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A comprehensive analysis of GATA3 expression in carcinomas of various origins with emphasis on lung carcinomas

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Abstract

GATA3 is a transcription factor involved in the embryogenesis of multiple human tissues and organs and in maintaining cell differentiation and tissue homeostasis in the adult organism. GATA3 is also involved in carcinogenesis and is regarded as a sensitive marker for urothelial and breast carcinomas, although its expression in carcinomas of non-breast/urothelial origin has been frequently reported. In this study, we sought to examine the extent and intensity of GATA3 expression in various carcinomas, mainly lung, urothelial, breast, and various other primary sites. Patients with breast carcinoma (n=40), carcinoma of the urinary bladder/renal pelvis (n=40), lung carcinoma (n=110), and various other origins (n=45) were included in the study. 165 patients had a primary tumor diagnosis, and 70 cases had a metastatic tumor diagnosis. Our results showed that GATA3 expression was significantly more common in carcinomas of the breast, urinary bladder, and renal pelvis compared to all other origins. All primary and 93% of metastatic urinary bladder carcinomas and 94% of primary and 80% of metastatic breast carcinomas expressed GATA3. Expression was lower in the non-urothelial histology of urinary primaries and in triple-negative breast carcinomas (TNBC). Focal staining, mostly faint, was seen in 5.6% of the primary lung adenocarcinomas and 35% of the primary lung squamous cell carcinomas. More extensive and intense staining was seen in 3.7% of the primary lung adenocarcinomas and 12% of the primary lung squamous cell carcinomas. Expression, mostly focal, was also seen in 30% of the metastatic lung carcinomas. Finally, high expression was seen in 12.5% of the other tumors (one metastatic pancreatic carcinoma, one metastatic salivary gland adenocarcinoma not otherwise specified, one metastatic squamous cell carcinoma of the skin, one primary uterine cervix serous carcinoma, and one squamous cell carcinoma of the head and neck), and focal expression was present in another 22% of them. No ideal cut-off for positivity for GATA3 staining could be identified, as increasing the cut-off in either the extent or the intensity of staining increased specificity but decreased sensitivity. In conclusion, our study shows that although GATA3 staining is very helpful in everyday practice in determining the breast/urothelial origin of carcinomas, there are two caveats to its use: the first is that nonclassical histologies of urothelial carcinomas and TNBC may be negative for the marker, and secondly, carcinomas of various origins may show (although rarely) intense positivity.

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Key words: GATA3, urothelial carcinoma, breast carcinoma, lung carcinoma, sensitivity, specificity.

Contributions: all the authors made a substantial intellectual contribution, read and approved the final version of the manuscript, and agreed to be accountable for all aspects of the work.

Conflict of interest: the authors declare no conflict of interest.

Ethics approval and consent to participate: the study protocol was approved by the University Hospital of Patras Committee for Research, Morality and Ethics (#246/14.05.2021).

Patient consent for publication: not applicable.

Funding: none.

Availability of data and materials: the datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Acknowledgments: the authors would like to thank Maria Roumelioti, Department of Pathology and Cytology, University Hospital of Patras, for her help with immunohistochemical staining of the slides.

Received: 17 May 2023.
Accepted: 27 July 2023.
Early view: 29 August 2023.

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Licensee PAGEPress, Italy
Monaldi Archives for Chest Disease 2024; 94:2641
doi: 10.4081/monaldi.2023.2641

Introduction

GATA3 belongs to the GATA family of transcription factors named after the DNA sequence (A/T)GATA(A/G) that is recognized by all members of the family [1]. A pioneer activity has been reported for the GATA family of transcription factors, meaning that they can bind heterochromatic areas in the DNA and enhance the recruitment of additional transcription factors, thus opening the chromatin to an euchromatin state [2,3]. GATA3 is involved in the embryoge-

nesis of multiple human tissues and organs [1,4], especially in the development of T-cells in the thymus [1,4], in trophoblastic differentiation [1], hematopoiesis [5], mammary gland morphogenesis and luminal cell differentiation [6,7], and kidney and urogenital duct development [8,9]. Deletion/mutations of the *GATA3* gene have been associated with hypoparathyroidism, sensorineural deafness, and renal anomaly syndrome, an autosomal dominant genetic disease also known as Barakat syndrome (OMIM#146255) [10]. A role for the GATA family of proteins in the maintenance of cell differentiation and tissue homeostasis in the adult organism [7,11,12] has also been proposed. In particular, GATA3 has been shown to be involved in various processes, including luminal cell differentiation [6], sympathetic neuron survival [13], and hematopoietic stem cell self-renewal [14].

