CELL MARQUE
a SIGMA-ALDRICH company

The Novel Marker GATA3 is Significantly More Sensitive than Traditional Markers Mammaglobin and GCDFP-15 for Identifying Breast Cancer in Surgical and Cytology Specimens of Metastatic and Matched Primary Tumors

George Yang, M.D.,[1] Mike Lacey, M.D.,[1] Kelsea Cumminings, B.S.,[1] Ourhay Mego, B.S.,[1] Ankur R Sangoi, M.D.,[2] Bijayee Shretha, M.D., Ph.D.,[2] Andrew H. Beck, M.D., Ph.D.[3]

[1]Cell Marque, Rocklin, CA; [2]El Camino Hospital, Mountain View, CA;

[3]Department of Pathology, Harvard Medical School and Beth Israel Deaconess Medical Center, Boston, MA

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.[2] 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 GCDFP-15 show good specificity but lack sensitivity[3] 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 GCDFP-15. In the first phase of our study, we compare mRNA expression levels of GATA3, GCDFP-15, and mammaglobin across a large and diverse set of over 6 thousand tumor samples and over 500 normal samples, spanning a wide range of tissue types. In the second phase of our study, we examined GATA3, GCDFP-15, 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, GCDFP-15 (PIP mRNA), and mammaglobin (SCGB2A2 mRNA) across a diverse range of tumor and normal samples, we downloaded the RNASeqv2 mRNA expression levels for GATA3, GCDFP-15 (PIP mRNA), and mammaglobin (SCGB2A2 mRNA) across a total of 6,318 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 was accessed and downloaded from the Broad Institute's Genome Data Analysis Center.[4] 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 Histopathological Sections: 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 (ARS) 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 GCDFP-15 (prediluted clone EP1582Y, Cell Marque) on one 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 *ultra*View 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 GCDFP-15 and mammaglobin. Staining intensity (0-3+) and extent (0-100%) were scored for all cases with an H-score calculated (range 0-300). Statistical analyses were performed using R software for statistical computing (http://www.r-project.org/).

Results

The Cancer Genome Atlas Pan-Tissue Dataset: To observe the expression of GATA3 mRNA levels 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, GCDFP-15, 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 (Figure 1A). Within normal breast tissue, GATA3, GCDFP-15, 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, GCDFP-15, 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 (Figure 1B). Each of the three markers showed highly specific expression for breast cancer, with the exception of GATA3, which was frequently expressed in bladder cancer (Figure 1B).

Table 1. Antibody sensitivity with minimum H-score cut-offs for a positive result in metastatic (M) and primary (P) carcinomas

H-SCORE (0-300)	GATA3	GCDFP-15	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 vs GCDFP-15 and GATA3 vs mammaglobin comparisons

Next, we compared the levels of mRNA expression of the three 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 GCDFP-15 (>20 fold increased) and mammaglobin (>10 fold increased) (Median GATA3 normalized expression units in breast cancer = 10.7K vs. 464 for GCDFP-15 and 912 for mammaglobin, both P < 2.2e16). These data suggest that GATA3 will be a more sensitive marker of breast cancer, than either GCDFP-15 or mammaglobin.

These mRNA-based data suggest that the novel marker GATA3 will outperform the traditional markers GCDFP-15 and mammaglobin for identifying breast cancer. In the next phase of our study, we evaluated this hypothesis by performing protein immunohistochemistry on a set of primary and metastatic breast cancer samples.

Breast Cancer Pathology Study Set: Patient ages (all women) ranged from 31-98 years (mean 62.3 years). 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 tumors 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 Outperforms Mammaglobin and GCDFP-15 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 GCDFP-15 and mammaglobin in a majority of the cases, including both poorly and well differentiated tumors, surgical specimens, and cytology specimens (Figures 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%, GCDFP-15=2.2/45%, mammaglobin=1.8/48%). Only 8 of the 166 metastatic cases (5%) were completely negative for GATA3 (H-score=0). GCDFP-15 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 vs. GCDFP-15 and Mammaglobin for the identification of metastatic breast cancer was sensitive to the H-Score cut-off used to classify positive staining, we compared the performance across a broad range of H-Scores (Table 1). At each H-Score cut-off, GATA3 showed significantly improved performance for the identification of metastatic breast cancer as compared with GCDFP-15 and Mammaglobin (Table 1). Similar results were also seen among primary tumors (Table 1). Significant staining differences by specimen type (surgical versus cytology), tumor subtype (including neoadjuvant therapy status), tumor grade (well versus poorly differentiated), patient age/gender, metastatic site, or ER/PR/HER2 status were not identified.

