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Intrinsic Subtype Switching and Acquired ERBB2/HER2 Amplifications and Mutations in Breast Cancer Brain Metastases

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Abstract

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IMPORTANCE—Patients with breast cancer (BrCa) brain metastases (BrM) have limited therapeutic options. A better understanding of molecular alterations acquired in BrM could identify clinically actionable metastatic dependencies.

OBJECTIVE—To determine whether there are intrinsic subtype differences between primary tumors and matched BrM and to uncover BrM-acquired alterations that are clinically actionable.

DESIGN, SETTING AND PARTICIPANTS—In total, 20 cases of primary breast cancer tissue and resected BrM (10 estrogen receptor [ER]-negative and 10 ER-positive) from 2 academic institutions were included. Eligible cases in the discovery cohort harbored patient-matched primary breast cancer tissue and resected BrM. Given the rarity of patient-matched samples, no exclusion criteria were enacted. Two validation sequencing cohorts were used—a published data set of 17 patient-matched cases of BrM and a cohort of 7884 BrCa tumors enriched for metastatic samples.

MAIN OUTCOMES AND MEASURES—Brain metastases expression changes in 127 genes within BrCa signatures, PAM50 assignments, and *ERBB2/HER2* DNA-level gains.

RESULTS—Overall, 17 of 20 BrM retained the PAM50 subtype of the primary BrCa. Despite this concordance, 17 of 20 BrM harbored expression changes (<2-fold or >2-fold) in clinically actionable genes including gains of *FGFR4* (n = 6 [30%]), *FLT1* (n = 4 [20%]), *AURKA* (n = 2 [10%]) and loss of *ESR1* expression (n = 9 [45%]). The most recurrent expression gain was *ERBB2/HER2*, which showed a greater than 2-fold expression increase in 7 of 20 BrM (35%). Three of these 7 cases were *ERBB2/HER2*-negative out of 13 *ERBB2/HER2*-negative in the primary BrCa cohort and became immunohistochemical positive (3+) in the paired BrM with metastasis-specific amplification of the *ERBB2/HER2* locus. In an independent data set, 2 of 9 (22.2%) *ERBB2/HER2*-negative BrCa switched to *ERBB2/HER2*-positive with 1 BrM acquiring *ERBB2/HER2* amplification and the other showing metastatic enrichment of the activating V777L *ERBB2/HER2* mutation. An expanded cohort revealed that *ERBB2/HER2* amplification and/or mutation frequency was unchanged between local disease and metastases across all sites; however, a significant enrichment was appreciated for BrM (13% local vs 24% BrM; *P* < .001).

CONCLUSIONS AND RELEVANCE—Breast cancer BrM commonly acquire alterations in clinically actionable genes, with metastasis-acquired *ERBB2/HER2* alterations in approximately 20% of *ERBB2/HER2*-negative cases. These observations have immediate clinical implications for patients with *ERBB2/HER2*-negative breast cancer and support comprehensive profiling of metastases to inform clinical care.

Keywords

Breast cancer; ErbB2; PAM50; brain metastasis; tumor profiling

INTRODUCTION

Brain metastases (BrM) occur in 10% to 15% of patients with metastatic breast cancer (BrCa) and present a major clinical challenge, highlighted by a poor 8.5-month median overall survival.¹ Limited therapeutic options exist for patients with BrM and current management consists of surgical resection, radiation therapy, and chemotherapy. *ERBB2/*

HER2-positive BrM have demonstrated encouraging responses to ERBB2/HER2-targeted therapies in recent clinical trials.² Unfortunately, in patients with ERBB2/HER2-negative BrM, no targeted therapies have shown even modest benefits.³

In this study, we performed targeted expression profiling on a molecularly diverse cohort of 20 primary breast tumors and their patient-matched BrM to determine transcriptional differences between primary cancers and BrM and to define metastasis-acquired alterations that may be clinically actionable.

METHODS

Eligible BrCa cases harbored paired formalin-fixed, paraffin-embedded tissue from primary BrCa and resected BrM (eTable 1 in Supplement 1). Two independent data sets reinforced findings—17 patient-matched BrCa cases of whole-exome sequencing data⁴ and targeted sequencing from 7884 BrCa tumors enriched for metastases. This study was approved by the institutional review boards of both participating institutions.

NanoString expression counts for target genes were generated from tumor RNA extracts. Hierarchical clusters, PAM50/Oncotype DX assignments,⁵ and fold-change expression values between patient-matched tumors (eFigure 1 in Supplement 1) were called using normalized expression counts.

Standard clinical ERBB2/HER2 and estrogen receptor (ER) immunohistochemistry (IHC) analyses were performed. DNA from case Pitt_62 was extracted and analyzed for *ERBB2* gains using a single-nucleotide polymorphism–based microarray. Metastasis-specific *ERBB2/HER2* copy number alteration and single-nucleotide variation (SNV) gains in whole-exome sequencing were interrogated with *CNVkit* and *GATK*.^{6,7} To test for site-specific enrichment of ERBB2/HER2 amplifications and SNVs in metastases, we analyzed FoundationOne (Foundation Medicine) targeted sequencing data.

