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The Novel Marker GATA3 is Significantly More Sensitive Than Traditional Markers Mammaglobin and GCDFP15 for Identifying Breast Cancer in Surgical and Cytology Specimens of Metastatic and Matched Primary Tumors

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Abstract

Traditional markers mammaglobin and GCDFP15 show good specificity but lack sensitivity and can be difficult to interpret in small tissue samples. We undertook a comparative study of the novel nuclear marker GATA3 (expression typically restricted to breast and urothelial carcinomas) and GCDFP15 and mammaglobin. We first compared quantitative mRNA expression levels of these 3 markers across a diverse set of over 6000 tumors and 500 normal samples from The Cancer Genome Atlas which showed dramatically higher GATA3 expression (> 10-fold higher) in breast cancer as compared with GCDFP15 or mammaglobin (both $P < 2.2e–16$), suggesting that GATA3 may represent a more sensitive marker of breast cancer than GCDFP15 or mammaglobin. We next examined protein expression by immunohistochemistry in 166 cases (including surgical and cytology specimens) of metastatic breast carcinoma and 54 cases with available matched primaries. One whole-slide section from each case was stained for monoclonal GATA3 (L50-823), monoclonal mammaglobin (31A5), and monoclonal GCDFP15 (EP1582Y). Staining intensity (0 to 3+) and extent (0% to 100%) were scored with an *H*-score calculated (range, 0 to 300). Sensitivities by varying *H*-score cutoffs for a positive result in metastatic breast carcinoma among GATA3/GCDFP15/mammaglobin, respectively, were as follows: any *H*-score = 95%/65%/78%, *H*-score > 50 = 93%/37%/47%, *H*-score > 100 = 90%/25%/27%, *H*-score > 150 = 86%/21%/19%, *H*-score > 200 = 73%/18%/9%, *H*-score > 250 = 66%/14%/6%. Significant staining differences by specimen type, tumor subtype/grade, or ER/PR/HER2 status were not identified. Significantly stronger correlation was observed between primary/metastatic GATA3 expression [Pearson's correlation = 0.81 (0.68–0.89)] as compared with the primary/metastatic correlations of GCDFP15 [Pearson's correlation = 0.57 (0.33–0.74)] and mammaglobin [Pearson's correlation = 0.50 (0.24–0.70)] (both $P < 0.05$). In conclusion, the novel marker GATA3 stains a significantly higher

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proportion of both primary and metastatic breast carcinomas than GCDFP15 or mammaglobin with stronger and more diffuse staining, helpful in cases with small tissue samples. The matched primary/metastatic expression of GATA3 is also more consistent. We propose that GATA3 be included among a panel of confirmatory markers for metastatic breast carcinoma.

Keywords

breast; carcinoma; metastatic; immunohistochemistry; GATA3; GATA-3; mammaglobin; GCDFP15

BACKGROUND

By early 2012, an estimated 2.9 million women carried a history of breast cancer, with an estimated 232,340 new cases of invasive breast cancer diagnosed in 2013.[1] Although similar statistics on the incidence of metastatic disease among these women is not collected, it has been estimated that 162,000 women in the United States are living with metastatic breast cancer (http://mbcn.org/education/category/prevalence). The magnitude of these estimates are consistent with the frequency by which surgical pathologists, both community and academic, encounter cases of metastatic tumor in patients with a history of breast cancer in daily practice. Of these specimens, either surgical or cytology, some may require confirmatory immunohistochemistry. Traditionally relied upon membranous/cytoplasmic markers, mammaglobin and GCDFP15, show good specificity but lack sensitivity[2] and can be difficult to interpret in small tissue samples or those with high background staining.

The goal of our study was to evaluate the sensitivity of the transcription factor GATA3 for identifying breast cancer in breast cancer specimens and to compare the performance of GATA3 with the conventional markers, mammaglobin and GCDFP15. In the first phase of our study, we compare quantitative mRNA expression levels of GATA3, GCDFP15, and mammaglobin across a large and diverse set of over 6000 samples and over 500 normal samples spanning a wide range of tissue types (including breast) from The Cancer Genome Atlas (TCGA). In the second phase of our study, we examined GATA3, GCDFP15, and mammaglobin expression by protein immunohistochemistry in both surgical and cytology specimens and primary and matched metastatic samples.