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 GCDFP-15 (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%, GCDFP-15=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). GCDFP-15 was also completely negative in these 3 cases (H-score=0) while mammaglobin was positive in 1 of these 3 cases (H-score 140).

Conclusion

The seminal study from 2007 comparing mammaglobin versus GCDFP-15 reported sensitivities of 55% and 23% (respectively) in breast carcinomas (n=121, included n=29 metastatic cases).[3] Despite these low staining sensitivities, mammaglobin and GCDFP-15 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 GCDFP-15 (respectively),[3] numbers which may seem surprisingly high on face value 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[5,6]) as a "novel" antibody, in fact GATA3 is a historical marker noted for its association with ER status and predictive hormone responsiveness in breast cancer.[7,8] While a few other large-series manuscripts have investigated GATA3 immunoreactivity in breast carcinomas (primary or metastatic) as a diagnostic confirmatory marker,[9-16] none to our knowledge have compared GATA3 staining to mammaglobin and GCDFP-15 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 our study, we first compare the mRNA expression levels of GATA3, GCDFP-15, and mammaglobin across a broad array of over 500 normal tissue samples and over 6,000 tumor samples collected as part of The Cancer Genome Atlas Project. These data show that although GATA3, GCDFP-15, 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.

Based on these results, we performed an immunohistochemistry-based study to evaluate these markers in breast cancer. Rather than using a tiered semi-quantitative 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 "positive" results. While GATA3 in both metastatic carcinoma and matched primary tumor showed superior sensitivity to both GCDFP-15 and mammaglobin, the value of GATA3 is demonstrated by raising the H-score threshold for a positive result (Table 1), useful in cases with scant tissue or those with problematic background staining. Moreover, relying on a nuclear marker (GATA3) versus membranous or cytoplasmic (GCDFP-15, mammaglobin) for a positivity staining result is much easier in these scenarios.

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.[9-16] The 1 manuscript that used both GATA3 clones reported staining sensitivities of 79% and 64% for the L50-823 clone and HGC-31 clone, respectively.[15] 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.[17] Although in our study only the GATA3 L50-823 clone was investigated, we chose this clone over the HGC-31 based on our own (unpublished) experiences during clinical validation while initially bringing up both of these markers in our labs. 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 to the more traditional markers GCDFP-15 and mammaglobin. In addition to breast carcinoma, GATA3 is an excellent marker for urothelial carcinoma.[10, 11, 18-22] 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 has emerged ranging from immunoreactivity in skin tumors (e.g., basal cell/squamous cell carcinomas and adnexal neoplasms), and germ cell tumors (e.g., choriocarcinoma and yolk sac tumor) to mesothelioma, salivary gland neoplasms, and paraganglioma.[10, 11, 16, 23, 24] As such, when utilizing GATA3 as a diagnostic confirmatory marker, consideration to other tumors of non-breast origin should be appreciated in the appropriate clinical context.

In summary, we report that the nuclear marker GATA3 stains a significantly higher proportion of both primary and metastatic breast carcinomas compared to GCDFP-15 and mammaglobin, and that the matched primary/metastatic expression of GATA3 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. Based on these findings, we propose that among a panel of diagnostic confirmatory markers for metastatic breast carcinoma, GATA3 (L50-823 clone) be selected as a first-line choice.

