of the square is inversely proportional to the SE; horizontal bars represent the 95% CIs of HRs. Statistically significant variables are shown in blue. Each gene signature was evaluated in a univariate analysis.

maintained in the metastatic setting, which is concordant with our findings using the basal-like, HER2-enriched, and luminal A/B intrinsic subtype classification. At the same time, prior gene expression-based studies with smaller number of patients are concordant with our findings (39–41). However, Lee and colleagues evaluated the PAM50 intrinsic subtypes in 17 paired samples of primary and brain metastasis, and subtype conversion was observed in 47.1% of the cases, which is higher than the 30.9% conversion rate observed in our study. However, similar to our study, a large proportion of luminal A primary tumors (1/6) changed to non-luminal A disease, and all basal-like primary tumors ($n = 6$) remaining basal-like at recurrence (42).

Other studies have evaluated changes in somatic mutations and gene copy-number aberrations (CNA) between primary and metastasis. For example, Meric-Bernstam and colleagues (43) performed targeted DNA sequencing of 3,320 exons of 182 cancer-related genes plus 37 introns from 14 genes in 74 tumors. In 33 matched primary and recurrent tumors, 97 of 112 (86.6%) somatic mutations were concordant. Of identified CNAs, 136 of 159 (85.5%) were concordant. There was an increased frequency of *CDK4/MDM2* amplifications in recurrences, as well as gains and losses of other actionable alterations. The authors concluded that analysis of recurrent tumors before treatment may provide additional insights, as both gains and losses of targets are observed. In another study, Ding and colleagues (41) described the genomic analyses of four DNA samples from an African-American patient with basal-like breast cancer: peripheral blood, the primary tumor, a brain metastasis, and a xenograft derived from the primary tumor. Of the 50 validated point mutations and small indels, 48 were detectable in all three tumors. Overall, while additional somatic mutations, copy number alterations, and structural variations occurred during the clinical course of the disease, most of the original mutations and structural variants present in the primary tumor were propagated.

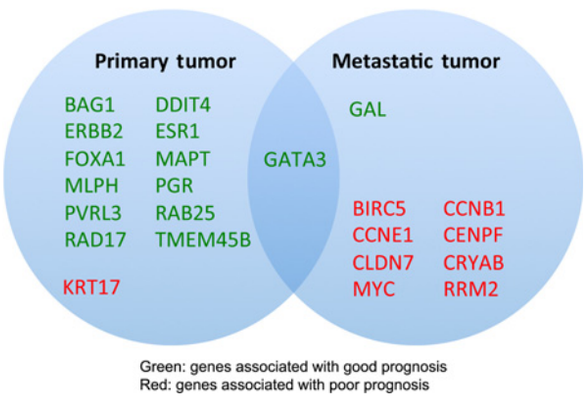

Figure 4.
Venn diagram of genes that predict overall survival from the data of recurrence when analyzed in primary versus metastatic disease. Green, genes associated with good prognosis; red, genes associated with poor prognosis.

Similar to prior studies looking at DNA alterations, we did not identify large absolute expression changes at the RNA level between primary and metastatic disease. Nonetheless, 47 genes were found differentially expressed, mostly within luminal A/B disease. Among them, *FGFR4* was detected as the top upregulated gene in metastatic disease. Interestingly, this gene is found in the PAM50 gene list and its overexpression is characteristic of the HER2-E intrinsic subtype. Fibroblast growth factor receptors are involved in development, differentiation, cell survival, migration, angiogenesis and carcinogenesis (44). Dimerization of the receptor leads to intracellular phosphorylation of receptor kinase domains and intracellular signal transduction, including *RAS/RAF/MEK* and *PI3K/AKT* pathways (45). These evidences suggest that *FGFR4* could drive the HER2-E phenotype in metastatic lesions with a HER2-negative/HER2-E profile. Indeed, we observed that the 8 patients whose tumors changed from luminal A/B in primary disease to HER2-E in metastasic disease showed an increase in *FGFR4* expression but not *ERBB2* expression (Supplementary Fig. S4). Of note, HER2-E subtype has been associated with estrogen-independent growth and poor outcome in patients with HR^+/HER2-negative breast cancer treated with anti-estrogens (46, 47). Further mechanistic studies are needed to elucidate the role of *FGFR4* in metastatic disease.