GATA3 is also involved in carcinogenesis. GATA3 mutations have been observed in breast carcinomas [15], and a tumor suppressor role has been attributed to GATA3 in breast and urothelial carcinomas [16-19]. The exact role of GATA3 in tumorigenesis may be site-dependent, as a tumor-promoting role has been described [20,21]. GATA3 has also been implicated in therapy resistance [22]. Pathologists across the world have been using GATA3 to support the urothelial or breast origin of carcinomas, based on studies that have identified the immunohistochemical expression of GATA3 as a sensitive marker for urothelial and breast carcinomas [23-25]. A positive prognostic role has also been attributed to GATA3 expression in breast carcinomas [26]. In the last years, an increasing number of studies have reported GATA3 expression in carcinomas of various other origins. Expression in carcinomas of non-breast/urothelial origin is usually focal and/or faint; thus, the utility of GATA3 in the clinical setting remains high. However, the extent and intensity of GATA3 expression in carcinomas of non-urothelial/breast origin and the cut-off value associated with better sensitivity and specificity in identifying a breast and urothelial origin have not been studied in detail before.

In this study, we sought to examine the extent and intensity of GATA3 expression in carcinomas of the lung, urothelial, breast, and various other primary sites to better describe the level of GATA3 expression across various tumors and define the cut-off value associated with higher sensitivity and specificity in identifying the primary site of the tumor. The objective of the study was to investigate the accuracy of GATA3 as a marker of breast and urothelial origin and highlight potential pitfalls in its use as a diagnostic marker.

Materials and Methods

Patients

The electronic database of the Department of Pathology of the University Hospital of Patras was searched for histopathological cases in which a GATA3 immunohistochemistry was performed as part of the diagnostic workup of the tumor in the years 2017-2020. Slides were retrieved from the archives of the Department of Pathology and reviewed by three pathologists (E-CM, PR, and VT). The electronic files of the patients were reviewed by FS to determine the primary site of the tumor. In total, 138 cases were retrieved. In addition, 97 cases with a primary lung carcinoma diagnosis, for which adequate material for immunohistochemical studies was available, were retrieved from the electronic files of the Department of Pathology from the years 2017-2020 and subjected to immunohistochemical staining with the same GATA3 antibody. The study has been approved by the University Hospital of Patras Committee for Research, Morality and Ethics (#246/14.05.2021).

In total, 235 cases were included in the study: 165 patients had a primary tumor diagnosis, and 70 cases had a metastatic tumor diagnosis. As expected, more samples from primary carcinomas, compared to metastatic carcinomas, were retrieved, as primary carcinomas are more commonly operated on and biopsied, and material from primary tumors is usually more abundant for further studies. The mean age of the patients was 64 years old [standard deviation (SD): 12] for the primary cases and 66 years old (SD: 11) for the metastatic cases. 89 cases were surgical excision specimens, 60 were surgical biopsies, 49 were endoscopic biopsies, 36 were fine needle aspirations/biopsies, and one was a bone marrow biopsy. The primary site and histologic type of the tumors are shown in *Supplementary Table 1*.

Methods

Immunohistochemistry was performed as previously described [27]. Briefly, 4 μm tissue sections were incubated with ethylenediamine tetraacetic acid pH 9 at 600 W in a microwave for 20 minutes. Then primary GATA3 (1:100, Cell Marque, clone) antibody was added. Envision (Dako, Carpentaria, CA, USA) was used as the detection system. Sections were counterstained with Harris' acidified hematoxylin.