METHODS AND MATERIALS

The Cancer Genome Atlas Dataset for Assessing mRNA Expression on Archival Fresh Frozen Tissue Samples

To assess the mRNA expression levels of GATA3, GCDFP15 (PIP mRNA), and mammaglobin (SCGB2A2 mRNA) across a diverse range of tumor and normal samples (including breast), we downloaded the RNASeqv2 mRNA expression levels for GATA3, GCDFP15 (PIP mRNA), and mammaglobin (SCGB2A2 mRNA) across a total of 6318 tumor samples spanning 24 cancer types and a total of 573 normal samples, spanning 15 tissue types. We used the level 3 RNASeqv2 RSEM genes normalized expression values for all analyses. The data were accessed and downloaded from the Broad Institute's Genome

Data Analysis Center (http://gdac.broadinstitute.org/). To visually display the distribution of mRNA levels across the samples stratified by tissue type, we created boxplots. To statistically assess the relative differences in expression levels across tissue types we performed the Wilcoxon signed-rank test.

Breast Cancer Study Set for Assessing Protein Expression on Histopathologic Sections

A total of 166 cases of metastatic breast carcinoma, including both surgical and cytology specimens with available cell blocks were retrieved from the pathology archives of one of archives of the author's (A.R.S.) institutions with diagnoses confirmed in all cases. Only cases with a documented history of breast carcinoma (either by review of prior lumpectomy/mastectomy slides or review of clinical charts) were included. Patients with any other malignancies were excluded from the study. Of these documented and unique 166 metastatic breast carcinoma cases from 140 different patients, 54 cases of various subtype with available matched primary tumor cell blocks were also retrieved. Immunohistochemical expression for monoclonal GATA3 (prediluted clone L50-823, Cell Marque, Rocklin, CA), monoclonal mammaglobin (prediluted clone 31A5, Cell Marque), and monoclonal GCDFP15 (prediluted clone EP1582Y, Cell Marque) on 1 whole-slide representative section per case was assessed. Four-millimeter-thick, formalin-fixed, paraffin- embedded freshly cut sections mounted on charged slides and baked at 60°C for 1 hour section were used on all cases and evaluated using the ultraView Universal DAB Detection kit on a Ventana BenchMark ULTRA from Ventana Medical Systems Inc. (Tucson, AZ). Separate positive and negative external controls were also used. Only nuclear staining was scored for GATA3 while either cytoplasmic or membranous staining was scored for GCDFP15 and mammaglobin. Staining intensity (0 to 3+) and extent (0%to 100%) were scored for all cases with an *H*-score calculated (range, 0 to 300). All cases were independently scored by 2 of the authors (A.R.S., B.S.) with the authors also blinded to metastasis/matched primary tumor data. The final *H*-score value for each case was determined as an average of the *H*-score from both authors. Cases with *H*-score disagreements of greater than 5 (or cases with *H*-scores <5) were rereviewed together by both authors with a consensus score agreed upon. Statistical analyses were performed using R software for statistical computing (http://www.r-project.org/).

RESULTS

The Cancer Genome Atlas Pan-Tissue Dataset

To compare the mRNA expression levels of GATA3, GCDFP15, and mammaglobin across a diverse range of tissues in tumor and normal samples, we used data provided by The Cancer Genome Atlas (TCGA).

Normal Tissues in TCGA

In normal tissues, GATA3, GCDFP15, and mammaglobin all showed the highest levels of expression in normal breast tissue, with relatively lower levels of expression in each of the other 14 tissue types (Fig. 1A). Within normal breast tissue, GATA3, GCDFP15, and mammaglobin were expressed at similar levels (median normalized expression units = 3.4k,

3.8k, and 4.3k, respectively), although GATA3 did show statistically significant lower expression in normal breast tissue as compared with mammaglobin (Wilcoxon $P = 0.01$).

Tumor Tissues in TCGA

In primary tumor tissues, GATA3, GCDFP15, and mammaglobin all showed the highest level of expression in breast cancer, with relatively lower levels of expression in each of the other 23 tumor types (Fig. 1B). Each of the 3 markers showed highly specific expression for breast cancer, with the exception of GATA3, which was frequently expressed in bladder cancer (Fig. 1B).

Next, we compared the levels of mRNA expression of the 3 markers within breast cancer. In contrast to the relatively similar pattern of expression observed in normal tissue, in breast cancer tissue GATA3 shows dramatically higher levels of expression compared with GCDFP15 (>20-fold increase) and mammaglobin (>10-fold increase) (median GATA3 normalized expression units in breast cancer = 10.7k vs. 464k for GCDFP15 and 912k for mammaglobin, both $P < 2.2e{-}16$). These data suggest that GATA3 will be a far more sensitive marker of breast cancer than either mammaglobin or GCDFP15, and in the next portion of the study, we evaluate this hypothesis through immunohistochemical studies on breast cancer pathology specimens.