References

1. Breast Cancer Facts and Figures 2013-2014. American Cancer Society, Inc 2013.
2. http://mbcn.org/education/category/prevalence.
3. Bhargava R, Beriwal S, Dabbs DJ. Mammaglobin vs GCDFP-15: an immunohistologic validation survey for sensitivity and specificity. Am J Clin Pathol. 2007;127:103-113.
4. http://gdac.broadinstitute.org/.
5. Asselin-Labat ML, Sutherland KD, Barker H, et al. Gata-3 is an essential regulator of mammary-gland morphogenesis and luminal-cell differentiation. Nat Cell Biol. 2007;9:201-209.
6. Kouros-Mehr H, Slorach EM, Sternlicht MD, et al. GATA-3 maintains the differentiation of the luminal cell fate in the mammary gland. Cell. 2006;127:1041-1055.
7. Hoch RV, Thompson DA, Baker RJ, et al. GATA-3 is expressed in association with estrogen receptor in breast cancer. Int J Cancer. 1999;84:122-128.
8. Parikh P, Palazzo JP, Rose LJ, et al. GATA-3 expression as a predictor of hormone response in breast cancer. J Am Coll Surg. 2005;200:705-710.
9. Liu H, Shi J, Prichard JW, et al. Immunohistochemical evaluation of GATA-3 expression in ER-negative breast carcinomas. Am J Clin Pathol. 2014;141:648-655.
10. Liu H, Shi J, Wilkerson ML, et al. Immunohistochemical evaluation of GATA3 expression in tumors and normal tissues: a useful immunomarker for breast and urothelial carcinomas. Am J Clin Pathol. 2012;138:57-64.
11. Miettinen M, McCue PA, Sarlomo-Rikala M, et al. GATA3: a multispecific but potentially useful marker in surgical pathology: a systematic analysis of 2500 epithelial and nonepithelial tumors. Am J Surg Pathol. 2014;38:13-22.
12. Shield PW, Papadimos DJ, Walsh MD. GATA3: a promising marker for metastatic breast carcinoma in serous effusion specimens. Cancer Cytopathol. 2014;122:307-312.
13. Braxton DR, Cohen C, Siddiqui MT. Utility of GATA3 immunohistochemistry for diagnosis of metastatic breast carcinoma in cytology specimens. Diagn Cytopathol. 2014;[epub ahead of print].
14. Cimino-Mathews A, Subhawong AP, Illei PB, et al. GATA3 expression in breast carcinoma: utility in triple-negative, sarcomatoid, and metastatic carcinomas. Hum Pathol. 2013;44:1341-1349.
15. Krings G, Nystrom M, Mehdi I, et al. Diagnostic utility and sensitivities of GATA3 antibodies in triple-negative breast cancer. Hum Pathol. 2014.
16. Ordonez NG, Sahin AA. Diagnostic utility of immunohistochemistry in distinguishing between epithelioid pleural mesotheliomas and breast carcinomas: a comparative study. Hum Pathol. 2014;45:1529-1540.
17. Kandalaft PL GA. Comparative sensitivies and specifities of antibodies to breast markers GCDFP-15, mammaglobin A, and different clones of GATA-3. USCAP abstract 225. 2013.
18. Chang A, Amin A, Gabrielson E, et al. Utility of GATA3 immunohistochemistry in differentiating urothelial carcinoma from prostate adenocarcinoma and squamous cell carcinomas of the uterine cervix, anus, and lung. Am J Surg Pathol. 2012;36:1472-1476.
19. Higgins JP, Kaygusuz G, Wang L, et al. Placental S100 (S100P) and GATA3: markers for transitional epithelium and urothelial carcinoma discovered by complementary DNA microarray. Am J Surg Pathol. 2007;31:673-680.
20. Liang Y, Heitzman J, Kamat AM, et al. Differential expression of GATA-3 in urothelial carcinoma variants. Hum Pathol. 2014;45:1466-1472.
21. Paner GP, Annaiah C, Gulmann C, et al. Immunohistochemical evaluation of novel and traditional markers associated with urothelial differentiation in a spectrum of variants of urothelial carcinoma of the urinary bladder. Hum Pathol. 2014;45:1473-1482.
22. Zhao L, Antic T, Witten D, et al. Is GATA3 expression maintained in regional metastases?: a study of paired primary and metastatic urothelial carcinomas. Am J Surg Pathol. 2013;37:1876-1881.
23. Schwartz LE, Begum S, Westra WH, et al. GATA3 immunohistochemical expression in salivary gland neoplasms. Head Neck Pathol. 2013;7:311-315.
24. So JS, Epstein JI. GATA3 expression in paragangliomas: a pitfall potentially leading to misdiagnosis of urothelial carcinoma. Mod Pathol. 2013;26:1365-1370.