Immunohistochemical evaluation

All slides were reviewed by two experienced pathologists (PR and VT) and one junior pathologist (E-CM). All reviewers were blinded to the primary site and histologic-type diagnosis of the tumor. Only nuclear staining was evaluated as GATA3 is a transcription factor and only nuclear expression is considered specific for diagnostic purposes. A weak cytoplasmic staining was seen in some cases but was considered non-specific and was not scored. The percentage of cells showing positive nuclear expression (1%, 5%, 10%, and then in increments of 10 up to 100%) and the intensity of staining (1+, 2+, and 3+) were assessed. The percentage of staining was divided into negative, 1:1-30%, 2:40-70%, and 3:>70%. The intensity of staining was multiplied by the level of staining to obtain a combined score (scale 0-9). Both the percentage of staining (labeled as extent) and the combined score were used for further analysis. In addition, for comparisons between the expression of GATA3, estrogen receptor (ER), and progesterone receptor (PR), the level of staining was multiplied by the intensity of staining for a total combined score of 0-300.

Statistical analysis

Patient characteristics and biomarker expression data were summarized with descriptive statistics (mean, median, and SD) and contingency tables. The chi-square test was used to compare the expression of GATA3 among the different histologic types and primary sites. Pearson's correlation was used to compare the relationship between the GATA3 and ER/PR. All reported p-values are two-sided at a significance level of 0.05. Analyses were performed using SPSS (released 2009; PASW Statistics for Windows, Version 18.0; SPSS Inc., Chicago, IL, USA).

Results

GATA3 expression was seen in 121 cases (51.5%), whereas 114 cases (48.5%) were negative. Faint to moderate staining was seen in adjacent lymphocytes, which served as a positive control in the negative cases. Among the positive cases, the extent of staining was 1

(1-30%) in 39 cases, 2 (40-70%) in 18 cases, and 3 (>70%) in 64 cases, and the intensity was 1+ in 37 cases, 2+ in 42 cases, and 3+ in 42 cases. GATA3 expression was significantly more common in carcinomas of the breast, urinary bladder, and renal pelvis compared to all other origins, both in the primary (p<0.001) and metastatic (p<0.001) settings (Table 1).

The mean and SD of expression levels in carcinomas of the breast, urinary bladder, renal pelvis, and lung according to histologic type are shown in *Supplementary Table 2*.

GATA3 expression in breast carcinomas

GATA3 was expressed in 16/17 primary carcinomas of no special type, 1/1 primary lobular carcinomas, and in 17/18 metastatic carcinomas of no special type. Expression levels and combined scores are shown in Table 2. Figure 1 shows representative photos of GATA3 staining in breast carcinomas. Mean expression (SD) levels in primary tumors were 82% (32%) in carcinomas of no special type, 78% (29%) in lobular carcinomas, and 100% in mucinous carcinomas.

Data regarding ER, PR, and human epidermal growth factor receptor 2 (cerbB2) expression in breast carcinomas was retrieved from patients' files. Among the 40 breast carcinomas, 14, 19, and 38 were ER, PR, and cerbB2 negative. 12 carcinomas were negative for all markers (triple negative). There was no difference in the expression of the markers between primary and metastatic breast carcinomas or in regard to the histologic type.

GATA3 expression showed a significant correlation with ER expression (p=0.002, r=0.476). In addition, GATA3 levels were higher in ER-positive tumors (mean 89) *versus* ER-negative tumors (mean 68) (p=0.034). When taking into account the intensity of staining, ER-negative tumors had a mean combined score of 138 (SD: 111) and ER-positive tumors of 241 (SD: 82), the difference between the two being statistically significant (p=0.002). The two GATA3-negative tumors were also ER (and PR and cerbB2) negative (triple negative), whereas 12 ER-negative, 17 PR-negative, 36 cerbB2-negative, and 10 triple-negative tumors were GATA3 positive.

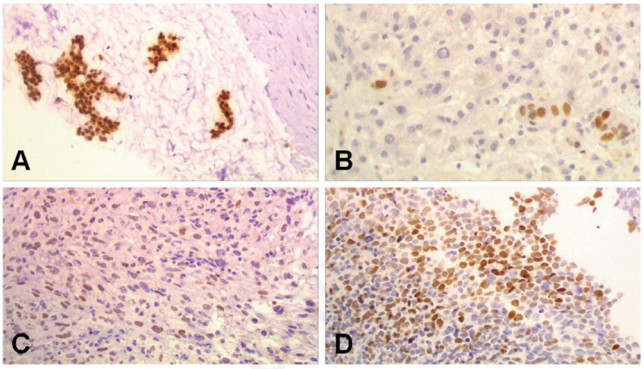

Figure 1. GATA3 expression in urothelial and breast carcinomas. A) High expression in a case of invasive mucinous breast carcinoma; B) triple-negative breast carcinoma of no special type with null GATA3. Trapped non-neoplastic lobules serve as an internal positive control; C) high expression in sarcomatoid urothelial carcinoma; D) GATA3 is highly expressed in this neuroendocrine (small cell) carcinoma of the bladder (original magnification ×200).

