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Published in:
The Breast Journal

DOI:
10.1111/tbj.12596

Publication date:
2016

Document version:
Final published version

Document license:
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Citation for pulished version (APA):
Krøigård, A. B., Larsen, M. J., Thomassen, M., & Kruse, T. A. (2016). Molecular Concordance Between Primary Breast Cancer and Matched Metastases. *The Breast Journal*, 22(4), 420-430. <https://doi.org/10.1111/tbj.12596>

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Molecular Concordance Between Primary Breast Cancer and Matched Metastases

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■ **Abstract:** Clinical management of breast cancer is increasingly personalized and based on molecular profiling. Often, primary tumors are used as proxies for systemic disease at the time of recurrence. However, recent studies have revealed substantial discordances between primary tumors and metastases, both with respect to traditional clinical treatment targets and on the genomic and transcriptomic level. With the increasing use of molecularly targeted therapy, discordance of actionable molecular targets between primary tumors and recurrences can result in nonoptimal treatment or unnecessary side effects. The purpose of this review is to illuminate the extent of cancer genome evolution through disease progression and the degree of molecular concordance between primary breast cancers and matched metastases. We present an overview of the most prominent studies investigating the expression of endocrine receptors, transcriptomics, and genome aberrations in primary tumors and metastases. In conclusion, biopsy of metastatic lesions at recurrence of breast cancer is encouraged to provide optimal treatment of the disease. Furthermore, molecular profiling of metastatic tissue provides invaluable mechanistic insight into the biology underlying metastatic progression and has the potential to identify novel, potentially druggable, drivers of progression. ■

Key Words: biopsy, breast cancer, cancer evolution, concordance, metastasis, targeted therapy

Breast cancer is the leading cause of cancer death in women worldwide (1). In spite of extensive treatment, about 30% of breast cancer patients will experience spread of the malignant disease to distant organs like liver, lungs, bone, and brain (2). The metastatic process of breast cancer is highly complex and to a large degree unexplored. Cancer cells are characterized by genomic instability and are believed to evolve in accordance with Darwinian positive selection and therefore further genetic evolution throughout progression might be expected (3). The term *oncogene addiction* (4) describes the cancer cell dependence of particular driver genes for the maintenance of the malignant phenotype and provides the rationale for targeted therapy. However, the distinction between

driver and passenger mutations is dynamic due to the shifting demands and challenges of a cancer cell, and a shift in oncogenic drivers can develop during progression (5). Genomic and transcriptomic discordances between primary tumors and metastases have the potential to reveal novel drivers of metastatic progression and, thus, biopsies of metastatic tissue are important for driving cancer research forward.

From the clinical perspective, the question of genetic disparity between a primary tumor and its recurrences becomes highly relevant not only to confirm the malignant diagnosis and preclude nonbreast malignancy but also due to the use of molecularly targeted therapy. Optimal management of cancer presupposes knowledge of actionable molecular targets in the malignant cells and therefore potential genomic discordance between primary tumors and recurrences poses a clinical challenge. Thus, genomic, transcriptomic, and proteomic concordance between primary tumors and metastases are highly relevant from both an academic and a clinical perspective.

Due to the extreme complexity of cancer biology, the complete overview of all genetic, epigenetic, tran-

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DOI: 10.1111/tbj.12596

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The Breast Journal, Volume 22 Number 4, 2016 420–430

scriptomic, and proteomic manifestations is very difficult to manage within one single study. Different technical approaches at each layer of biology result in a varying degree of detail and each study typically uncovers only a fraction of the complete picture.

The aim of this study was to provide an overview of the research field of concordance between primary breast tumors and matched metastases. The current review is not exhaustive of all published studies in the field, but presents a selection of the most prominent work. Comprehensive literature searches were conducted in PubMed with combinations of the following search terms “breast cancer”, “breast carcinoma”, “biopsy”, “metastasis”, “metastases”, “recurrence”, “concordance”, “discordance”, “ER, HER2”, “gene expression profiling” “CGH”, “aCGH”, “next generation sequencing”, and “epigenetics”. Only studies comparing matched primary tumors and metastases were considered. Studies were screened by titles and abstracts and evaluated according to relevance, scientific method, and impact in the research field.