More detailed methods can be found in Supplement 1, and more detailed results, including unprocessed raw count data, can be found in Supplement 2.

RESULTS

To determine the transcriptional similarity between primary tumors and patient-matched BrM, we performed unsupervised hierarchical clustering on normalized gene expression values. This produced 3 major clades broadly classified as ER-positive, ERBB2/HER2-positive, and ER-negative (Figure 1A). Overall, 12 of 20 (65%) patient-matched pairs clustered within a single doublet clade. PAM50 assignments were concordant in 17 of 20 pairs (Figure 1B). OncotypeDX (Genomic Health) scores were also largely unchanged between primary and metastatic tumors, retaining their clinical risk score in 15 of 20 cases (eTable 2 in Supplement 1)

Despite a large degree of similarity between patient-matched pairs, 100 genes were recurrently altered (eFigure 2A in Supplement 1); 55 genes harbored 2-fold expression gains and/or losses in at least 25% of cases (Figure 2A). The most recurrently down-regulated

genes were cytokeratins—*KRT17* in 14 of 20 pairs, *KRT5* and *KRT14* in 15 pairs (eFigure 2B in Supplement 1). The most recurrently up-regulated genes were *RAB6B* and *GRB7* (eFigure 2B in Supplement 1).

Ten genes in the panel are defined as clinically actionable,⁸ and all but 3 cases had at least 1 gene with a BrM-specific change (Figure 2B). *ERBB2/HER2* was the most recurrent alteration showing at least a 2-fold expression increase in 35% of BrM. Notably, 3 cases were classified as ERBB2/HER2-negative in the primary tumor. *FGFR4* showed increased expression in 30% of BrM, with 3 cases showing a greater than 4-fold increase. Other recurrent gains included *FLT1*, *AURKA*, and *EGFR*. The most recurrently down-regulated gene was *ESR1*, showing a 2-fold expression decrease in 4 samples and a greater than 4-fold decrease in 5 samples. Two samples with the greatest fold-change in *ESR1* switched expression from ER-positive to ER-negative levels, with 1 case showing substantial protein level changes (eFigure 3A and B in Supplement 1).

ERBB2/HER2 IHC was performed in 3 ERBB2/HER2-negative samples with the greatest mRNA gains in *ERBB2/HER2* (Figure 3A). All 3 tumors showed clinical ERBB2/HER2-status switching via IHC scores—RCS_4 and RCS_6; 1+ in primary, 3+ in BrM (data not shown), Pitt_62; 0 in primary, 3+ in BrM (eFigure 4A in Supplement 1). Single-nucleotide polymorphism array copy number alteration analysis revealed that ERBB2/HER2 status switching is driven by canonical amplification of the *ERBB2/HER2* locus (eFigure 4B in Supplement 1).

Given that 3 of 13 ERBB2/HER2-negative tumors showed ERBB2/HER2 gains, we then examined *ERBB2/HER2* amplification and SNVs in an independent whole-exome sequencing cohort (n = 17; 9 ERBB2/HER2-negative and 8 ERBB2/HER2-positive) of patient-matched cases (dbGaP phs000730.v1.p).⁹ One ERBB2/HER2-negative case (Broad_PB0150) showed a metastasis-specific copy number gain in *ERBB2/HER2*, which was consistent with the case switching to ERBB2/HER2-positive clinically in the BrM (eFigure 5 in Supplement 1). Another case (Broad_PB0049) switched from ERBB2/HER2-negative to ERBB2/HER2-positive clinically, but no metastasis-specific copy number alteration gain was appreciated within *ERBB2/HER2*; however, an enrichment—from an allele frequency of 39% to 69%—of a somatic V777L activating mutation in the metastasis was identified (eFigure 5 in Supplement 1).

To generalize these observations and test whether ERBB2/HER2 gains are enriched in BrM, we analyzed a cohort of 7884 breast cancers (7265 with unambiguous tissue site information) representing 3135 cases of local disease and 4130 cases of metastases for amplifications and/or SNV in *ERBB2/HER2*. Comparing all local and metastatic tumors from all sites (Figure 3B) showed no significant difference; however, there was a strong and significant enrichment of *ERBB2/HER2* alterations specifically in brain metastases (24%) compared with local disease (13%) (Fisher exact $P < .001$) (Figure 3B).

DISCUSSION

The brain is a common and catastrophic site of metastasis for patients with breast cancer and an understanding of metastasis-acquired alterations is limited. In this study, we found that patient-matched primary BrCa and BrM have similar intrinsic subtypes; however, when examining on a gene level, clinically actionable alterations were identified in all but 3 pairs.

