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# GATA3 expression in gestational trophoblastic tissues and tumours

Jelena Mirkovic,[1,2] Kevin Elias,[2,3] Ronny Drapkin,[4*] Justine A Barletta,[1,2] Bradley Quade[1,2] & Michelle S Hirsch[1,2]

[1]*Department of Pathology, Women's and Perinatal Pathology Division, Brigham and Women's Hospital, Boston, MA, USA,* [2]*Harvard Medical School, Boston, MA, USA,* [3]*Division of Gynecologic Oncology, Brigham and Women's Hospital, Boston, MA, USA, and* [4]*Department of Medical Oncology, Dana Farber Cancer Institute, Boston, MA, USA*

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## GATA3 expression in gestational trophoblastic tissues and tumours

*Aims*: GATA3 is a zinc-finger transcription factor that is important for trophoblast differentiation. GATA3 is sensitive for urothelial and breast carcinomas, but the specificity is low. The aim of this study was to investigate the expression of GATA3 in trophoblast-related tissues and neoplasia.

*Methods and results*: GATA3 immunohistochemistry was performed on 33 placentas, one atypical placental site nodule, 25 hydatidiform moles (HMs), and 13 gestational trophoblastic tumours (GTTs). One hundred and sixty endometrial adenocarcinomas were also stained. Western blotting was performed on trophoblastic cell lines and compared to other cancer cell lines. Immature placentas were characterized by strong, diffuse nuclear GATA3 staining. Mature placentas showed less expression with scattered positive cells in the villous cytotrophoblast. HMs showed diffuse expression in cytotrophoblast and implantation site trophoblast, and heterogeneous expression in extravillous trophoblast. All GTTs were positive for GATA3. All endometrial adenocarcinomas were GATA3-negative. Western blotting demonstrated GATA3 in choriocarcinoma, whereas the placenta, and cervical and endometrial cancer cell lines, were negative.

*Conclusions*: All trophoblast lineages were positive for GATA3. The extent of GATA3 expression varied between immature and mature placentas, suggesting a role in trophoblast maturation. GATA3 does not distinguish normal placenta, HMs, or GTTs. Nevertheless, GATA3 may help in distinguishing trophoblastic tumors from Mullerian epithelial malignancies and a subset of tumours of unknown origin.

Keywords: choriocarcinoma, endometrial adenocarcinoma, GATA3, hydatidiform mole, immunohistochemistry, Mullerian, placenta, trophoblast

## Introduction

GATA3 is a zinc-finger transcription factor with a wide variety of functions, including the development and differentiation of breast tissue, the urothelial/renal systems, T-cell lymphocytes, skin, and parts of the nervous system.[1] GATA3 is also a well-known regulator of trophoblast-specific gene expression and placental function. In fact, transcriptome analysis of term placentas by RNA sequencing has shown that *GATA3* is one of the most highly expressed developmental genes, and that it is enriched in placenta as compared with other tissues.[2] In embryonic stem cells, GATA3 is capable of overriding pluripotency and directing the expression of a multitude of

Address for correspondence: MS Hirsch, MD, PhD, Department of Pathology, Brigham and Women's Hospital, 75 Francis Street, Amory-3, Boston, MA 02115, USA. e-mail: mhirsch1@partners.org
*Present address: Department of Obstetrics and Gynecology, Ovarian Cancer Research Center, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA

trophoblast-related genes. In trophoblastic stem cells, *GATA3* promotes differentiation both via independent induction of trophoblast genes and in synchrony with other placental development gene regulators such as *CDX2*.[3,4] GATA3 has also been shown to be involved in the regulation of the synthesis of placental hormones, such as placental lactogen I and proliferin.[5]

In tumours, GATA3 was recently described as a sensitive biomarker for urothelial and breast carcinomas.[6–9] The use of GATA3 in combination with PAX8 is both sensitive and specific for distinguishing breast and Mullerian primary tumours. Similarly, the use of GATA3 in combination with NKX3.1 is both sensitive and specific for distinguishing urothelial and prostatic primaries. GATA3 is also useful for evaluating metastatic lesions when breast and urothelial carcinomas are in the clinical differential diagnosis; however, secondary-line biomarkers, such as gross cystic disease fluid protein (GCDFP) and/or mammaglobin and p63, would be required to distinguish these two tumour types.[6,10]