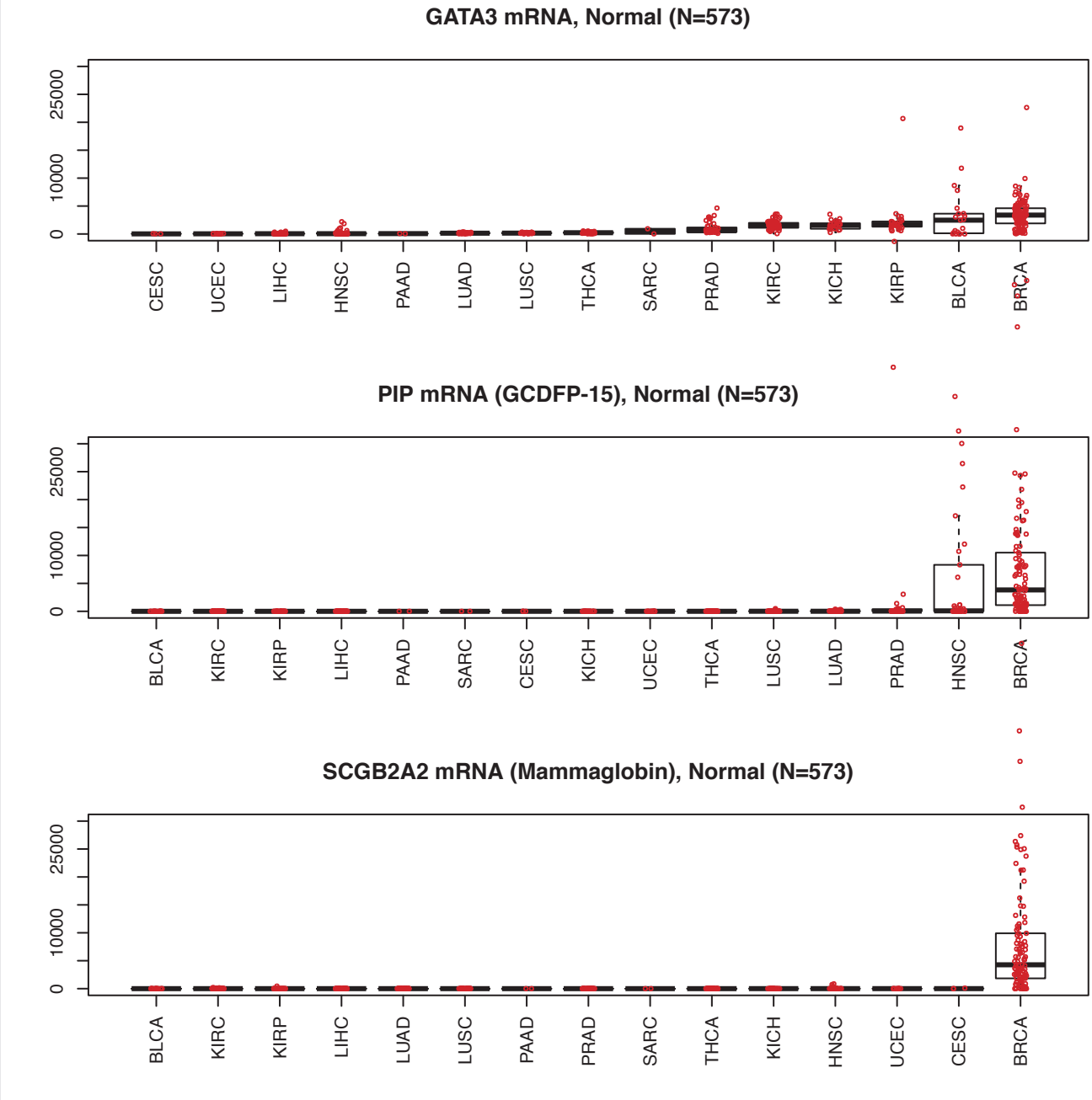

GATA3 mRNA, Normal (N=573)
0
10000
25000
CESC
UCEC
LIHC
HNSC
PAAD
LUAD
LUSC
THCA
SARC
PRAD
KIRC
KICH
KIRP
BLCA
BRCA
PIP mRNA (GCDFP-15), Normal (N=573)
0
10000
25000
BLCA
KIRC
KIRP
LIHC
PAAD
SARC
CESC
KICH
UCEC
THCA
LUSC
LUAD
PRAD
HNSC
BRCA
SCGB2A2 mRNA (Mammaglobin), Normal (N=573)
0
10000
25000
BLCA
KIRC
KIRP
LIHC
LUAD
LUSC
PAAD
PRAD
SARC
THCA
KICH
HNSC
UCEC
CESC
BRCA