ENDOCRINE RECEPTORS

From a clinical point of view, focused investigations of targetable molecular markers are highly relevant. Tables 1 and 2 display selections of studies evaluating concordance of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), respectively, between paired primary tumors and metastases. The studies quite consistently show surprisingly high dis-

cordance rates. It has been debated whether receptor discordance is due to technical issues such as poor reproducibility of the immunohistochemical technique, inter- or intraobserver variance, or reflect a true biological phenomenon. Obviously, correct tissue handling and fixation is crucial and the current recommendations for clinical testing should be met (6,7), however, analytical errors cannot account for all of the discordances. Aurilio et al. performed a meta-analysis of 48 articles evaluating ER, PR, and HER2 receptor discordance between primary breast cancer and metastases (8). The meta-analysis included 4200 patients evaluating ER status and found pooled proportions of tumors shifting from positive to negative and negative to positive ER status of 24% and 14%, respectively. For PR status the same figures were 46% and 15%, and for HER2 status 13% and 5%, and these data were based on a total of 2,739 and 2,987 patients, respectively. This clearly signals that a loss of receptor is more frequent than gain of receptor, confirming a biological phenomenon.

Most of the studied asynchronous metastases have been subject to adjuvant treatment, which may explain the high discordance rates. Cancer cell populations evolve dynamically and may be molded by the selective pressures provided by treatment. Stratification of patients allows insight into the phenomenon. Lindström et al. reported that the proportion of patients losing ER was highest in the group of patients treated with endocrine therapy alone or in combination with chemotherapy, lower in the group treated with chemotherapy alone, and lowest in the

Table 1. Selection of Studies Analyzing Discordance of ER and PR Receptors Between Primary Tumors and Matched Metastases

Author (ref)	Year	Method	No. patients	Analyzed metastatic site	Intervening treatment between primary tumor and metastases	Gain of ER, %	Loss of ER, %	Gain of PR, %	Loss of PR, %
Simmons et al. (51)	2009	IHC	25	Asynchronous metastases	Yes, in some patients	0	12	0	28
Amir et al.* (52)	2012	IHC	117	Asynchronous metastases	Yes, in some patients	16	15	8	74
Lindström et al.* (9)	2012	IHC	459/430	Asynchronous metastases	Yes, in some patients	7	24	7	33
Jensen et al. (53)	2012	IHC	118	Asynchronous metastases	Yes, in some patients	3	8	–	–
Heitz et al. (54)	2013	IHC	411	Asynchronous metastases	Yes, in some patients	19	22	16	41
Curtit et al. (55)	2013	IHC	235	Asynchronous metastases	Yes, in some patients	5	12	7	22
Aurilio et al. (56)	2013	IHC	107	Asynchronous metastases	Yes, in some patients	3	18	3	40
Ibrahim et al. (57)	2013	IHC	120	Asynchronous metastases	Not specified	7	9	8	33
Aurilio et al. (8)	2013	MA	4,200/2,739	Asynchronous metastases	Yes, in some patients	14	24	15	46
Hoefnagel et al. (15)	2013	IHC	55	Several asynchronous metastases	Not specified	–	–	–	–
Yang et al. (12)	2014	IHC	133	Asynchronous metastases	Yes, in some patients	3	15	6	27
Qu et al. (58)	2014	IHC	48	Asynchronous metastases	Yes, in some patients	4	10	2	14

*Included in the meta-analysis by Aurilio et al.
IHC, immune histochemistry; MA, meta-analysis.

Table 2. Selection of Studies Analyzing Discordance of the HER2 Receptor Between Primary Tumors and Matched Metastases