Table 1. GATA3 expression in carcinomas of breast/bladder/pelvis *versus* other sites.

GATA3	Extent	0	1	2	3	Total				p
Primary	Breast/bladder/pelvis	1	3	6	33	43				<0.001
	Other sites	90	24	6	2	122				
Metastatic	Breast/bladder/pelvis	3	5	4	25	37				<0.001
	Other sites	20	7	2	4	33				
	Combined score	**0**	**1**	**2**	**3**	**4**	**6**	**9**	**Total**	**p**
Primary	Breast/bladder/pelvis	1	1	5	2	1	13	20	43	<0.001
	Other sites	90	19	8	0	3	1	1	122	
Metastatic	Breast/bladder/pelvis	3	3	2	0	3	10	16	37	<0.001
	Other sites	20	5	3	0	1	2	2	33	

Table 2. GATA3 expression in breast carcinomas.

GATA3	Extent	0	1	2	3	Total			
Primary	Carcinoma no special type	1	0	1	15	16			
	Lobular	0	0	0	1	2			
	Mucinous carcinoma	0	0	0	1	1			
Metastatic	Carcinoma no special type	1	3	2	12	18			
	Lobular	0	0	2	1	3			
	Combined score	**0**	**1**	**2**	**3**	**4**	**6**	**9**	**Total**
Primary	Carcinoma no special type	0	0	1	2	0	3	10	16
	Lobular	1	0	0	0	0	0	1	2
	Mucinous carcinoma	0	0	0	0	0	0	1	1
Metastatic	Carcinoma no special type	1	2	1	0	2	6	6	18
	Lobular	0	0	0	0	1	1	1	3

GATA3 expression in urinary bladder and renal pelvis tumors

GATA3 was expressed in 16/16 primary urothelial carcinomas, in 2/2 primary small cell carcinomas, and in 2/2 primary sarcomatoid carcinomas of the urinary bladder. Mean expression (SD) levels in primary tumors were 92% (13%) in urothelial carcinomas, 22.5% (25%) in neuroendocrine carcinoma, and 85% (21%) in sarcomatoid carcinomas.

Regarding metastatic bladder tumors, expression was seen in 12/13 urothelial carcinomas and 0/1 squamous carcinomas (SqCC). Mean expression levels were 81% (SD: 36%) for metastatic urothelial carcinomas.

In carcinomas of the renal pelvis, GATA3 was expressed in 3/3 primary, 2/2 metastatic urothelial carcinomas, and 1/1 primary small cell carcinomas. Mean expression levels were 60% (SD: 51%) for primary urothelial carcinomas and 65% (SD: 49%) for metastatic tumors.

Expression levels and the combined score of GATA3 expression in tumors of the urinary bladder and renal pelvis are shown in Table 3.

GATA3 expression in lung carcinomas

In primary lung carcinomas, GATA3 expression was seen in 5/53 adenocarcinomas, 20/42 SqCC, and 0/2 non-small cell carcinomas (NSCLC), not otherwise specified (NOS). In metastatic lung carcinomas, GATA3 expression was seen in 4/8 adenocarcinomas, 1/2 SqCC, 2/2 large cell carcinomas, and 0/1 neuroendocrine tumors (atypical carcinoid).

Expression levels and combined score of GATA3 expression in tumors of the lung are shown in Table 4.

GATA3 expression in other tumors

Significant (>40%) GATA3 expression was seen in one metastatic pancreatic carcinoma (extent 3, combined score 9), one metastatic salivary gland adenocarcinoma NOS (extent 3, combined score 6), one metastatic SqCC of the skin (extent 2, combined score 4), one primary uterine cervix serous carcinoma (extent 3, combined score 9), and one SqCC of the head and neck (extent 3, combined score 6) (Figure 2). Focal staining (5-10%) was seen in five cases: two gastric adenocarcinomas, one ovarian transitional cell carcinoma, one ovarian mucinous carcinoma, and one renal angiomyolipoma. Rare positive cells (<5%) were seen in four cases: two ovarian serous carcinomas, one cervical SqCC, and one esophageal SqCC.