Despite the known association of GATA3 with trophoblast-specific gene expression and placental function, its expression in a wide variety of trophoblast-related tissues and neoplasia has not been evaluated until recently. Miettinen *et al.* examined GATA3 expression in developing tissues, and found strong nuclear GATA3 positivity in a variety of fetal structures at 7 and 10 weeks of gestational age.[11] Almost all trophoblastic cells and amnion epithelia in a 7-week-old embryo showed GATA3 expression. However, in this study, GATA3 expression during placental maturation was not examined. In tumours, Miettinen demonstrated GATA3 positivity in pure choriocarcinoma (11/11, 100%), the choriocarcinoma component in mixed germ cell tumours, syncytiotrophoblastic giant cells present in two seminomas, and endodermal sinus tumours (6/6, 100%).[11] GATA3 positivity was also detected in one placental site trophoblastic tumour (PSTT) involving the myometrium. Prior to publication of the Miettinen study, the authors of this study incidentally noticed GATA3 expression in a metastatic trophoblastic tumour. This finding prompted further evaluation of GATA3 in a more comprehensive study of gestational trophoblastic tissues and tumours.

The goals of this study were to: (i) evaluate GATA3 expression in placenta as a function of gestational age; (ii) evaluate GATA3 in hydatidiform molar gestations; (iii) expand our understanding of GATA3 expression in gestational trophoblastic tumours; and (iv) confirm the previously reported sensitivity and specificity of GATA3 by evaluating it in a larger cohort of uterine carcinomas.

## Materials and methods

### TISSUE SELECTION

Following approval from the Institutional Review Board at Brigham and Women's Hospital, cases were retrieved from the archival files in the Department of Pathology at Brigham and Women's Hospital and the consulting files of one author (M.S.H.). These cases include: 33 placentas (11 first-trimester immature placentas and 22 third-trimester mature placentas), one atypical placental site nodule, 11 partial hydatidiform moles (HMs), 14 complete HMs, and 13 gestational trophoblastic tumours (GTTs) [six choriocarcinomas, four PSTTs, and three epithelioid trophoblastic tumours (ETTs)], and 160 endometrial adenocarcinomas (153 endometrioid, three serous, and four clear cell). Thirty (30) cervical adenocarcinomas from another study performed by our group were also re-evaluated.[12] All endometrial adenocarcinomas were evaluated on a tissue microarray; the remaining cases were all whole mount tissue sections. Haematoxylin and eosin-stained slides generated from formalin-fixed paraffin-embedded tissue were reviewed to confirm the diagnoses prior to inclusion in the study.

### IMMUNOHISTOCHEMISTRY

Immunohistochemistry was performed with the Envision Plus/Horseradish Peroxidase system (Dako, Carpinteria, CA, USA), and a monoclonal antibody against GATA3 (Biocare, Concord, CA, USA; clone L50-823; 1:500 dilution). Briefly, paraffin-embedded sections were incubated in hydrogen peroxide and absolute alcohol for 30 min to block endogenous peroxidase activity. Antigen retrieval was performed with pressure cooker pretreatment in citrate buffer (pH 6.0). Tissue sections were subsequently incubated with the primary antibody for 40 min at 25°C. Following Tris-buffered saline rinses, the tissue was incubated with the Envision Plus secondary antibody for 30 min, and then with diaminobenzidine for 5 min. Appropriate positive (urothelial carcinoma) and negative (incubation with secondary antibody only) controls were stained in parallel for each round of immunohistochemistry.

GATA3 was evaluated for nuclear staining. Strong nuclear immunoreactivity was considered to be a positive result. Internal positive controls (T-cell lymphocytes) were noted and used as an intensity reference when present.

