EXPRESSION OF GATA 3 IN EPITHELIAL TUMORS

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ABSTRACT

Objective: To assess the expression of GATA-3 among epithelial tumors of four organs including invasive mammary carcinoma, prostatic adenocarcinoma, colorectal carcinoma and high grade serous carcinoma of ovary.
Study Design: Case series study.
Place and Duration of Study: The study was performed at Shaukat Khanum Memorial Cancer Hospital and Research Center, from Jul 2017 to Dec 2017.
Methodology: Twenty cases of each of these tumors were collected and stained with ready to use GATA-3 immunohistochemical stain (Cell marque (L50-823) mouse monoclonal antibody) using BOND III Leica automated immunostainer. The expression of the antibody was assessed by two histopathologists. Diffuse nuclear staining in more than 30% of tumor cells was considered as positive.
Results: All the breast carcinomas showed diffuse strong positivity in tumor cells, while all the cases of prostatic adenocarcinoma showed negative staining. Aberrant and focal staining was observed in a few cases of high grade serous and colorectal carcinomas. Three cases of high grade serous carcinoma showed weak nuclear staining in 10% of cells. Only one case of colorectal carcinoma showed weak nuclear blush in 10 to 20% of tumor cells.
Conclusion: GATA-3 was found a sensitive and specific marker for mammary carcinomas. It is a useful diagnostic tool and should be included in a panel of immunohistochemical markers when working on a metastatic tumor of unknown origin.

Keywords: Colorectal carcinoma, GATA-3, High grade serous carcinoma, Invasive mammary carcinoma, Prostatic adenocarcinoma.

INTRODUCTION

Specification and maintenance of differentiated cell types that arise from multipotent stem cells is the basic feature of development. This function is mediated by a series of transcription factors and regulator proteins that activate target genes of specific cell fates and repress genes of alternative cell fates. GATA family of transcription factors is one such family which acts by binding to a consensus DNA sequence in the promoters of genes and directly activates or represses expression of target genes. GATA also remodel gene loci by recruitment of chromatin remodeling complexes[1].

There are atleast fifty seven members of GATA family, of which only six, GATA 1-6 are found in mammalia. All these proteins share certain common features including two transactivation domains at amino terminus, two zinc fingers at carboxyl terminus and a conserved basic region immediately following the zinc finger motifs. All the family members show various degrees of homology with each other[2,3].

The distribution of GATA proteins in human body is very tissue specific. GATA 1-2 are expressed in hematopoietic cells while GATA 4-6 are expressed in tissues such as heart liver and intestine. We are mainly concerned with GATA-3 which has a both hematopoietic (where it affects the development of T-cells) and non-hematopoietic (kidney, CNS, skin and mammary glands) distribution[2,3].

The tissue specific distribution of GATA-3 has found great utility in pathology in determining lineages of certain tumors in the correct clinical context[3]. Many site specific markers have been introduced for the last many years with different specificity and sensitivity for different

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organs. Few of these markers show nuclear and few show cytoplasmic expression. Usually when a new immunohistochemical marker is introduced the researcher claims that the particular marker has highest sensitivity for a particular tumor but on extensive research we finally came to know that every immunohistochemical marker shows expression for other tumors as well and this knowledge is important in order to prevent misdiagnosis. Same is the case with GATA-3. It is considered a very sensitive and specific marker for breast and urothelial carcinomas and initial studies also confirm this finding. However to test its sensitivity and specificity, we thought of applying GATA-3 not only in breast tumors but also in other epithelial tumors being diagnosed in our institute[3,4].

The objective of this study was to determine the expression of GATA-3 in epithelial tumors that most commonly present with metastasis i.e. Invasive ductal carcinoma, high grade serous carcinoma, colorectal carcinoma and prostatic adenocarcinoma. Determining sensitivity and specificity of the protein might help in excluding false positive results.

METHODOLOGY

This case series study was conducted at department of Histopathology Shaukat Khanum Memorial Cancer Hospital and Research centre. Twenty cases each of invasive ductal carcinoma of breast, prostatic adenocarcinoma, high grade serous carcinoma of ovary and colorectal carcinoma were selected through non-probability purposive sampling during the period of July 2017 to December 2017. Tissues with poor processing and post treatment specimen were excluded from the study. GATA-3 Immunohistochemical stain Cellmarque (L50-823) mouse monoclonal antibody, ready to use dilution was applied on all the selected tissues using BOND III Leica automated immunostainer.