Breast Cancer Pathology Study Set

Patient ages (all women) ranged from 31 to 98 years (mean, 62.3 y). Of the 166 cases of metastatic breast carcinoma, 133 cases were surgical specimens (55 biopsies, 78 resections) and 33 were cytology specimens. The categorized sites of these metastases (as a percentage of total) included lymph node (39.8%), bone/soft tissue (24.7%), cardiovascular/lung (19.3%), gastrointestinal tract (12.7%), gynecological tract (1.8%), brain (1.2%), and adrenal (0.6%). Of these 166 cases of metastatic breast carcinoma, 54 had available matched primary tumor cells for an assessment of staining constancy, with subtypes including invasive ductal carcinoma (n = 38, including 5 that underwent neoadjuvant therapy), invasive lobular carcinoma (n = 10, including 1 that underwent neoadjuvant therapy), invasive micropapillary carcinoma (n = 2), invasive pleomorphic lobular carcinoma (n = 2), invasive mucinous carcinoma (n = 1), and invasive secretory carcinoma (n = 1). Of these 54-matched primaries, 85% were ER or PR positive (n = 46), 15% were HER2 positive (n = 8), and 9% were negative for ER/PR/HER2 (n = 5).

GATA3 Protein Expression Outperforms Mammaglobin and GCDFP15 for the Identification of Primary and Metastatic Breast Cancer

Raw *H*-score data for every case of metastatic breast carcinoma is listed in Figure 2 (including cases with available match primary tumors). GATA3 performed superior to both GCDFP15 and mammaglobin in a majority of the cases, including both poorly and well-differentiated tumors, surgical specimens, and cytology specimens (Figs. 3, 4). Among the positive cases for each of the 3 markers, GATA3 showed the strongest and most diffuse staining (average staining intensity/staining extent: GATA3 = 2.8/88%, GCDFP15 = 2.2/45%, mammaglobin = 1.8/48%). Only 8 of the 166 metastatic cases (5%) were completely negative for GATA3 (*H*-score = 0). GCDFP15 was also completely negative in

these 8 cases (*H*-score = 0) while mammaglobin was positive in 4 of 8 of these cases (average *H*-score of 48 among the positive cases). To assess whether the improved performance of GATA3 versus GCDFP15 and mammaglobin for the identification of metastatic breast cancer was sensitive to the *H*-score cutoff used to classify positive staining, we compared the performance across a broad range of *H*-scores (Table 1). At each *H*-score cutoff, GATA3 showed significantly improved performance for the identification of metastatic breast cancer as compared with GCDFP15 and mammaglobin (Table 1). Similar results were also seen among primary tumors (Table 1). We did not observe significant staining differences by specimen type (surgical vs. cytology), tumor subtype (including neoadjuvant therapy status), tumor grade (well vs. poorly differentiated), patient age/sex, metastatic site, or ER/PR/HER2 status.

For cases with matched primary and metastatic samples (n = 54), significantly stronger correlation was observed between primary and metastatic GATA3 expression [Pearson's Correlation = 0.81 (0.68–0.89)] as compared with the primary to metastatic correlation of GCDFP15 [Pearson's correlation = 0.57 (0.33–0.74)] and as compared with the primary to metastatic correlation of mammaglobin [Pearson's correlation = 0.50 (0.24–0.70)] (both $P < 0.05$). Among the positive cases of matched primary tumors, GATA3 showed the strongest and most diffuse staining (average staining intensity/staining extent: GATA3 = 2.8/92%, GCDFP15 = 2.1/40%, mammaglobin = 1.7/44%). Three cases of matched primary tumors were completely negative for GATA3 (matched metastatic carcinomas were also GATA3 negative, *H*-score = 0). GCDFP15 was also completely negative in these 3 cases (*H*-score = 0) while mammaglobin was positive in 1 of these 3 cases (*H*-score = 140).

CONCLUSIONS

The seminal study from 2007 comparing mammaglobin versus GCDFP15 reported sensitivities of 55% and 23%, respectively, in breast carcinomas (n = 121, included n = 29 metastatic cases).[2] Despite these low-staining sensitivities, mammaglobin and GCDFP15 remain the traditionally relied upon confirmatory markers in the assessment of metastatic breast carcinoma. Importantly, this 2007 study also noted "equivocal" staining results (focal and/or weak staining was also considered equivocal) of 20% and 27% for mammaglobin and GCDFP15, respectively,[2] numbers which seem surprisingly high but in fact are in keeping with our own experience with these 2 markers in interpreting cases with background staining or scant tissue samples. Although we describe GATA3 (GATA binding protein 3, a zinc finger transcription factor critical to the development and maintenance of breast ductal epithelium[3,4]) as a "novel" antibody, in fact GATA3 has previously been evaluated in several studies of breast cancer, where expression has been noted to correlate with ER expression and hormone resposiveness.[5,6] Although a few other large-series manuscripts have investigated GATA3 immunoreactivity in breast carcinomas (primary or metastatic) as a diagnostic confirmatory marker,[7–14] none to our knowledge have compared GATA3 expression to mammaglobin and GCDFP15 at both the mRNA and protein levels, and none have compared protein expression in both surgical and cytology specimens. Similarly, none to our knowledge have concurrently examined staining constancy of these 3 markers with the matched primary tumor. Therefore, the current study was undertaken.