Sensitivity and specificity of GATA3 expression in identifying breast/urothelial origin of a tumor

We calculated the sensitivity and specificity of GATA3 expression in identifying a breast/urothelial origin and breast/urothelial his-

Table 3. GATA3 expression in carcinomas of urothelial origin (urinary bladder and renal pelvis).

GATA3	Extent	0	1	2	3	Total
Primary	Urothelial carcinoma	0	1	3	15	19
	NECA	0	2	1	0	3
	Sarcomatoid carcinoma	0	0	1	1	2
Metastatic	Urothelial carcinoma	1	2	0	12	15
	Squamous cell carcinoma	1	0	0	0	1

	Combined score	0	1	2	3	4	6	9	Total
Primary	Urothelial carcinoma	0	1	1	0	1	9	7	19
	NECA	0	0	2	0	0	1	0	3
	Sarcomatoid carcinoma	0	0	1	0	0	0	1	2
Metastatic	Urothelial carcinoma	1	1	1	0	0	3	9	15
	Squamous cell carcinoma	1	0	0	0	0	0	0	1

NECA, neuroendocrine carcinoma.

Table 4. GATA3 expression in carcinomas of the lung.

GATA3	Extent	0	1	2	3	Total
Primary	Adenocarcinoma	48	3	2	0	53
	Squamous cell carcinoma	22	15	4	1	42
	NSCLC, NOS	2	0	0	0	2
Metastatic	Adenocarcinoma	4	2	1	1	8
	Squamous cell carcinoma	1	1	0	0	2
	Large cell carcinoma	1	1	0	0	2
	NET	1	0	0	0	1

	Combined score	0	1	2	3	4	6	9	Total
Primary	Adenocarcinoma	48	2	3	0	0	0	0	53
	Squamous cell carcinoma	22	11	5	0	3	1	0	42
	Undifferentiated carcinoma	2	0	0	0	0	0	0	2
Metastatic	Adenocarcinoma	4	0	3	0	0	0	1	8
	Squamous cell carcinoma	1	1	0	0	0	0	0	2
	Undifferentiated carcinoma	1	1	0	0	0	0	0	2
	NET	1	0	0	0	0	0	0	1

NSCLC, non-small cell carcinomas; NOS, not otherwise specified; NET, neuroendocrine tumors.

tology of a tumor in our entire cohort for various cut-offs (Tables 5 and 6). Our results showed that sensitivity and specificity were generally higher for primary tumors compared to metastatic carcinomas. Both sensitivity and specificity were higher in defining the histology (urothelial and breast-NOS/lobular, *versus* other histologies), compared to defining the primary site (urinary/bladder/pelvis/breast *versus* other sites) in metastatic tumors. Most importantly our study shows that even though GATA3 expression is an indication of the breast/urothelial origin of the tumor, high expression can be noted in other tumors and there is no single cut-off that can be used to provide confidence of the tumor's origin. The incorporation of the intensity of staining into the scoring system increased sensitivity and specificity for both origin and histology in the metastatic samples.

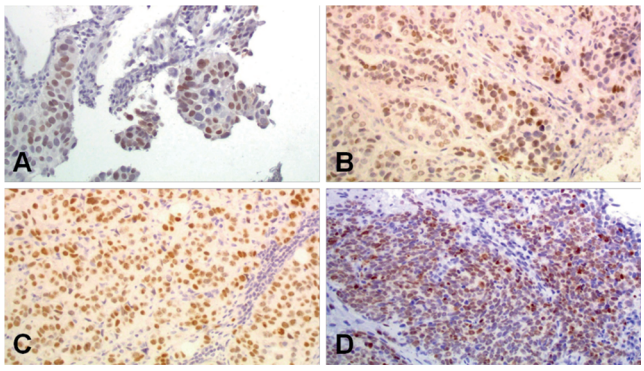

Figure 2. GATA3 expression in carcinomas of non-urothelial/non-breast origin. A) High expression in squamous cell carcinoma of the lung; B) moderate to intense expression in a lung adenocarcinoma; C) high expression in a lymph node metastatic pancreatic carcinoma; D) high expression in a lymph node metastatic squamous cell carcinoma of the head and neck (original magnification ×200).