### WESTERN BLOT ANALYSIS

Established breast (T47D), urothelial (T24), cervical (HeLa), T-cell leukaemia (Jurkat) and endometrial (HEC1A) cancer cell lines were evaluated for GATA3 protein expression, and compared with an SV40 immortalized normal mature placenta cell line [CRL1584 (3A-Sub E), referred to henceforth as NP], two choriocarcinoma cell lines (JEG3 and JAR), and fresh mature placental tissue. All cell lines were purchased from the American Type Culture Collection (Manassas, VA, USA). Cells were grown under standard tissue culture conditions in a 37°C incubator with 5% $CO_2$. Prior to lysis, cells were washed twice in warm phosphate-buffered saline (PBS), and then detached from tissue culture plates with trypsin. Cell pellets were washed twice in complete medium with 5% fetal bovine serum to neutralize the trypsin, and then twice in ice-cold PBS to remove any trace serum. Cell pellets were lysed by a 30-min incubation on ice with RIPA buffer (Boston BioProducts, Ashland, MA, USA) containing PhosStop phosphatase inhibitor and complete EDTA-free protease inhibitor tablets (both from Roche Life Science, Indianapolis, IN, USA). Fresh placentas were obtained directly from the delivery suite. Full-thickness sections of placenta were snap-frozen in liquid nitrogen, and then pulverized into a powder by shaving the frozen tissue pieces with a sterile scalpel. The powder was then homogenized in RIPA buffer with protease and phosphatase inhibitors by the use of a glass dounce. Protein amounts were quantified with the Bio-Rad DC Protein Assay (Bio-Rad, Hercules, CA, USA), and 20 μg of total protein for each cell line and the fresh frozen placenta was loaded on a 4–12% NuPAGE Bis-Tris gradient gel (Life Technologies, Grand Island, NY, USA). Protein gel electrophoresis was performed in MOPS-SDS running buffer (Life Technologies). After separation, the proteins were transferred to a nitrocellulose membrane with the iBlot system (Life Technologies). The membrane was blocked overnight at 4°C in PBS containing Tween-20 with 5% bovine serum albumin. The membrane was incubated with the primary monoclonal anti-GATA3 antibody (Biocare; clone L50-823) at a 1:500 dilution in blocking buffer. Mouse monoclonal anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody (1:2000 dilution in blocking buffer; Sigma, St Louis, MO, USA) was used as a loading control. Anti-GATA3 antibody was incubated overnight at 4°C, and anti-GAPDH antibody for 1 h at room temperature. Membranes were incubated in anti-mouse IgG, horseradish peroxidase-linked secondary antibody (Cell Signaling, Beverly, MA, USA) in blocking buffer for 1 h at room temperature. GAPDH was detected with ECL 2 Western Blotting Substrate (Thermo Scientific, Waltham, MA, USA), and GATA3 was detected with Supersignal West Femto Max Sensitivity Substrate (Thermo Scientific). Both were analysed on the FluorChem HD2 imaging system (Alpha Innotech, San Leandro, CA, USA).

## Results

### IMMUNOHISTOCHEMICAL RESULTS

The immunohistochemical results are summarized in Table 1.

**Table 1.** Immunohistochemical expression of GATA3 in placenta, hydatidiform molar gestations, and gestational trophoblastic tumours

| Diagnosis | GATA3 expression |
|---|---|
| Placenta | |
| Immature | Strong and diffuse in cytotrophoblast and implantation site trophoblast; variable in syncytiotrophoblast |
| Mature | Reactive in scattered villous cytotrophoblast; strong and diffuse in the amnion layer and intermediate trophoblast |
| Implantation site | |
| Normal | Strong diffuse (all cases) |
| APSN | 1/1 |
| Hydatidiform moles | |
| PHM | 11/11 |
| CHM | 14/14 |
| Gestational trophoblastic tumours | |
| CHORIOCA | 6/6 |
| PSTT | 4/4 |
| ETT | 3/3 |
| Endometrium | |
| EMCA | 0/160 |

APSN, Atypical placental site nodule; CHM, Complete hydatidiform mole; CHORIOCA, Choriocarcinoma; EMCA, Endometrial carcinoma; ETT, Epithelioid trophoblastic tumour; PHM, Partial hydatidiform mole; PSTT, Placental site trophoblastic tumour.