Currently, large-phase III clinical trials, especially within HR+/HER2-negative disease, are not taking into account this

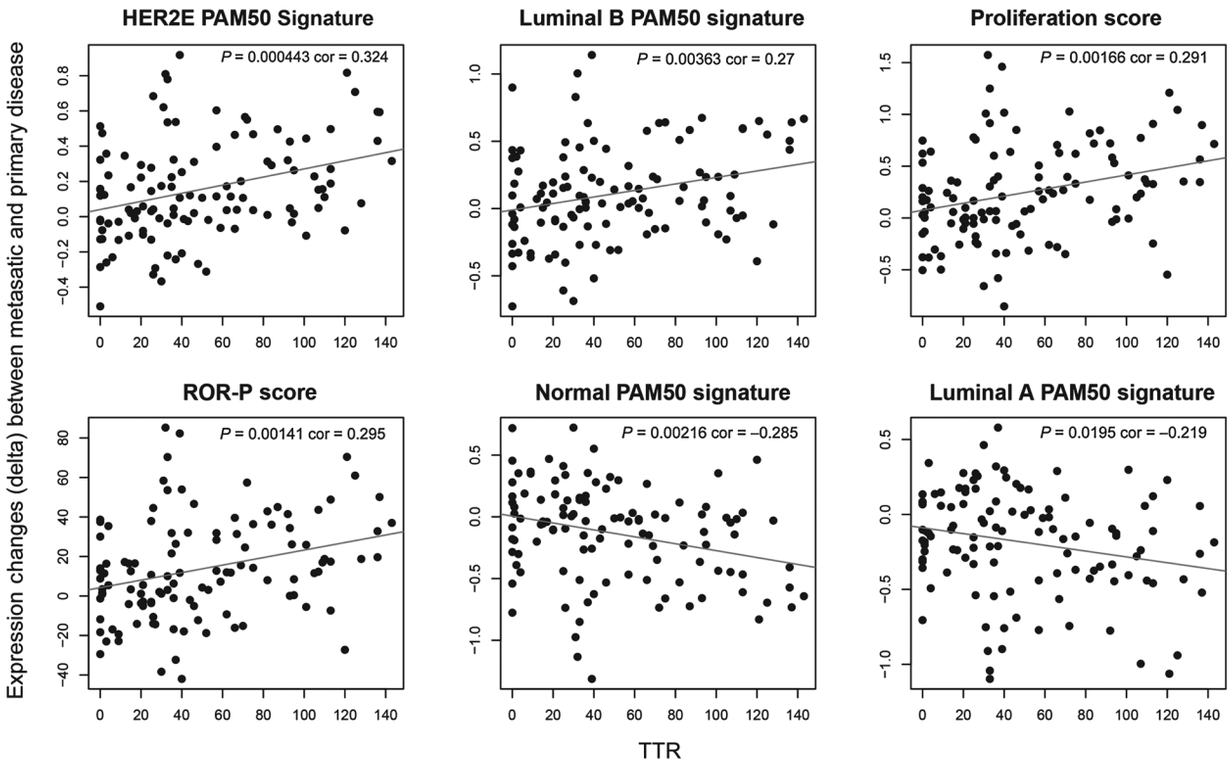

Figure 5.
Correlation between time to tumor recurrence (TTR) and the magnitude of gene/signature expression changes between primary and metastatic disease.

biological heterogeneity, such as proliferation, which is not well captured by HR and HER2 statuses. For example, patients with a luminal A profile following endocrine therapy might be treated with second-line endocrine therapy while those that change to a HER2-negative/HER2-E or luminal B profile might be treated with chemotherapy or other novel combinatory strategies such as endocrine therapy and *CDK4/6* inhibition. Overall, this result suggests that, although there is some stability of the intrinsic subtype, approximately 40% of the tumors will change subtype, highlighting the need to biopsy metastatic disease to better understand the clinical and biological evolution of a tumor.