In the first phase of this study, we compared the mRNA expression levels of GATA3, GCDFP15, and mammaglobin across a broad array of over 500 normal tissue samples using RNA-Seq data obtained from over 6000 tumor samples collected as part of The Caner Genome Atlas Project. An advantage of RNA-Seq data, as compared with protein immunohistochemistry data, is that RNA-Seq provides a more quantitative estimate of expression levels, enabling more precise estimation of magnitudes in expression differences between competing markers. Further, RNA-Seq and immunohistochemistry are complementary, as RNA-Seq provides a measure of expression at the mRNA level, which can then be further studied, confirmed, and extended by immunohistochemistry at the protein level. Analysis of the TCGA RNA-Seq data show that although GATA3, GCDFP15, and mammaglobin show relatively similar levels of expression in normal breast tissue samples, GATA3 shows dramatically higher levels of mRNA expression in invasive breast cancer (10- to 20-fold higher expression), suggesting that it will outperform GCDFP15 and mammaglobin as a sensitive biomarker of breast cancer for use in diagnostic pathology.

To build on this observation and more fully evaluate its clinical significance, we performed an immunohistochemistry- based study to evaluate these 3 markers in breast cancer. Rather than using a tiered semiquantitative assessment of staining results, we chose to use *H*-scores to independently incorporate precise staining intensity and extent score into a single numerical value and also to better analyze antibody sensitivity by varying minimum *H*-score cut-offs for a “positive” results. GATA3 showed superior sensitivity to both GCDFP15 and mammaglobin in both metastatic carcinoma and matched primary tumor. And the added value GATA3 was further demonstrated by raising the *H*-score threshold for a positive result (Table 1), which showed that GATA3 outperformed mammaglobin and GCDFP15 at all thresholds, suggesting that the increased sensitivity and diffuseness of staining will make GATA3 especially useful in cases with scant tissue or those with problematic background staining. Moreover, on scant specimens evaluating positivity of nuclear markers (such as GATA3) tends to be much easier than relying on membranous or cytoplasmic markers (such as GCDFP15 and mammaglobin).

Of the aforementioned manuscripts that have investigated GATA3 as a diagnostic marker for breast carcinomas, 2 GATA3 clones were used: 6 used the GATA3 L50-823 clone, 2 used the GATA3 HGC-31 clone, and 1 used both clones.[7–14] The one manuscript that used both GATA3 clones reported staining sensitivities of 79% and 64% for the L50-823 clone and HGC-31 clone, respectively.[13] Another study (in abstract format) that also compared these 2 GATA3 clones in breast carcinomas reported staining sensitivities of 96% and 89% for the L50-823 clone and HGC-31 clone, respectively. [15] Although in our study only the GATA3 L50-823 clone was investigated, we chose this clone over the HGC-31 based on our own (A.R.S., G.Y.; unpublished data) experiences during clinical validation while initially bringing up both of these markers in our laboratories. Results from these 2 comparative GATA3 clone studies support our own notion of L50-823 as a more sensitive diagnostic marker for breast carcinoma.

It should also be mentioned that the goal of this study was to investigate the sensitivity, not specificity, of GATA3 compared with the more traditional markers GCDFP15 and mammaglobin. In addition to breast carcinoma, GATA3 is an excellent marker for urothelial

carcinoma.[8,9,16–20] However, as is often the inevitable fate for a marker touted as "specific" for a particular category of tumor, subsequent reports of GATA3 expression in a wide variety of neoplasms have emerged, ranging from immunoreactivity in skin tumors (eg, basal cell/squamous cell carcinomas and adnexal neoplasms) and germ cell tumors (eg, choriocarcinoma and yolk sac tumor) to mesothelioma, salivary gland neoplasms, and paraganglioma. [8,9,14,21,22] As such, when utilizing GATA3 as a diagnostic confirmatory marker, consideration should also be given to other tumors of nonbreast origin that may express GATA3.