Figure 1A

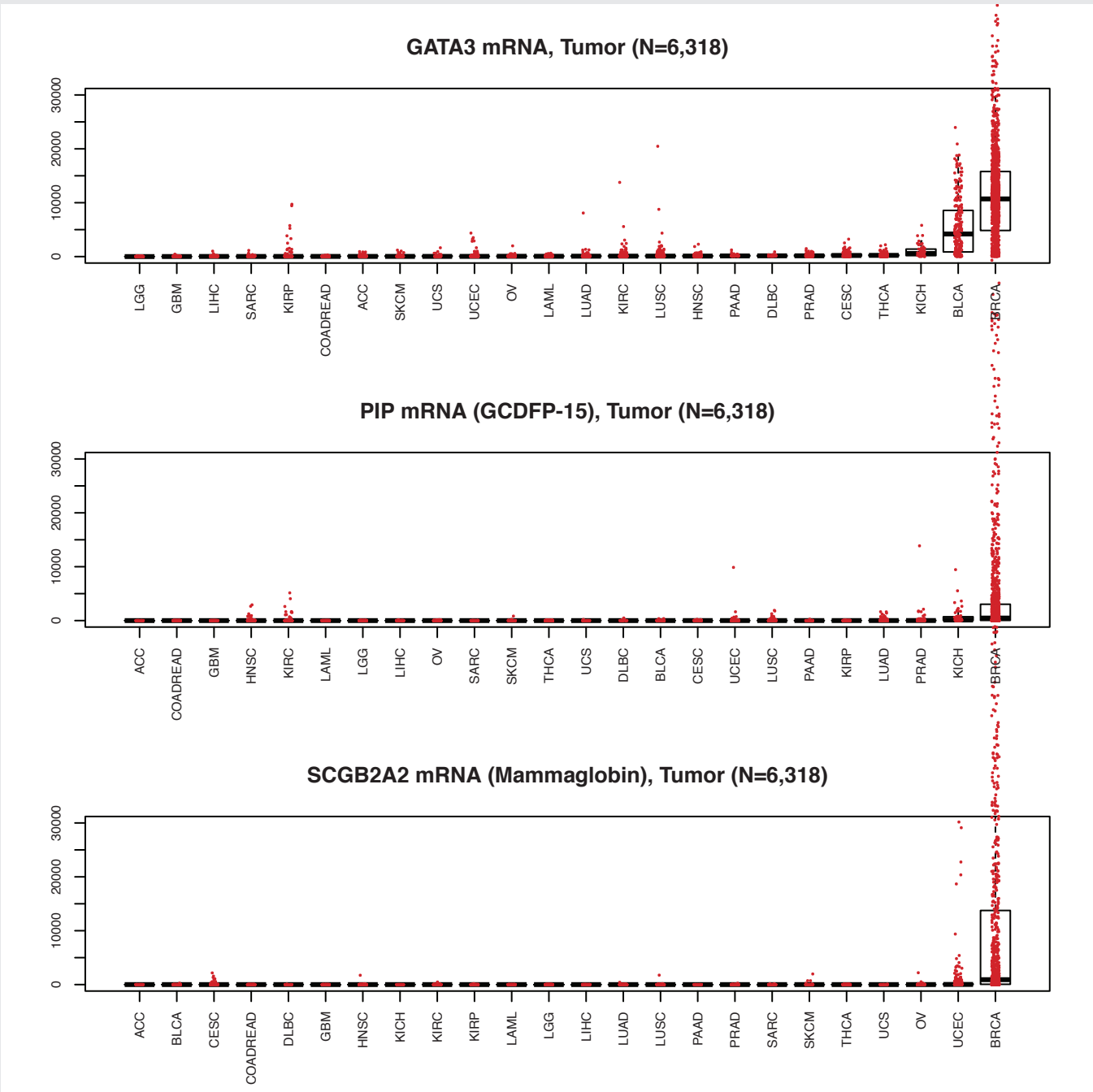

GATA3 mRNA, Tumor (N=6,318)
0
10000
20000
30000
LGG
GBM
LIHC
SARC
KIRP
COADREAD
ACC
SKCM
UCS
UCEC
OV
LAML
LUAD
KIRC
LUSC
HNSC
PAAD
DLBC
PRAD
CESC
THCA
KICH
BLCA
BRCA
PIP mRNA (GCDFP-15), Tumor (N=6,318)
0
10000
20000
30000
ACC
COADREAD
GBM
HNSC
KIRC
LAML
LGG
LIHC
OV
SARC
SKCM
THCA
UCS
DLBC
BLCA
CESC
UCEC
LUSC
PAAD
KIRP
LUAD
PRAD
KICH
BRCA
SCGB2A2 mRNA (Mammaglobin), Tumor (N=6,318)
0
10000
20000
30000
ACC
BLCA
CESC
COADREAD
DLBC
GBM
HNSC
KICH
KIRC
KIRP
LAML
LGG
LIHC
LUAD
LUSC
PAAD
PRAD
SARC
SKCM
THCA
UCS
OV
UCEC
BRCA