Author (Ref)	Year	Method	No. patients	Analyzed metastatic site	Intervening treatment between primary tumor and metastases	Gain of HER2, %	Loss of HER2, %
Leni et al. (11)	2014	IHC, FISH	148	Synchronous ALN metastases	No	2	3
Simmons et al. (51)	2009	FISH	25	Asynchronous metastases	2% of patients received trastuzumab	8	0
Amir et al.* (52)	2012	IHC, FISH	117	Asynchronous metastases	4% of patients received trastuzumab	8	20
Lindström et al.* (9)	2012	IHC, FISH	104	Asynchronous metastases	Not specified	5	8
Jensen et al. (53)	2012	IHC, FISH, CISH	114	Asynchronous metastases	One patient received adjuvant trastuzumab. The two patients that lost HER2 positivity had not received trastuzumab	7	2
Chan et al. (59)	2012	SISH, FISH	116	Asynchronous metastases	8% of patients had received adjuvant trastuzumab, including the patient who was HER2 discordant	0%	1%
Niikura et al. (13)	2012	IHC, FISH	182	Asynchronous metastases	41% of patients had received adjuvant trastuzumab	–	24
Nakamura et al. (10)	2013	IHC, FISH	156	Asynchronous metastases	Adjuvant trastuzumab/no treatment	5	3
Heitz et al. (54)	2013	IHC, FISH	411	Asynchronous metastases	3% of patients had received adjuvant trastuzumab	11	40
Curtit et al. (55)	2013	IHC, FISH	235	Asynchronous metastases	3% of patients had received adjuvant trastuzumab	1	3
Aurilio et al. (56)	2013	IHC, FISH	86	Asynchronous metastases	One patient received adjuvant trastuzumab	4	2
Ibrahim et al. (57)	2013	IHC, FISH	120	Asynchronous metastases	None of the patients had received adjuvant trastuzumab	12	5
Aurilio et al. (8)	2013	MA	2,987	Asynchronous metastases	Not specified	5	13
Hoefnagel et al. (15)	2013	IHC, SISH	55	Several asynchronous metastases	Not specified	–	–
Yang et al. (12)	2014	IHC, FISH	133	Asynchronous metastases	9% of patients received trastuzumab	3	3
Qu et al. (58)	2014	IHC	48	Asynchronous metastases	Not specified	4	4

*Included in the meta-analysis by Aurilio et al.

IHC, immune histochemistry; CISH, chromogenic in situ hybridization; FISH, fluorescence in situ hybridization; SISH, silver in situ hybridization; MA, meta-analysis.

group of patients who received no treatment ($p < 0.01$) (9). A similar effect for HER2 expression was reported by Nakamura et al. showing that the negative conversion rate of HER2 expression in metastatic lesions was 37% in patients treated with trastuzumab and only 6% in patients not treated with trastuzumab ($p < 0.05$) (10). This illustrates how receptor positive cancers develop therapy-resistant metastases due to positive selection of receptor negative cancer cells. That receptor discordance is highly influenced by selective pressures of treatment is supported by the low number of discordant cases in a study by Leni et al. comparing primary tumors and synchronous metastases, i.e., without intervening treatment (11).

Recently, Yang et al. found that among 105 patients ER discordance and HER2 discordance between primary tumors and distant metastases resulted in a worse overall survival and postrecurrence survival ($p < 0.05$) compared with concordant cases (12). Concordantly, Niikura et al., in a study includ-

ing only patients with HER2-positive primary tumors, found that patients with discordance of HER2 status had shorter overall survival than did patients with concordant HER2 status ($p = 0.003$) (13). Lindström et al. reported that women with ER-positive primary tumors that changed to ER-negative tumors had a significant 48% increased risk of death compared with women with stable ER-positive tumors (9). This intuitively makes sense, as tumors that are able to evade targeted treatment by altering the expression of receptors are more difficult to eradicate.

Conclusively, changes in ER and HER2 receptor expression seem to be a frequent phenomenon in breast cancer progression correlating with worse prognosis and, thus, having both prognostic and clinical implications. American guidelines recommend HER2 receptor status to be reevaluated at recurrence by biopsy at the metastatic site (7). The European Society for Medical Oncology (ESMO) second international consensus guideline for advanced breast cancer (Nov. 2013) states that a biopsy of a metastatic lesion should be per-

formed, if clinically feasible, to reassess biomarkers (especially ER and HER2) at least once in the metastatic setting (14). Addressing the issue of discordance between primary tumors and metastases, it is recommended at the level of an expert opinion, to consider use of targeted therapy when receptors are positive in at least one biopsy, regardless of timing (14).

It is, however, important to stress that biopsies from different metastatic sites may be discordant. A study by Hoefnagel et al. reports significant discordance in ER status and PR status across different metastases within the same patient (15). Thus, a single metastatic site may not be representative of all disseminated cancer cells and therefore, e.g., loss of ER expression in a single metastatic biopsy does not necessarily indicate that the patient will no longer benefit from anti-estrogen treatment. Expression of the ER receptor is in many cases epigenetically, thus reversibly regulated and influenced by anti-estrogen treatment and therefore re-expression of the receptor is possible if endocrine treatment is discontinued. The effect of changing the treatment according to biopsy results at recurrence needs to be evaluated in large patient cohorts.