In summary, we report that GATA3 is expressed at dramatically higher levels than mammaglobin and GCDFP15 at the mRNA level in breast cancer, and GATA3 immunohistochemistry stains a significantly higher proportion of both primary and metastatic breast carcinomas compared with GCDFP15 and mammaglobin, and the matched primary/metastatic expression of GATA3 immunohistochemistry is more consistent. GATA3 positivity is also stronger and more diffuse than the other 2 markers which can be helpful in cases with small tissue samples or problematic background staining. On the basis of these findings, we propose that GATA3 (L50-823 clone) be included among a panel of diagnostic confirmatory markers for metastatic breast carcinoma.

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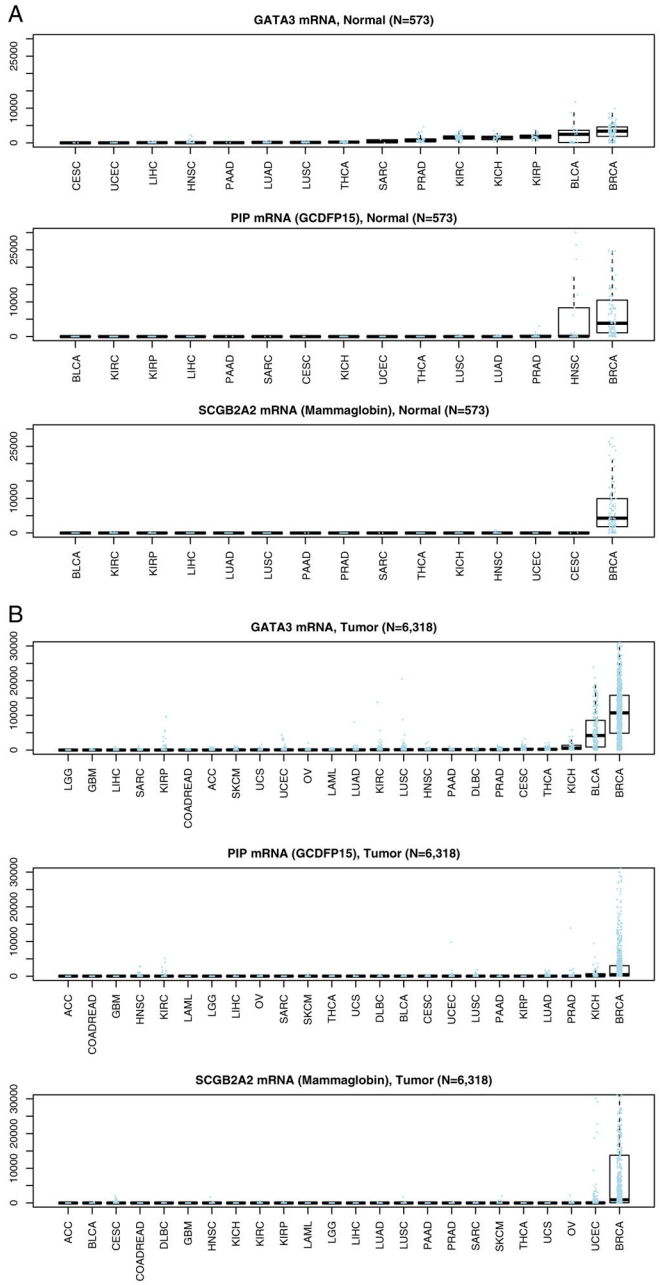

FIGURE 1.

GATA3, GCDFP15, and mammaglobin expression in The Cancer Genome Atlas. A, The boxplots display mRNA expression levels in normal tissue samples. Each blue circle represents 1 patient sample. Each box contains patients between the 25th and 75th percentile of the indicated marker's expression, and the median is indicated by a thick black line. Tissue types are ordered according to the median level of marker expression. The *y*-axis indicates the mRNA normalized expression units. B, Boxplots of mRNA expression levels in tumor tissues, ordered according to the median marker expression level. Tissue types are indicated by the following codes: ACC, adrenocortical carcinoma; BLCA, bladder urothelial

carcinoma; BRCA, breast invasive carcinoma; CESC, cervical and endocervical cancers; COADREAD, colorectal adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, brain lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PRAD, prostate adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneousmelanoma; THCA, thyroid carcinoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma.