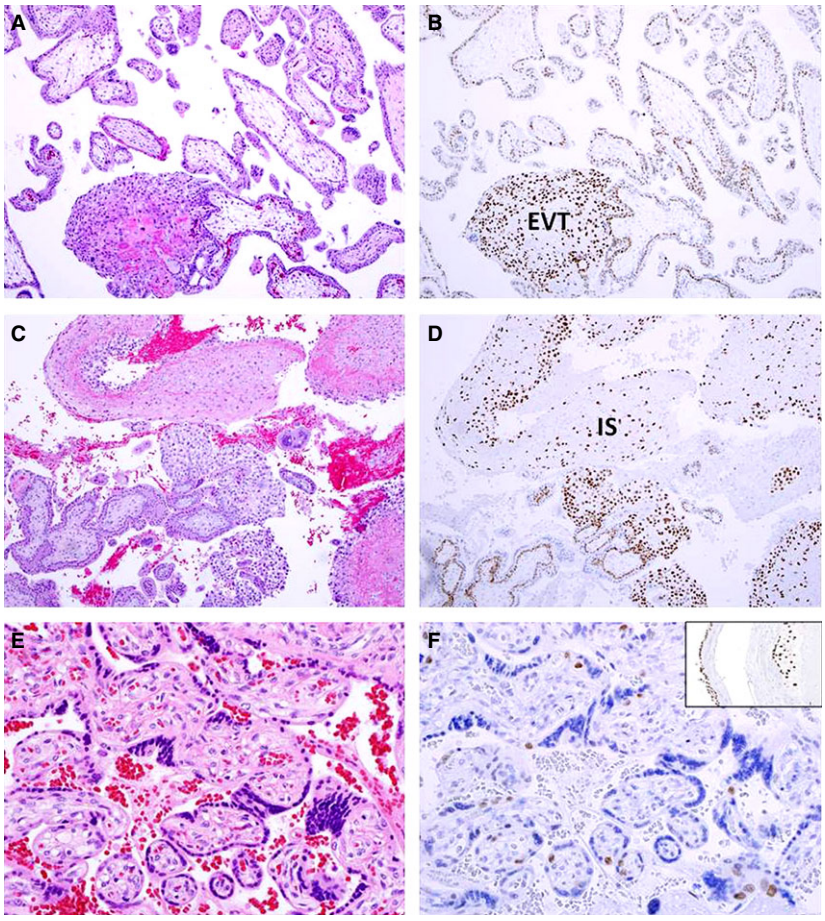


**Figure 1.** Immunohistochemical expression of GATA3 in placentas. **A**,**B**, Immature placenta (first-trimester gestation); strong and diffuse staining was seen in both the cytotrophoblast cell layer and the extravillous trophoblast (EVT), and variable staining was seen in the syncytiotrophoblast. **C**,**D**, The intermediate trophoblast in the placental implantation site (IS) was consistently positive for GATA3. **E**,**F**, Mature placenta (third-trimester gestation); only scattered positive cells were seen in the villous cytotrophoblast, and there was little to no expression in syncytial knots. The amniotic cell layer was GATA3-positive (inset).

*GATA3 expression in placenta*

Immature placentas were characterized by strong and diffuse nuclear GATA3 staining of the implantation site (intermediate trophoblast) and cytotrophoblast layer, with variable staining in the syncytiotrophoblast layer (Figure 1A–D). Mature placentas showed less expression of GATA3, with scattered positivity in villous cytotrophoblast, and little to no expression in syncytial knots (Figure 1E,F). The amnion layer was invariably positive for GATA3, as were the intermediate trophoblast associated with fibrin located in the chorionic plate and in intervillous spaces (Figure 1F, insert). The implantation site in mature placentas was strongly positive for GATA3 (not shown).

*GATA3 expression in HMs*

Complete and partial HMs both showed diffuse expression in the cytotrophoblast and implantation site trophoblast; heterogeneous GATA3 expression was seen in areas of extravillous trophoblast hyperplasia (Figure 2A–D).