GENE EXPRESSION PROFILING

Few studies have performed gene expression profiling of primary breast cancers and matched axillary lymph node metastases (16–19) as seen in Table 3.

Most studies have found gene expression patterns of primary tumors and matched lymph node metastases to be strikingly similar. Despite the high similarity, some studies conclude that breast cancer metastases are molecularly distinct from their primary tumors, and genes differentially expressed between primary tumors and metastases are reported. However, a very small overlap exists between the lists of genes reported to be associated with the metastasis phenotype between each study.

Very few studies have performed the comparison using asynchronous distant metastatic tissue. Weigelt et al. compared pairs of primary tumors and matched distant metastases and reported highly similar gene expression patterns (20), a metastasis-specific gene expression profile was not found, and the study concluded that metastatic capabilities were an inherent feature of the primary tumor.

The contradictory results might reflect the shortcomings of gene expression assays to resolve the puz-

zle. Breast cancer is an extremely heterogeneous disease and displays both intertumor heterogeneity and intratumor heterogeneity. The pattern of somatic mutations and copy number alterations as well as epigenetic modifications is highly unique to each cancer genome and, thus, the transcriptional differences between two breast cancer patients, even within the same subtype, may be greater than the transcriptional differences arising through progression of the disease and therefore remain elusive in many gene expression studies. Moreover, gene expression profiling does not allow identification of subpopulations within the cancer tissue and report the mixed picture of transcriptomes of cancer cells and stromal cells. Hao et al. show that the expression at the transcript level does not always correspond to expression at the protein level (17), thus emphasizing the caution to be taken when conclusions are drawn from gene expression studies.

Woditschka et al. performed gene expression profiling of matched primary tumors and brain metastases and found two genes to be differentially expressed (21). Studies of this nature address the issue of organotropism, the phenomenon of special affinity of cancer cells to particular organs, and have the potential to reveal novel drivers of metastatic progression.

NONCODING RNA AND EPIGENETICS

Gene expression regulation by noncoding RNAs and epigenetic mechanisms are likely to play key roles in the metastatic process as the identified metastasis suppressor genes are known to be transcriptionally downregulated, rather than being hit by inactivating mutations (22). Very few studies have explored differences in the expression of noncoding RNAs (23,24) and epigenetic mechanisms including methylation patterns (25–28) or histone acetylation comparing primary tumors and metastases. Differences in the expression levels of miRNAs between matched primary tumors and metastases have been found (23) and also long noncoding RNAs like HOTAIR have been shown to be differentially expressed (24). HOTAIR promotes metastasis by inducing chromatin conformational changes and methylation changes leading to gene expression patterns favorable for metastasis. Thus, the effect of increased transcription of a single long noncoding RNA can influence the expression of many genes and thereby set an entirely altered cell state. Also, DNA methylome changes during can-

Table 3. Selection of Studies Based on Gene Expression Profiling and DNA Methylation Profiling

Author (Ref)	Year	Method	No. patients	Analyzed metastatic site	Result
Weigelt et al. (20)	2003	GEP	8	Asynchronous distant metastases	Highly similar gene expression profiles of primary tumors and metastases
Hao et al. (17)	2004	GEP, TMA	9	Synchronous ALN metastases	Some genes differentially expressed between primary tumors and metastases
Suzuki and Tarin (18)	2007	GEP	10	Synchronous ALN metastases	Very similar gene expression profiles. A small number of genes were differentially expressed between primary tumors and metastases
Vecchi et al. (19)	2008	TMA	26	Synchronous ALN metastases	Some genes differentially expressed between primary tumors and metastases
Ellsworth et al. (16)	2008	GEP	20	Synchronous ALN metastases	51 differentially expressed genes
Feng et al. (27)	2010	MP	38	Synchronous ALN metastases	Investigation of six putative tumor suppressor genes suggests that hypermethylation of tumor suppressor genes is extended from primary tumors to metastases
Barekati et al. (28)	2012	MP	24	Synchronous ALN metastases	Investigation of 12 breast cancer candidate genes reveals methylation heterogeneity between primary tumors and metastases
Gravgaard et al. (23)	2012	miRNA microarray	14	ALN metastases, distant metastases	15 miRNAs were differentially expressed between primary tumors and corresponding distant metastases
Chisholm et al. (24)	2012	lncRNA in situ hybridization	54	Unspecified metastases	The lncRNAs HOTAIR and EZH2 had increased expression in metastases compared with matched primary tumors
Woditschka et al. (21)	2014	GEP of 502 cancer-related genes	23	Brain metastases	Two genes, <i>BARD</i> and <i>RAD51</i> , were overexpressed in brain metastases compared with both matched primary tumors and compared with unlinked systemic metastases
Reyngold et al. (25)	2014	MP, GEP	44	Regional metastases	Integrated analysis of methylation and gene expression identified genes whose expression correlated with metastasis-specific methylation
Moarii et al. (26)	2014	MP	48	Synchronous ALN metastases, local recurrences, contralateral breast carcinomas	Methylation patterns can help to distinguish local recurrences from new primary tumors