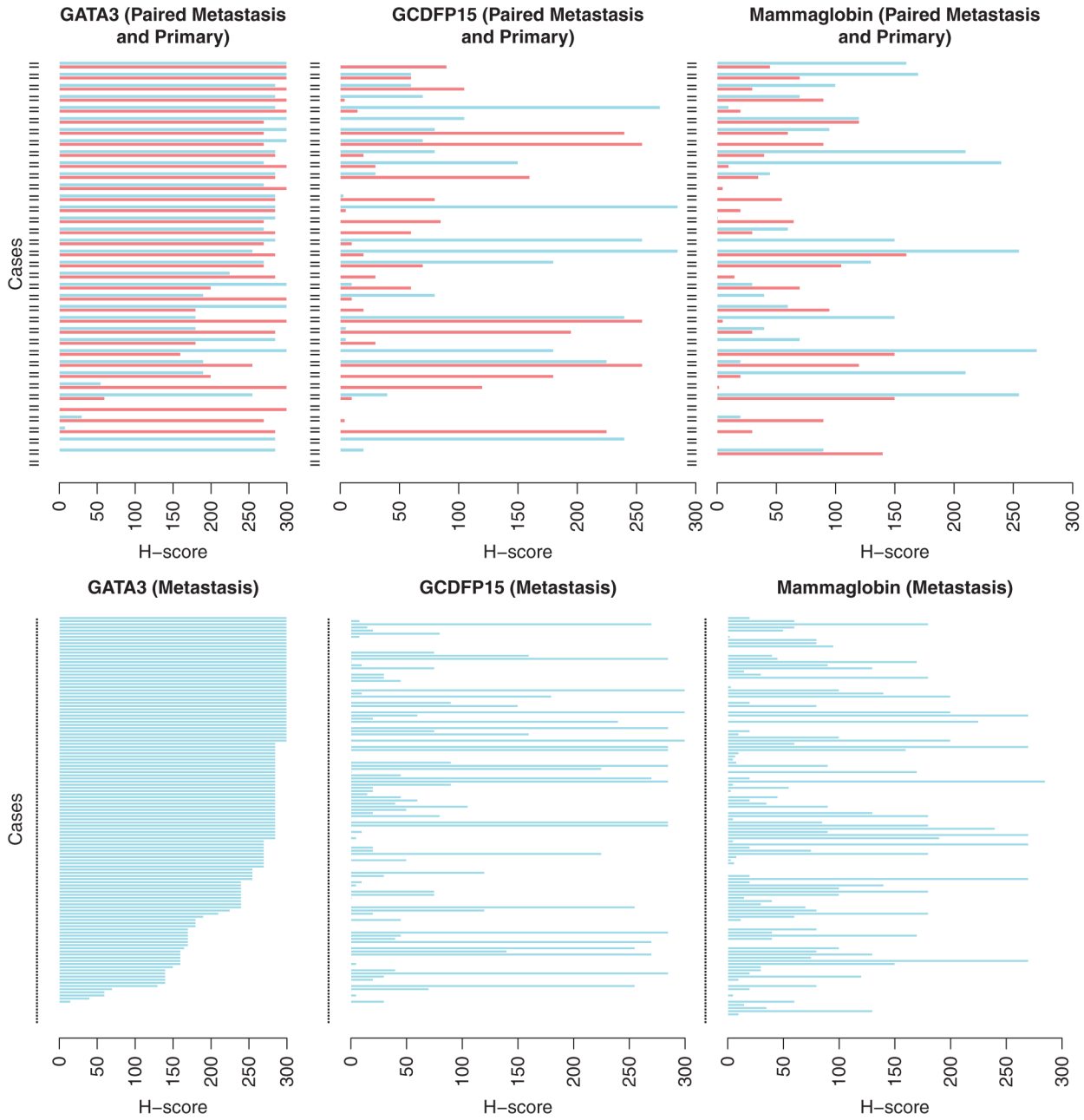

FIGURE 2.

Raw data of GATA3, GCDFP15, and mammaglobin expression for all study cases. Individual cases are indicated on the *y*-axis. *H*-scores (0 to 300) are indicated on the *x*-axis. The top 3 graphs display data for cases of metastatic breast carcinoma (blue) with paired primary tumor (red). The bottom 3 graphs display data for cases of metastatic breast carcinoma without paired primary tumor.