Discussion

Previous studies have shown that GATA3 is a sensitive and specific marker for breast [28], and urothelial carcinomas [23]. However, an increasing number of studies have shown positivity in various tumors of non-urothelial, non-breast origin. Most of the studies have considered a single cut-off [*i.e.*, <1% (28), 20%] [23] and/or did not take the intensity of staining into account. When extent and intensity were taken into account, most of the tumors of non-breast/non-urothelial origin, *i.e.*, anal and uterine SqCC, showed weak and/or focal staining [23].

In this study, we examined the extent and intensity of GATA3 immunohistochemical staining in breast, lung, and urothelial carcinomas and a variety of other carcinomas to evaluate its utility in clinical practice in both primary and metastatic settings. In agreement with the literature and the current routine use of GATA3 [24,28], its expression was more common in breast and urothelial carcinomas, compared to carcinomas of other tissue origins.

All primary urothelial carcinomas and 93% of metastatic urothelial carcinomas expressed GATA3 in our study. In 94% of the primary tumors and 80% of the metastatic tumors, expression was seen in >40% of the cells. These findings are in agreement with previous studies that have shown non-focal (cut-offs varying, the most common being >5% and >20%) staining of GATA3 in 77-99% of urothelial carcinomas [23,29-31]. Staining is more common in low-grade carcinomas [30] and in the luminal molecular subtype [32]. Similar to our results, most tumors show moderate to strong staining [23,31]. In addition, our results showed that GATA3 expression is higher in primary compared to metastatic tumors. This is in line with previous findings that focal staining is somewhat more common in metastatic

Table 5. Sensitivity and specificity of GATA3 expression for urinary bladder/renal pelvis/breast origin in the whole cohort.

	Primary tumors		Metastatic tumors	
Cut-off	Sensitivity	Specificity	Sensitivity	Specificity
0	97.6	73.2	91.9	60.6
1	92.9	93.5	78.4	81.8
2	78.6	98.4	67.6	87.8
1	95.2	88.6	83.8	75.6
2	85.7	95.9	78.4	84.9
3	80.9	95.9	78.4	84.9
4	78.6	98.4	70.3	87.9

Table 6. Sensitivity and specificity of GATA3 expression for urothelial/breast histology in the whole cohort.

	Primary tumors		Metastatic tumors	
Cut-off	Sensitivity	Specificity	Sensitivity	Specificity
0	97.4	71.6	94.4	61.8
1	94.7	91.3	80.6	82.3
2	84.2	97.6	69.4	88.2
1	94.7	85.8	86.1	76.5
2	89.5	94.5	80.5	85.3
3	84.2	94.5	80.5	85.3
4	81.6	96.9	76.5	88.2

urothelial carcinomas compared to primary tumors, with the latter showing non-focal staining [23] and that loss of GATA3 expression is seen in 25% of urothelial carcinomas that express the marker in the primary setting [31]. In addition, a higher sensitivity of GATA3 staining has been shown in primary urothelial carcinoma compared to cell blocks from metastatic foci [33].

Expression of GATA3 in urinary bladder tumors with a non-urothelial morphology, *i.e.*, adenocarcinoma [34], and sarcomatoid carcinoma [35], has been noted, although at a lower frequency and with a lower extent and intensity of staining, whereas expression in small cell and squamous carcinomas is quite rare [36]. Our study did not focus on urothelial carcinoma variants, and the number of tumors with such histologies was very small. However, we did find intense GATA3 staining in neuroendocrine and sarcomatoid carcinomas, supporting the notion that GATA3 can be used when evaluating bladder tumors of non-classic histology and can be very helpful in cases of such metastatic carcinomas of unknown primary to support a urothelial primary. However, a larger series with these types of carcinomas from various origins should be evaluated for more valuable results.