GEP, gene expression profiling; TMA, tissue microarray analysis; ALN, axillary lymph node; lncRNA, long noncoding RNA; MP, methylation profiling.

cer progression are still highly unexplored, but likely contribute massively to the metastatic phenotype. A recent study profiling the DNA methylome of 44 matched primary tumors and regional metastases was able to identify a metastasis-specific methylation signature (25).

TARGETED GENOMIC APPROACHES

Different targeted approaches have been employed to address the issue of genomic concordance between primary tumors and metastases, as seen in Table 4.

Moelans et al. investigated the copy number concordance between 55 primary tumors and corresponding distant metastases of 21 established oncogenes and tumor suppressor genes using multiplex ligation-dependent probe amplification (29). The study found overall no significant difference in copy numbers, concluding that there was overall little genomic progression from

primary breast tumors to their distant metastases. Conversely, other targeted studies have reported discordances. A targeted study based on Snapshot genotyping of 100 primary tumors and paired recurrences evaluating concordance of *PIK3CA* mutations found a net gain mutation rate in metastatic disease (30) as 21 patients changed genotype from wild type to mutant, compared with 11 patients who lost the mutation allele in the metastasis. Moreover, laser capture microdissection revealed microheterogeneity for the *PIK3CA* mutation in primary tumors, confirming subclonality among cancer cells. A recent study including 73 patients with matched primary tumors and asynchronous metastases identified five pairs with *PIK3CA* mutation in metastatic tissue but not primary tumor and two patients with mutation in primary tumor but not in the metastatic sample (31).

Using targeted next-generation sequencing (NGS), Meric-Bernstam et al. investigated the concordance of

Table 4. Selection of Studies Comparing Primary Tumors and Metastatic Lesions Using Targeted Genomic Approaches

Author (Ref)	Year	Method	No. patients	Analyzed metastatic site	Target	Result
Jensen et al. (30)	2011	TMA, SNaPshot gt, IHC	100	Asynchronous metastases	<i>PIK3CA</i> , <i>PTEN</i> , pAKT, Ki67, ER, HER2	<i>PIK3CA</i> mutation in 45% of primary tumors and 53% of metastases, showing a net gain in <i>PIK3CA</i> mutations. <i>PTEN</i> deficiency dropped from 26% in primary tumors to 20% in the metastases
Moelans et al. (29)	2014	MLPA	55	Asynchronous metastases	21 cancer-related genes	Overall no significant difference in mean MLPA copy numbers between primary tumors and metastases
Meric-Bernstam et al. (32)	2014	NGS	33	Asynchronous metastases	182 cancer-related genes	86.6% of the 112 somatic mutations detected were concordant. 62.3% of the 159 CNAs detected were concordant
Schleifman et al. (31)	2014	IHC, SNP gt	73	Asynchronous metastases	<i>PIK3CA</i> , <i>AKT1</i> , <i>PTEN</i> , Ki67.	Five pairs had <i>PIK3CA</i> mutation in metastatic tissue but not primary tumor and two pairs had <i>PIK3CA</i> mutation in the primary tumor but not in the metastatic sample
Deng et al. (47)	2014	Sanger s	17	CTCs, DTCs, and metastases	<i>PIK3CA</i>	Mutational discordance between CTCs, DTCs, and metastases, and among CTCs isolated at different time points
Rothé et al. (48)	2014	NGS	17	Metastases, cfDNA in plasma	50 cancer-related genes	In some cases, analysis of cfDNA in plasma seemed more representative of the mutational spectrum of the metastatic disease than a single biopsy of a metastatic lesion
De Mattos Arruda et al. (49)	2014	NGS	1	Synchronous bone and liver metastases, cfDNA in plasma	300 cancer-related genes	A longitudinal monitoring of the patient with liquid biopsies the mutant allele fractions identified in the cfDNA varied over time and mirrored the pharmacodynamic response

TMA, tissue microarrays; IHC, immune histochemistry; ALN, axillary lymph node; CNA, copy number aberration; CTCs, circulating tumor cells; DTCs, disseminated tumor cells; MLPA, multiplex ligation probe amplification; NGS, next-generation sequencing; Gt, genotyping; Ds, direct sequencing; S, sequencing.