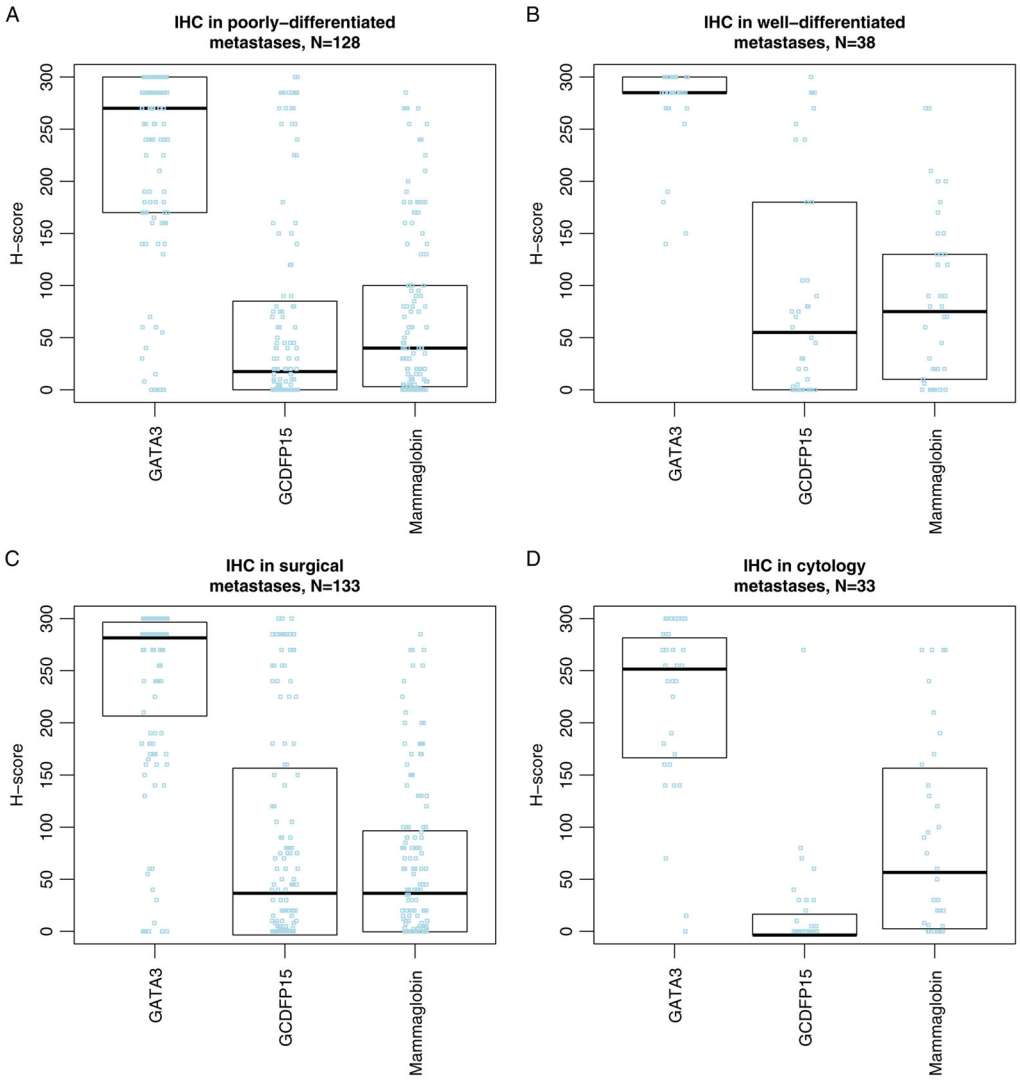

FIGURE 3.

GATA3, GCDFP15, and mammaglobin expression sorted by tumor grade and case type. Each tumor sample is indicated by a blue circle. Each box contains patients between the 25th and 75th percentile of the indicated marker's expression, and the median is indicated by a thick black line. The boxplots on the top-left (A) include all poorly differentiated metastatic samples, the boxplots on the top-right (B) include all well-differentiated metastatic samples, the boxplots on the bottom-left (C) include all surgical metastatic specimens, and the boxplots on the bottom-right (D) include all cytology metastatic specimens.