The cases were evaluated for immunohistochemical staining by two pathologists. A positive staining was defined as nuclear staining in more than 30% of the tumor cells[5]. Cytoplasmic staining or very faint focal nuclear staining was considered as negative. Data were analyzed in SPSS version 21. Descriptive statistics like frequency, percentage, mean and standard deviation were calculated.

RESULTS

Out of twenty cases of prostatic adenocarcinomas, sixteen biopsies were of transurethral resection of prostate followed by two trucut

Table-I: Clinical and Morphological features of patients of Prostatic adenocarcinoma included in the study.

Average age	70.35 ± 10.54 yrs
Types of Biopsies	
TURP	16 (80%)
Trucut	2 (10%)
TVP	2 (10%)
Gleason grade	
Grade 6,7	5 (25%)
Grade 8,9,10	15 (75%)
WHO group	
Group 1	-
Group 2	4 (20%)
Group 3	1 (5%)
Group 4	4 (20%)
Group 5	11 (55%)
Perineural invasion	8 (40%)
Intraductal carcinoma	1 (5%)
High grade PIN	-
Extraprostatic extension	-
Extent of tissue involvement	69.50 ± 20.64%

Table-II: Important clinical and morphological features of breast carcinoma in the study.

Age	47.85 ± 11.92 yrs
Laterality	
Left side	8 (40%)
Right side	12 (60%)
Receptor status (Available in 14 cases)	
Luminal type A	2 (14%)
Luminal type B	8 (57%)
Her 2 neu positive	3 (21%)
Triple negative	1 (07%)
Subtype	
Invasive ductal carcinoma	18 (90%)
Invasive lobular carcinoma	2 (10%)
Grade	
Grade II	8 (40%)
Grade III	12 (60%)

biopsies and two transvesical prostatectomy specimes. Twenty five percent of tumors had a Gleason grade 6, 7 and 75 percent were of Gleason grade 8-10. Majority of the tumors belonged to WHO group 5. Perineural invasion was seen in forty percent of cases. One case also showed intraductal carcinoma. All the cases of prostatic adenocarcinomas regardless of Gleason grade were negative for GATA-3. No nuclear or even aberrant cytoplasmic expression was noted in tumor cells. However, GATA-3 highlighted the basal cells of non-neoplastic prostatic glands and

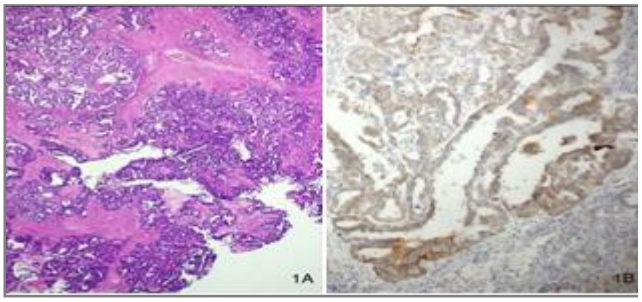

Figure-1: 1A: High grade serous carcinoma (x10), 1B: Non specific cytoplasmic staining of GATA-3.

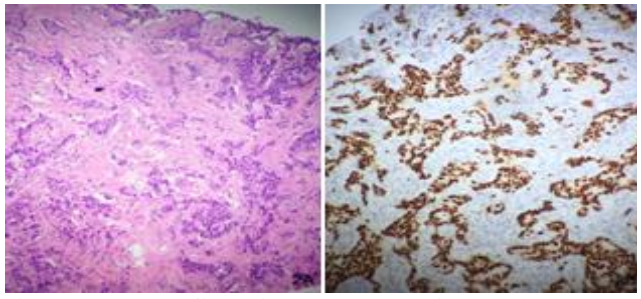

Figure-2: 2A: Infiltrating ductal carcinoma breast (x10), 2B: Strong nuclear labelling of GATA-3.

normal urothelium of the urethra. Basal cells in intraductal carcinoma and basal cell hyperplasia also showed nuclear positivity for GATA-3. The clinical and important morphological aspects of these cases were summarized in (table-I).

Twenty cases of invasive mammary carcinomas were analyzed for expression of GATA-3. Average age of patients was 47.8 years. Important clinical and morphological features of breast carcinoma in the study were summarized in (table-II). There were eighteen cases (90%) of invasive ductal carcinomas and two cases (10%) of invasive lobular carcinomas. Forty percent of tumors were grade II and sixty percent were of grade III. ER, PR and HER2Neu receptor status was available in fourteen cases. Two cases (14%) were luminal type A, eight cases (57%) were luminal type B, three cases (21%) were HER2Neu positive only and one case (7%) was triple negative. All the cases of carcinoma breast showed strong diffuse staining in more than ninety percent of tumor cells regardless of subtype, grade or receptor status. Positive IHC staining was also identified in the normal breast ducts and lobules.