182 cancer-related genes in 33 paired primary tumors and recurrences (32). The study found that 86.6% of the somatic mutations and 62.3% of the copy number aberrations were concordant between primary tumors and recurrences and that actionable molecular targets to be lost or gained in the metastases compared with matched primary tumors.

GLOBAL GENOMIC APPROACHES

A number of studies have applied comparative genomic hybridization (CGH) to detect copy number discordances between primary breast tumors and matched metastases, as seen in Table 5, with rather contradictory results. Some studies found a significantly higher number of aberrations in metastases compared with primary tumors (33,34), whereas

others did not (35–38). Using CGH, Torres et al. found extensive clonal divergence between primary tumors and metastases and higher frequency of alterations in primary breast cancers with metastases compared with tumors with no metastatic spread (38). This finding is also reported by Desouki et al. using array CGH (39). Conversely, also using array CGH, Poplawski et al. found 20% higher number of aberrations in primary tumors compared with metastases (40). This result is quite surprising as the general assumption is that additional genetic aberrations can accumulate through cancer progression due to the inherent genomic instability underlying cancer. One explanation might be the heterogeneity within the primary tumor, where several subclones with adverse aberrations coexist, whereas metastases are seeded from one of these subclones and therefore contains

Table 5. Selection of Studies Comparing Primary Tumors and Metastatic Lesions Using Global Genomic Approaches

Author (Ref)	Year	Method	No. patients	Analyzed metastatic site	Result
Nishizaki et al. (34)	1997	CGH	16	Local recurrences, ALN metastases, or distant metastases	The total number of aberrations detected exclusively in the lymph node metastases or distant metastases was higher than that for the primary tumors (2.5 versus 0.7 $p < 0.05$)
Kuukasjärvi et al. (35)	1997	CGH	29	Asynchronous metastases	Mean number of changes detected by CGH was 8.7 (range 0–20) in primary tumors and 9.0 (range 1–24) in metastases—no significant difference. Pairwise clonality analysis of matched primary tumors and metastases revealed that 69% of the metastases were highly similar to their primary tumor and 31% of the metastases were genetically different from their primary tumor
Torres et al. (38)	2007	CGH	12	ALN metastases	No significant difference in the number of genomic imbalances between primary tumors and metastases. Clustering analysis showed extensive clonal divergence between primary tumors and metastases. Higher frequency of alterations in breast cancers with metastases than in tumors without metastases
Friedrich et al. (33)	2008	CGH	40	Local recurrences, ALN metastases, systemic metastases	Significant higher number of chromosomal imbalances in local recurrences (22.6 versus 16.2 $p = 0.02$), ALN metastases (21.5 versus 18.0 $p = 0.03$) and systemic metastases (28.1 versus 22.4 $p = 0.02$) compared with primary tumors
Li et al. (36)	2008	CGH	29	ALN metastases	26 of 29 pairs clustered together in unsupervised hierarchical clustering
Santos et al. (37)	2008	CGH	20	SLN metastases	No significant difference in copy number aberrations between primary tumors and SLN
Shah et al. (41)	2009	NGS	1	Asynchronous distant metastasis	Of 32 coding, nonsynonymous mutations detected in the distant metastasis, 5 mutations were present in the primary tumor, 6 mutations were subclonally present in the primary tumor, 19 mutations were not detected in the primary tumor, and 2 were undetermined
Poplawski et al. (40)	2010	aCGH	13	Synchronous ALN metastases	20% higher number of aberrations in primary tumors (mean 33) compared with metastases (mean 27)
Desouki et al. (39)	2010	aCGH	30	Synchronous ALN metastases	Higher frequency of copy number alterations in breast cancers with metastases than in tumors without metastases. Similar number of copy number alterations in primary tumors and ALN metastases
Ding et al. (42)	2010	NGS	1	Asynchronous distant metastasis and xenograft	Two de novo mutations and a large deletion exclusively found in the distant metastasis, 20 shared mutations at higher frequency in the distant metastasis
Kroigård et al. (43)	2015	NGS	1	Asynchronous distant metastasis	In the distant metastasis, 17 additional nonsynonymous point mutations were found. The distant metastasis retained all previous copy number aberrations and displayed 18 copy number loss and 18 copy number gain events in addition to the early acquired copy number events