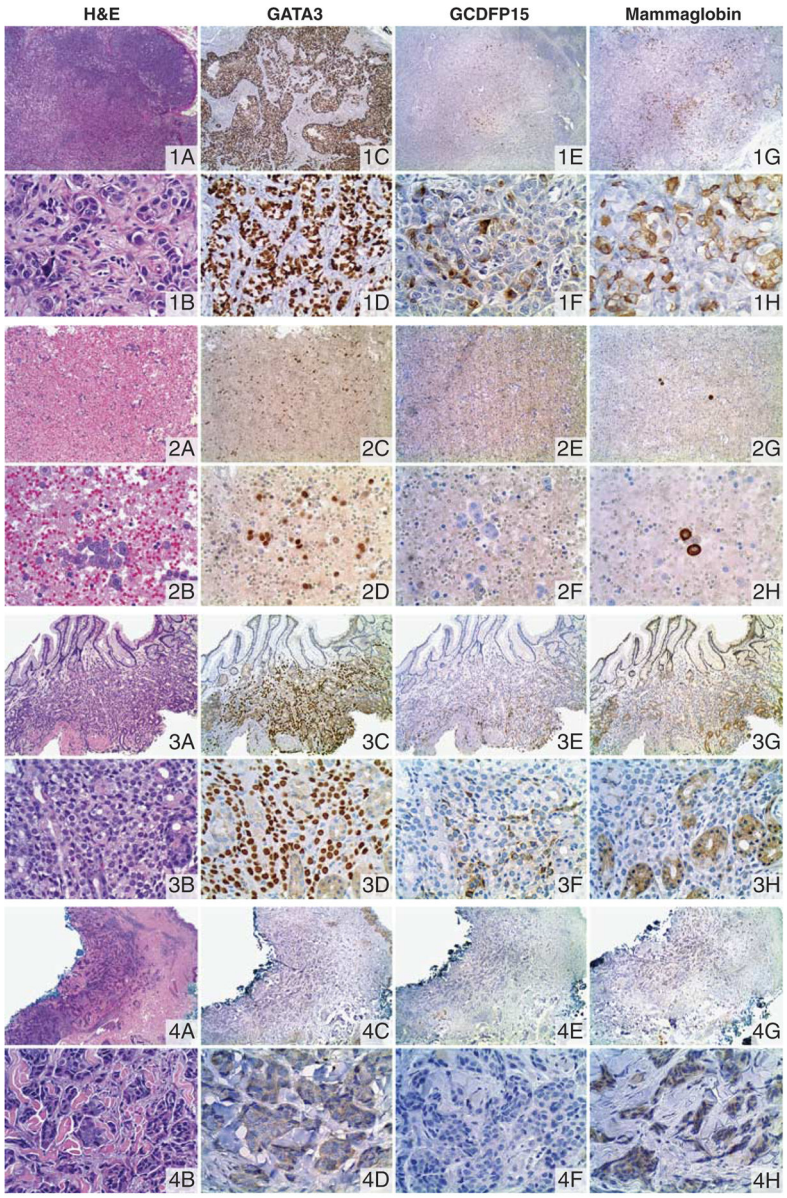

FIGURE 4.

Representative photomicrographs of study cases. Paired low/high power images (×40/×400) from 4 cases with H&E stain, GATA3, GCDFP15, and mammaglobin. Lymph node resection with extensive involvement by metastatic ductal carcinoma (1A/1B). GATA3 shows diffuse strong staining (1C/1D), whereas GCDFP15 and mammaglobin both show patchy moderate staining (1E/1F, 1G/1H). Pleural fluid cytology specimen with scattered involvement by metastatic ductal carcinoma (2A/2B). Diffuse moderate/strong staining with GATA3 highlights more tumor cells appreciated than by H&E alone (2C/2D). GCDFP15 is negative (2E/2F) while mammaglobin is strongly but very focally positive (2G/2H). Gastric biopsy with subtle involvement by metastatic lobular carcinoma (3A/3B). GATA3 shows diffuse strong staining (3C/3D) while GCDFP15 shows patchy weak staining (3E/3F). Mammaglobin is negative in the tumor and shows background staining within gastric glands

(3G/3H). Skin nodule resection ofmetastatic ductal carcinoma (4A/4B). Both GATA3 and GCDFP15 are negative (4C/4D, 4E/4F) while mammaglobin shows moderately diffuse but weak staining (4G/4H). Only nuclear staining was scored as positive for GATA3 while membranous or cytoplasmic staining was scored as positive for GCDFP15 and mammaglobin. Image sizes: 1A, 1C, 1E, 1G, 4A, 4C, 4E, 4G = ×40; 2A, 2C, 2E, 2G, 3A, 3C, 3E, 3G = ×100; 1B, 1D, 1F, 1H, 2B, 2D, 2F, 2H, 3B, 3D, 3F, 3H = ×400.

TABLE 1

Antibody Sensitivity With Minimum *H*-Score Cutoffs for a Positive Result in Metastatic (M) and Primary (P) Carcinomas

H-Score (0–300)	GATA3	GCDFP15	Mammaglobin
Any	M = 95%*, *P* = 94%	M = 65%, *P* = 83%	M = 78%, *P* = 89%
>50	M = 93%*, *P* = 94%*	M = 37%, *P* = 50%	M = 47%, *P* = 48%
>100	M = 90%*, *P* = 93%*	M = 25%, *P* = 33%	M = 27%, *P* = 22%
>150	M = 86%*, *P* = 91%*	M = 21%, *P* = 24%	M = 19%, *P* = 7%
>200	M = 73%*, *P* = 78%*	M = 18%, *P* = 17%	M = 9%, *P* = 4%
>250	M = 66%*, *P* = 74%*	M = 14%, *P* = 11%	M = 6%, *P* = 0%

*$P < 0.0001$ for both GATA3 versus GCDPF15 and GATA3 versus mammaglobin comparisons.