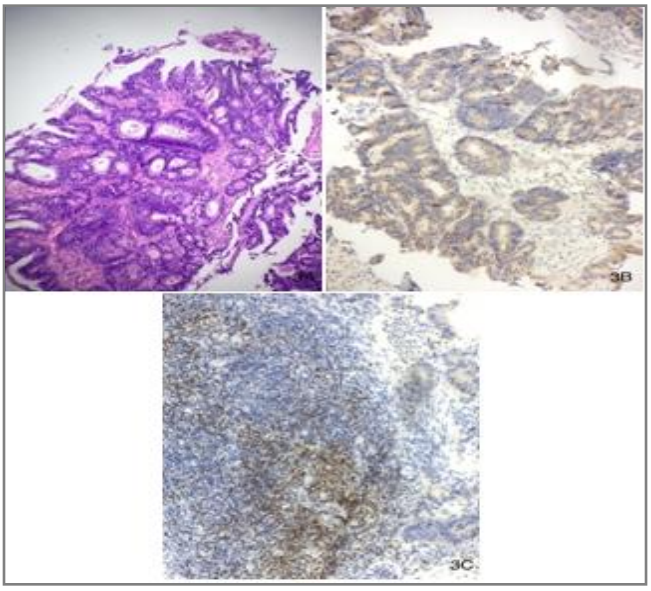

Figure-3: 3A: Colorectal adenocarcinoma showing moderate differentiation (x10), 3B: Non specific cytoplasmic staining of GATA-3 in tumor cells of colonic adenocarcinoma, 3C: Nuclear staining of GATA-3 in lymphocytes.

Although all twenty cases of high grade serous carcinoma showed negative expression, three cases (15%) cases showed weak nuclear staining in 10% of cells and eleven cases (55%) showed non-specific cytoplasmic staining in tumor cells.

In twenty cases of colorectal carcinomas, only one case showed weak nuclear blush in 10-20% of tumor cells. Non-specific cytoplasmic staining was observed in a single case. None of the cases showed staining pattern that met our criteria for positivity. Most of the lymphoid

follicles (T-cells) showed positive nuclear labelling with GATA-3 which can be used as a useful internal control while technically validating individual cases (figure-1 to 4).

DISCUSSION

Metastatic carcinoma of unknown primary causes a challenge to both the clinician and the pathologist. A number of immunohistochemistry algorithms have been developed to determine the site of primary tumor. These immunohistochemical stains include but not limited to CK 7, CK 20, CK 19, TTF 1, CDX 2, NKX 3.1, PSA, PAX 8, WT 1, SATB 2, HMB 45, Melan A, Calretinin, etc. To classify tumors into different groups. Site specific markers such as TTF-1, PSA, Hepar-1, PAX 8, CDX 2, and SATB 2 are used to accurately point out the primary site[6].

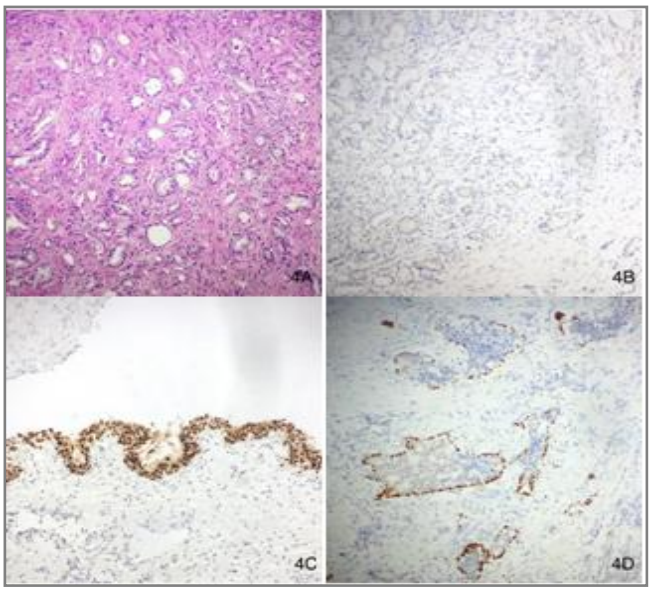

Figure-4: 4A: H&E Prostatic Adenocarcinoma (x10), 4B: Negative staining of GATA 3 in tumor cells of prostatic adenocarcinoma, 4C: GATA-3 showing strong nuclear staining in urothelium of prostatic urethra, 4D: GATA-3 staining in basal layer of non-neoplastic prostatic glands.