CGH, comparative genomic hybridization; aCGH, array comparative genomic hybridization; ALN, axillary lymph node; SLN, sentinel lymph node; CSFTC, cerebrospinal fluid-derived tumor cells; DTCs, disseminated tumor cells; NGS, next-generation sequencing.

only a subset of aberrations. Another explanation may be a higher degree of normal cell admixture within the metastases, resulting in lower sensitivity. The inconclusiveness of the studies is likely due to the relatively poor resolution of the CGH technique which renders this assay obsolete compared with copy number analysis using NGS, or arrayCGH/SNP-array technology.

Using whole-genome sequencing, Shah et al. sequenced a distant metastasis, biopsied from a pleural effusion, of one breast cancer patient with ER-positive, HER2-negative lobular breast cancer, and found 32 somatic nonsynonymous coding mutations (41). Five of the mutations were present in the corresponding primary tumor, 6 mutations were present subclonally in the primary tumor, 19 were not

detected in the primary tumor, and 2 were undetermined. The study confirms molecular heterogeneity within the primary tumor and substantial further genomic evolution of the metastasis. The patient had been treated with adjuvant anti-estrogen therapies, but received no neo-adjuvant or adjuvant chemotherapy. A mediating factor for the genomic discordance between primary tumor and recurrence may be the great time span of 9 years between the primary tumor and the recurrence in addition to the selective pressures provided by anti-estrogen therapies.

A study by Ding et al. based on a single breast cancer patient performed whole-genome sequencing of a basal-like (ER negative, PR negative, and HER2 negative) primary breast cancer, a brain metastasis, a xenograft, and matched peripheral blood (42). The

patient had been treated with neo-adjuvant chemotherapy. The time interval from the primary surgery till appearance of the brain metastasis was 8 months and in this time span the patient received radiation therapy. The xenograft tumor line was generated from a sample of the primary tumor biopsied before treatment. Of the 50 validated point mutations and small indels detected, 48 were present in all three tumors. The metastasis contained two *de novo* mutations and a large deletion not present in the primary tumor and significant enrichment of 20 shared mutations. The two *de novo* mutations private to the metastasis were not likely to be essential to the metastatic process, as one of them was a silent mutation and both mutations were absent in the xenograft, which nevertheless displayed full metastatic abilities. Hence, the metastasis-enabling mutations were most likely to be present already within the genome of the primary tumor. The study found that 96.11% and 93.98% of copy number aberrations found in the primary tumor were retained in the metastasis and the xenograft, respectively, indicating that most primary tumor copy number aberrations are preserved during disease progression. The quite high percentages imply that very few copy number events are evolved in the primary tumor after dissemination, rendering them private to the primary tumor. This suggests that dissemination of the successful metastatic cell most likely occurred relatively late in molecular time from the most advanced clone of the primary tumor. Conversely, only 80.65% of metastasis and 61.29% of xenograft copy number aberrations were found in the primary tumor, revealing substantial additional development of copy number events after dissemination from the primary tumor. Thus, copy number aberrations constitute the largest degree of genomic discordance between the primary tumor and the metastasis in this study. All five detected translocations were concordant between primary tumor and metastasis. The relatively small genomic discordance between the primary tumor and metastasis in this study may be explained by the relatively short time span of 8 months between the primary tumor and metastasis.

A recent study reports the genome evolution of one breast cancer patient with ER-positive, HER2-negative invasive ductal carcinoma including two pre-invasive regions, a primary tumor, and a distant metastasis located in a contralateral periclavicular lymph node, representing recurrence after 4 years (43). The patient had been treated with neo-adjuvant and adjuvant

chemotherapy and adjuvant anti-estrogen therapy. This study similarly found the metastasis to be seeded from the most advanced clone in the primary tumor, thus relatively late in molecular time. The study revealed substantial mutational discordance between the primary tumor and metastasis with 17 additional somatic nonsynonymous point mutations and 36 additional copy number aberrations in the metastasis, whereas early acquired copy number aberrations were kept as imprints in the genome. The genomic evolution presented in this study may be strongly influenced by the selective pressures provided by the adjuvant treatment.