Another interesting observation was the significant correlation between the magnitude of gene expression changes of various signatures between primary and metastasis disease and the time from diagnosis to tumor recurrence. Specifically, we observed that the longer the time to recurrence, the more aggressive the tumors become based on proliferation and expression of luminal genes. This suggests that there is an intrinsic evolution of tumor cells towards a more aggressive phenotype as time elapses. However, the correlation coefficients were weak and thus the magnitude of gene expression changes might also be explained by other variables such as the treatments received in (neo)adjuvant setting.

This study has several limitations worth noting. First, this is a retrospective study using tumor samples from different hospitals and a selection bias is plausible. Second, patients received different adjuvant and/or metastatic systemic treatments and thus we could not evaluate treatment effects on tumor biology or survival. However, subtype conversion of the 14 patients with *de novo* metastatic disease was found to be 57.1%, suggesting that subtype conversion is independent of treatment effects. More studies are needed to address this particular question. Third, metastatic tumor biopsies were not always collected at the time of the diagnosis of recurrent disease. Fourth, we did not analyze DNA mutations such as *ESR1* whose incidence is known to increase during tumor progression (12). Further studies will be able to evaluate if the gene expression changes observed during progression of luminal breast cancer are related to the appearance of *ESR1* mutations.

To conclude, most biological changes occurring during metastatic progression of breast cancer are largely unknown today. Here, we compared intrinsic molecular subtype and expression of individual genes in paired primary and metastatic tissues. Our results suggest that although intrinsic subtype is largely maintained during metastatic progression, luminal/HER2-negative tumors acquire a luminal B or HER2-E profile during metastatic progression, likely reflecting tumor evolution and/or acquisition of estrogen-independency. Moreover, our study highlights the importance of molecular characterization of metastatic disease.

Disclosure of Potential Conflicts of Interest

C.M. Perou is a Board of Directors Member, has ownership interest (including patents), and is a consultant/advisory board member for Bioclassifier LLC. A. Prat is a consultant/advisory board member for NanoString Technologies. No potential conflicts of interest were disclosed.

Disclaimer

The funders did not have any role in the study design, conduct, or decision to submit the manuscript for publication (CERCA Programme/Generalitat de Catalunya).

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Grant Support

This work was supported by funds from Instituto de Salud Carlos III - PI13/01718 (A. Prat), by a Career Catalyst Grant from the Susan Komen Foundation (A. Prat), by Banco Bilbao Vizcaya Argentaria (BBVA) Foundation (A. Prat), and by the Breast Cancer Research Foundation. This work was also supported by funds from FEDER (RETICC): RD12/0036/0051 to J. Albanell and F. Rojo, RD12/0036/0070 to A. Lluch and RD12/0036/0076 to M. Muñoz, CIBERONC-CB16/12/00241 to J. Albanell and F. Rojo, and CIBERONC-CB16/12/00481 to A. Lluch. J.M. Cejalvo holds a fellowship from "PhD4MD," a Collaborative Research Training Programme for Medical Doctors (IDIBAPS, August Pi i Sunyer Institute for Biomedical Research and IRB Barcelona, Institute for Research in Biomedicine) partially funded by Instituto de Salud Carlos III, (ISCIII, project: II14/00019). J.M. Cejalvo and R.R. Gomis were supported by the Spanish Ministry of Science and Innovation grant SAF2013-46196 (FEDER Funds) and the Generalitat de Catalunya AGAUR 2014-SGR grant 535.

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Received October 13, 2016; revised November 23, 2016; accepted February 17, 2017; published OnlineFirst March 1, 2017.

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