Figure 1B

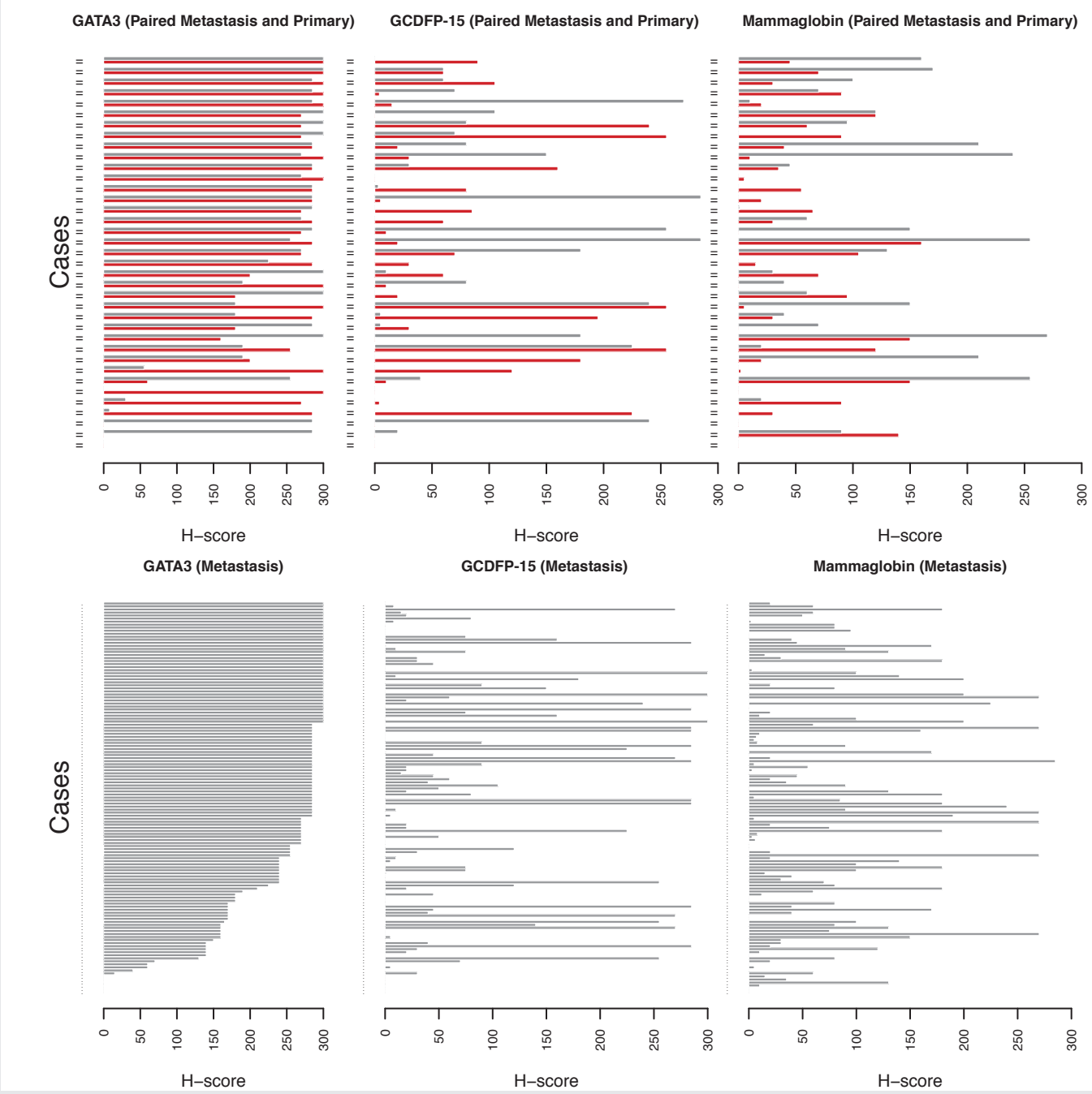

GATA3 (Paired Metastasis and Primary)
GCDFP-15 (Paired Metastasis and Primary)
Mammaglobin (Paired Metastasis and Primary)
Cases
0
50
100
150
200
250
300
H–score
GATA3 (Metastasis)
GCDFP-15 (Metastasis)
Mammaglobin (Metastasis)
Cases
H–score