The most recurrent gain in BrM was *ERBB2/HER2*, which showed expression increases in 7 of 20 (35%) of BrM and changes from *ERBB2/HER2*-negative to *ERBB2/HER2*-positive levels. Duchnowska et al¹⁰ and Thomson et al¹¹ reported *ERBB2/HER2*-negative to *ERBB2/HER2*-positive switching frequencies of 16% and 18% respectively in BrM via IHC. Gutierrez et al¹² reported an *ERBB2/HER2*-switching frequency of 11% in tamoxifen-resistant tumors, and notably, 6 of 7 cases with *ERBB2/HER2* expression gains were ER-positive.¹² Our analysis of *ERBB2/HER2*-switching samples reinforces these observations and shows expression gains are partially driven by classical amplification of the *ERBB2* locus. Interestingly, the *ERBB2/HER2*-switching Broad_PB0049 case showed no copy number gains specific to the BrM, yet harbored an enrichment of the activating *V777L ERBB2/HER2* mutation,¹³ suggesting another DNA-level mechanism of *ERBB2/HER2* gain in BrM.

Novel recurrent targetable alterations beyond *ERBB2/HER2* were also discovered, including expression increases in *FGFR4* (30% of pairs), *FLT2* (20%), *AURKA* (10%) and *EGFR* (10%). Each one of these targets have clinical trials ongoing and our results suggest that trial eligibility requiring expression of these markers (NCT02325739) should assess metastatic tumors if available, especially given greater than 4-fold mRNA expression gains over primary tumors in patient-matched metastases. A limitation of this study, however, is the small number of patient-matched pairs.

Significant loss of gene expression from the primary to metastatic lesions was also observed. The most recurrent expression losses involved cytokeratins. Cytokeratins have shown a complex role in oncogenesis and breast cancer metastasis, with loss of cytokeratin expression being a hallmark of epithelial-mesenchymal transition and metastasis.^{14,15} Intermediate steps of metastasis—such as processes taking place within circulating tumor cells—may be masked in our paired analyses. The most recurrently lost clinically actionable gene was *ESR1*. Importantly, loss of ER expression is an established mediator of therapy resistance.¹⁶

CONCLUSIONS

Breast cancer BrM are remarkably similar transcriptionally to patient-matched primary tumors; yet, recurrent expression changes in clinically actionable genes are common. Given this evolution, metastasis-acquired features should inform targeted therapy selection and trial eligibilities in advanced cancer settings. Furthermore, approximately 20% of *ERBB2/HER2*-negative patients with BrCa show copy number alteration and/or SNV gains in *ERBB2/HER2* across multiple cohorts, which warrants immediate clinical attention as many of these patients will not be provided *ERBB2/HER2*-targeted therapies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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KEY POINTS**Question**

Do brain metastases gain clinically actionable molecular alterations when compared with patient-matched primary breast cancers?

Findings

Overall, 17 of 20 brain metastases in this study retained the PAM50 subtype of the primary tumor; yet, clinically actionable gains are common, with approximately 20% of ERBB2/HER2-negative primary tumors switching to ERBB2/HER2-positive.

Meaning

Breast cancer brain metastases commonly acquire alterations in clinically actionable genes, which has immediate clinical implications and supports comprehensive profiling of metastases to inform clinical care.

Figure 1. Expression Profile Similarity in Patient-Matched Breast Cancer Metastases to the Brain

(A) Unsupervised hierarchical clustering heatmap of 20 patient-matched cases with hormone status (green indicates positive; black, negative), tissue site source or institution (yellow indicates Royal College of Surgeons [RCS], Ireland; purple, University of Pittsburgh [Pitt], United States), and tumor site (blue indicates primary; red, metastasis) of each sample indicated. The asterisks below the plots indicate patient-matched pairs that clustered in the same doublet of a clade in the dendrogram. (B) PAM50 intrinsic molecular subtype calls in patient-matched cases (red indicates basal; green, *ERBB2/HER2*; blue, LumA; yellow, LumB). Discordant pairs are marked with a delta symbol. BP indicates breast primary; BrM, brain metastasis.

Figure 2. Recurrent Expression Alterations in Breast Cancer Brain Metastases
(A) OncoPrint plot of highly recurrent (>25% of cases) expression alterations in 20 cases, ranked by frequency of alteration by gene. Blue tile represents a greater than 2-fold decrease in the patient-matched brain metastasis relative to the primary, while a red tile represents a greater than 2-fold increase. (B) Tile plot visualizing expression alterations in clinically actionable genes. Top panel consists of recurrent increases in expression (light red indicates a >2-fold increase; dark red, >4-fold increase), bottom panel are recurrent decreases in expression (light blue indicates a >2-fold decrease; dark blue, >4-fold decrease) between patient-matched pairs. Pitt indicates University of Pittsburgh; RSC, Royal College of Surgeons.

Figure 3. *ERBB2/HER2* Gains in Breast Cancer Brain Metastases

(A) Paired ladder plot of *ERBB2/HER2* expression in patient-matched cases. Gray dots represent samples with suspected hormone status switching; P values are from Wilcoxon signed-rank tests (primaries vs metastases). (B) The top panel shows *ERBB2/HER2* alterations (amplification or mutation) in 3135 local tumors and 4130 metastatic tumors, and the bottom panel shows *ERBB2/HER2* alterations in local tumors and 167 brain metastases (mets). NS indicates not significant.