In breast carcinoma cases, GATA3 displayed non-focal and moderate to strong immunoreaction in 94% of the primary carcinomas and 80% of metastatic tumors. One primary and one metastatic carcinoma were completely negative for the marker. Both tumors were triple-negative carcinomas. Expression of GATA3 in breast carcinoma varies in the literature, depending on the cut-off used, the histologic grade, and the hormonal status/molecular subtype of the tumors [24-26,28,37,38]. For ER-positive tumors, staining has been shown in 72-96.6% of the cases studied [24,28,37,38]. Expression in metastatic carcinoma is somewhat lower [39], similar to our findings, albeit a high concordance in GATA3 expression has been shown among matched primary and metastatic carcinomas [37]. Expression in triple-negative breast carcinomas (TNBC) is much lower (5.7-48%) [25,28,37,38]. However, in our study, a higher percentage of TNBC with GATA3 positivity was seen (80%). Thus, GATA3 expression remains a useful marker to determine a breast primary regardless of its molecular subtype, keeping in mind that its sensitivity may be lower in TNBC and in the metastatic setting.

GATA3 expression was seen in a minority of lung carcinoma cases in our series. In SqCC, expression was more common than in adenocarcinomas. Focal staining, mostly faint, was seen in 5.6% of the primary lung adenocarcinomas and 35% of the primary SqCC. More extensive and intense staining was seen in 3.7% of the primary lung adenocarcinomas and 12% of the primary lung SqCC. Previous studies have shown a null phenotype [15,32], or rare (1-8% of adenocarcinomas and 6-23% of SqCC) GATA3 staining in NSCLC [37,39-42]. The pattern of staining is frequently not adequately described in these studies but is usually focal and of low intensity. Importantly, our study adds to these findings that i) the number of NSCLC cases staining positive for GATA3 is higher than previously reported, especially amongst the SqCC; and ii) even though staining of these cases is usually weak and focal, extensive and intense staining may, albeit rarely, be seen. In these cases, attention to morphology (*i.e.*, features of SqCC), evaluation of other markers (thyroid transcription factor 1, ER), clinical history, and radiologic correlation are important for the correct diagnosis to be rendered.

Perhaps the most important finding of our study was that intense and non-focal GATA3 expression was seen in a few cases of non-breast/non-urothelial origin (one metastatic pancreatic carcinoma, one metastatic salivary gland adenocarcinoma NOS, one metastatic SqCC of the skin, one primary uterine cervix serous carcinoma, and one SqCC of the head and neck). This is an important finding, rarely highlighted in previous studies but not uncommonly seen in routine practice and confirmed in our study, that needs to be considered when evaluating this marker in the diagnostic setting. This is particularly important in metastatic tumors of unknown origin, where immunohistochemistry is used to determine the primary site of the tumor. Expression in SqCC of various origins, especially the skin, was not surprising as it has been reported before [41]. The strong GATA3 expression in skin SqCC is probably correlated with its expression in the normal epidermis [41], a finding also noted in our cases.

Similarly, expression in salivary gland and pancreatic ductal adenocarcinomas has been seen in 43% and 37% of the cases, with the number of positive cells varying from 10 to 100%. Our study confirms these findings and highlights the pancreas and the salivary gland as potential origins of GATA3-positive metastatic adenocarcinomas.

The best cut-off for GATA3 positivity remains to be determined. A combined score of 2 (more than 40% positive cells, or any positive cells with at least moderate intensity) seems like an acceptable cut-off value based on our results, with 78.4 sensitivity and 84.9 specificity in determining the site of origin and 80.5 sensitivity and 85.3 specificity in determining the classic breast and urothelial histologies in the metastatic setting. However, our study highlights the fact that there is no ideal cut-off for positivity for GATA3 staining, as increasing the cut-off in either the extent or the intensity of staining increases specificity but decreases sensitivity. In addition, sensitivity and specificity were lower for the metastatic tumors compared to the primary carcinomas, and sensitivity was lower, and specificity was higher when non-classic histologies of breast and urothelial origin were included.

Conclusions

Our study shows that although GATA3 staining is very helpful in everyday practice in determining the breast/urothelial origin of carcinomas, there are two caveats to its use: the first is that non-classical histologies of urothelial carcinomas and TNBC may be negative for the marker, and secondly, carcinomas of various origins may show (although rarely) intense positivity.

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Online supplementary material:

Supplementary Table 1. Primary site and histologic type of the tumors.

Supplementary Table 2. Mean and standard deviation of expression levels in carcinomas of the breast, urinary bladder, renal pelvis, and lung according to histologic type.