GATA-3 is a tissue specific marker and its application has recently been recognized in identifying carcinomas of breast, urothelium and tumors of trophoblastic origin. GATA-3 is localized in the cytoplasm. To regulate gene expression and access its target genes it is transported across the nuclear membrane. Therefore, GATA-3 shows nuclear staining[7].

GATA-3 is usually expressed in breast and urothelial carcinomas. Its expression has also been noted in ovarian Brenner tumors. New studies showed expression in squamous cell carcinoma, skin adnexal tumors and choriocarcinomas[8].

One of the most common problems a pathologist faces in urogenital pathology is differentiating prostatic and bladder tumors. Both the tumors have different treatment protocols including surgery, adjuvant and neoadjuvant radiotherapy or chemotherapy. It is relatively easy to differentiate low grade prostatic adenocarcinoma from urothelial carcinoma. However, higher grade prostatic tumors share many overlapping morphological features with high grade urothelial carcinoma. The current study demonstrated that none of the prostatic adenocarcinomas of any grade showed GATA-3 expression. GATA-3 only highlighted basal cells of non-neoplastic prostatic glands and urothelium of prostatic urethra. Chang *et al,* performed GATA-3 on n=38 cases of High grade prostatic adenocarcinomas and found similar results. Almost 80% of High grade urothelial carcinomas expressed GATA-3 in the same study. A panel of immunohistochemical stains including urothelial markers (*p* 63, HMWCK and GATA-3) and prostatic markers (PSA and NKX 3.1) is generally recommended, but in most cases a reduced panel of GATA-3 and NKX 3.1 can give equivalent results[9].

In breast, GATA-3 is expressed by luminal epithelial cells. Most primary carcinomas of breast show expression of GATA-3 (80-90%)[10]. However, GATA-3 is also retained in most metastatic mammary carcinomas which makes it a very useful marker in evaluating metastatic carcinomas, especially when they present at unusual sites. We found GATA-3 expression in all 20 cases (100%) including 18 invasive ductal carcinomas and 2 invasive lobular carcinomas. Our study showed expression of GATA-3 in all the cases of breast carcinoma irrespective of histological type,

grade and receptor status. Expression of GATA-3 is reportedly lower in triple negative tumors[11]. Only one case in our study was triple negative but the tumor cells in this case also showed strong nuclear expression for GATA-3. In a larger study, Deftereos *et al*, showed that all the non-triple negative tumors stained with GATA-3 while more than 60% tumors in triple negative subtype were GATA-3 positive[12]. Sensitivity of GATA-3 was found to be far more than other breast lineage specific markers including Mammaglobin and GCDFP-15.[12,13].

Adenocarcinoma of colon and High grade serous carcinomas are among the most common tumors that metastasize to different locations and have to be separated from other tumors with poorly differentiated morphology. Carcinoma of breast and ovary might also occur simultaneously in patients having BRCA 1 and BRCA 2 gene mutation. A panel of immunohistochemical stains such as PAX 8, GATA-3, *p* 53, WT 1 can be applied to differentiate tumors of breast and ovary. Therefore, the differential staining of GATA-3 in breast and ovarian carcinoma might help to identify and stage individual tumors[14,15].

In our study, both the cancers of ovary and colon showed negative staining in majority of patients. Only few cases showed weak nuclear staining in only a few cells, not more than 30%, and were considered negative.

In summary, we examined immunohistochemically four common epithelial tumors for GATA-3 including mammary carcinomas. This marker was a sensitive although not totally specific for breast carcinomas[16,17]. Other three epithelial tumors were either negative or showed non specific staining. Therefore GATA-3 can be used as a practical tool for characterization of carcinomas[18-20]. We think that a single immunostain is never enough to diagnose a tumor with absolute accuracy. Histology is a gold standard and immunohistochemical stains should always be used in a panel as every marker, no matter how sensitive and specific it is, can show false positive and false negative results.

CONCLUSION

GATA-3 is a sensitive and specific marker for mammary carcinomas. It is a useful diagnostic tool and should be included in a panel of immunohistochemical markers when working on a metastatic tumor of unknown origin.

CONFLICTS OF INTEREST

This study was no conflict of interest to be declare by any author.

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