Figure 2

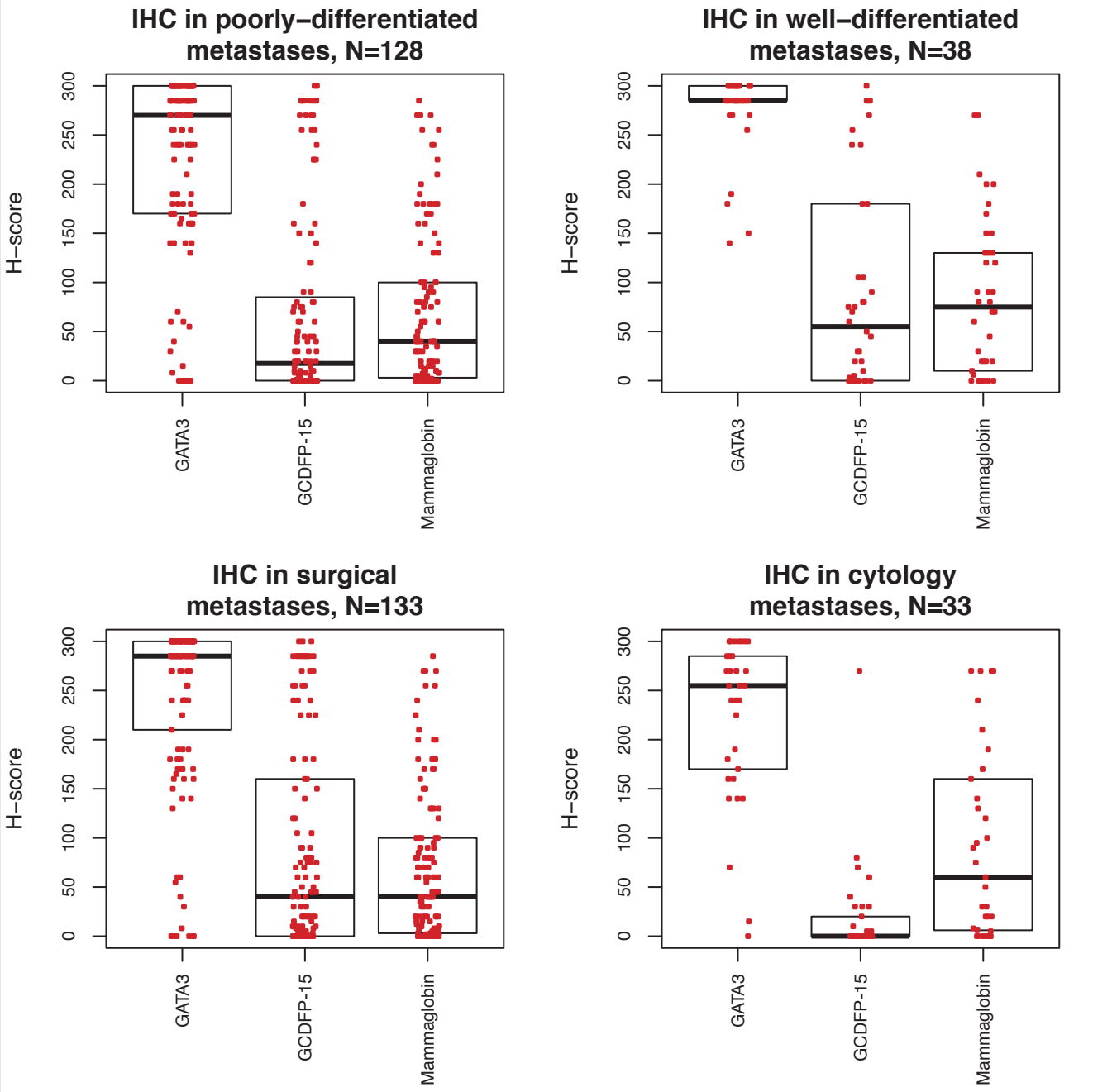
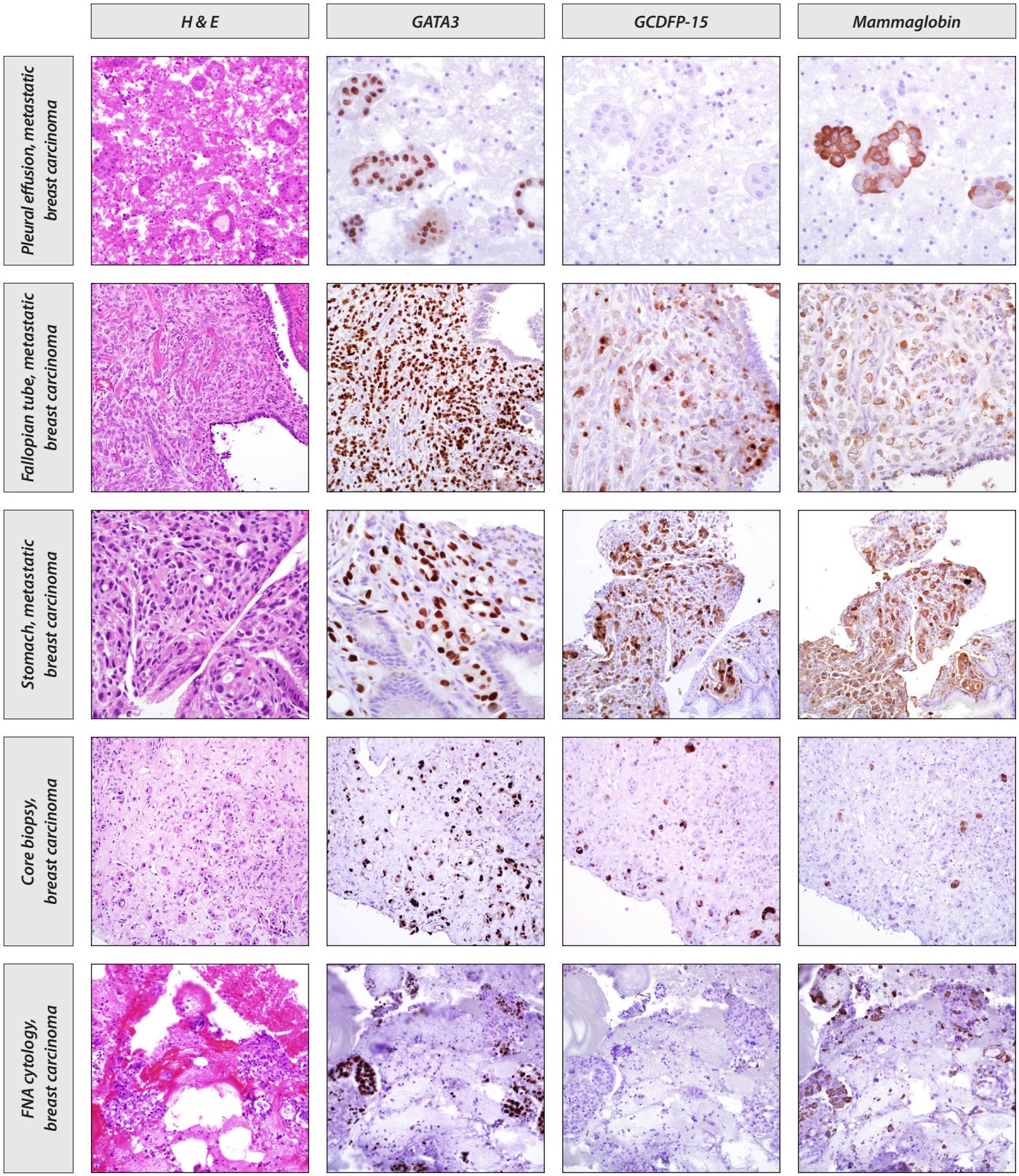

IHC in poorly–differentiated metastases, N=128
IHC in well–differentiated metastases, N=38
IHC in surgical metastases, N=133
IHC in cytology metastases, N=33
H–score
0
50
100
150
200
250
300
GATA3
GCDFP-15
Mammaglobin

Figure 3

The Novel Marker GATA3 is Significantly More Sensitive than Traditional Markers Mammaglobin and GCDFP-15 for Identifying Breast Cancer in Surgical and Cytology Specimens of Metastatic and Matched Primary Tumors



CELL MARQUE
a SIGMA-ALDRICH company
• Toll-Free: 800.665.7284 • Direct: 916.746.8900 • service@cellmarque.com • www.cellmarque.com