Studies with broad or global approaches detecting discordances between primary tumors and metastases may provide novel insight into genes or pathways playing a role in the metastatic process.

LIQUID BIOPSY

An appealing alternative for biopsy of metastatic lesions is the minimally invasive “liquid biopsy” with molecular characterization of circulating tumor cells (CTCs) (44) or tumor cell derived cell-free DNA (cfDNA) in plasma (45). Circulating tumor DNA levels are found to correlate with tumor burden (46) and may be used to monitor treatment response, detect subclinical residual disease, and for directing therapy decisions.

These methods actually address the heterogeneity of breast cancer as the CTCs and cfDNA in plasma originates from all malignant lesions within the patient. This is supported in recent studies comparing primary tumors, metastases, and CTCs or cfDNA (47–49). However, the methods are dependent on the detection of cancer-specific genetic changes and rely on the assumption that the cancer genomes does not evolve to an extent where it loses identifiable markers. Another concern is that the cell-free DNA originates from apoptotic cancer cells, i.e., the cancer cells succumbing to therapy, whereas the viable subset of cancer cells may be more difficult to detect (50), thus demanding a high sensitivity of the assay.

CONCLUSION

The increasing use of molecularly targeted therapy in breast cancer treatment emphasizes the importance of establishing whether primary tumors may be used as surrogates of disseminated disease. Discordance of

actionable molecular targets has great clinical implications for optimal patient care and the avoidance of unnecessary side effects. Naturally, influencing factors of genomic, transcriptomic, and proteomic discordance between primary tumors and metastases include selective forces provided by treatment, the degree of genomic instability of the cancer genome, and the time span in which genomic alterations can take place. Explanations of the inconsistencies of the reported studies include methodological issues, relatively low numbers of studied patients, and maybe most importantly the heterogeneity of breast cancer. Due to clonal heterogeneity a small section of a primary tumor might not be representative of the entire clonality of the tumor, leaving small subclones undetected, and the selective pressures provided by treatment can entail positive selection of initially overlooked cancer cell populations. Moreover, it is likely that the different subtypes of breast cancer may be more or less prone to further genomic, transcriptomic, and proteomic evolution.

Overall, the studies reveal substantial discordances between primary tumors and metastases, which stresses the need for analysis of metastatic tissue at recurrences. International guidelines state that a biopsy of a metastatic lesion should be performed, if clinically feasible. It should, however, be kept in mind that evidence of the clinical impact of changes in treatment based on biopsy of metastases have yet to be evaluated and that one biopsy may not be representative of all disseminated cancer cells.

In addition to the direct clinical advantages of biopsy of recurrences, the molecular characterization of metastatic lesions and the molecular differences between matched primary tumors and metastatic lesions have the potential to reveal novel, potentially targetable, drivers of metastatic progression. The extreme complexity of cancer biology necessitates analysis not only on a single level, as the novel targets for molecularly targeted therapy can be revealed at the DNA, RNA, protein level, or epigenetically. Liquid biopsies with analysis of CTCs or tumor-derived cfDNA are likely to play an increasing role in cancer patient monitoring and have the advantage of addressing the issue of genetic heterogeneity.

FUNDING

Odense University Hospital Free Research Fund, Harboefonden, Aase og Ejnar Danielsen Fond, Fabrikant Einar Willumsens Mindelegat, Grosserer M.

Brogaard og Hustrus Mindefond, Kong Christian Den Tiendes Fond, Dagmar Marshalls Fond, Axel Muusfeldts Fond, Kræftfonden, Raimond og Dagmar Ringgård-Bohns Fond, Grete og Sigurd Pedersens Fond, Syddansk Universitets Forskningsfond, Poul og Ellen Hertz' Fond, Fonden til Lægevidenskabens Fremme, Grosserer A.V. Lykfeldt og Hustrus Legat, Familien Hede Nielsens Fond, Lykfeldts Legat, Dansk Kræftforskningsfond, Ulla og Mogens Folmer Andersens Fond, Ingeniør K. A. Rohde og Hustrus Legat, Krista og Viggo Petersens Fond, Danish Strategic Research Council, DBCG-TIBCAT.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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