*GATA3 expression in GTTs*

All ETTs, PSTTs, and choriocarcinomas, as well as the atypical placental nodule, were positive for GATA3 (Figure 3). All three examples of ETT were characterized by diffuse strong GATA3 positivity (Figure 3A,B). Three of four PSTTs were diffusely and strongly immunoreactive for GATA3 (Figure 3C,D), whereas one showed a weak to moderate scattered staining pattern. Five of six choriocarcinomas showed diffuse and strong GATA3 staining (Figure 3E,F), whereas one showed a multifocal strong staining pattern. Both mononuclear (cytotrophoblast) and multinucleated (syncytiotrophoblast) cells in the choriocarcinomas were immunoreactive (Figure 3E,F).

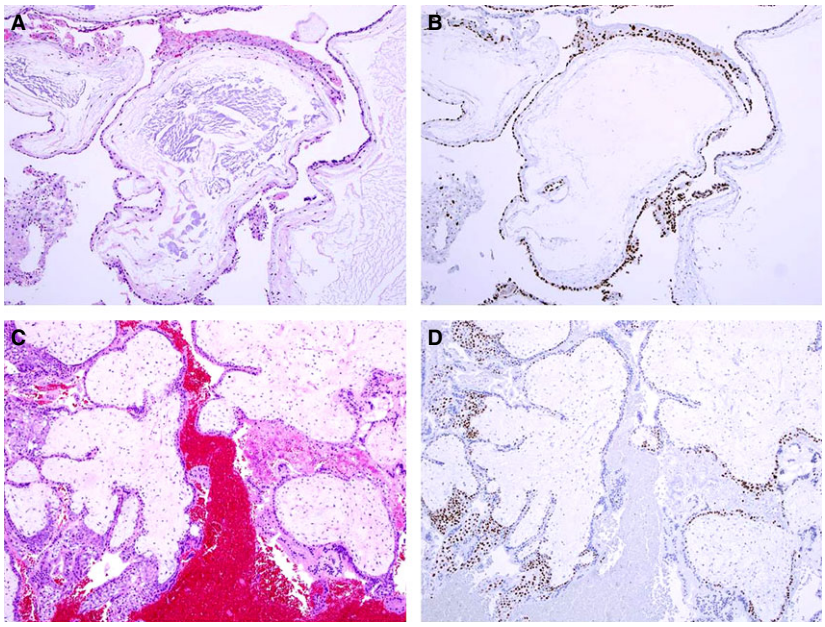


**Figure 2.** Immunohistochemical expression of GATA3 in hydatidiform moles. **A**,**B**, Partial hydatidiform mole. **C**,**D**, Complete hydatidiform mole. Partial and complete moles were characterized by diffuse expression in the cytotrophoblast and implantation site intermediate trophoblast (latter not shown); heterogeneous expression was seen in extravillous trophoblastic hyperplasia.

The atypical placental site nodule showed a weaker staining pattern in scattered cells when compared to other trophoblastic neoplasms (not shown).

*GATA3 expression in non-trophoblastic tissues*

All 160 endometrial adenocarcinomas, including endometrioid, serous and clear cell types, were negative for GATA3. Additionally, 30 cervical adenocarcinomas evaluated in another study from our laboratory were also negative for GATA3.[12]

### WESTERN BLOT ANALYSIS

Consistent with our immunohistochemical findings in formalin-fixed paraffin-embedded human tissues, western blot analysis of human trophoblastic tissues and cancer cell lines showed that GATA3 expression was stronger in the choriocarcinoma cell lines (JEG3 and JAR) than in mature placenta tissue or an immortalized normal placenta cell line (Figure 4). As with the tissue sections, the endometrial and cervical cancer cell lines were negative for GATA3. The T-cell leukaemia, breast and urothelial carcinoma cell lines were also positive for GATA3, and served as positive controls (Figure 4). These findings confirm the sensitivity and specificity for GATA3 when trophoblastic tumours are compared with lower genital tract tumours. Interestingly, the low but present expression of GATA3 in the normal mature placental tissues (NP and fresh frozen term placenta) was consistent with the lower expression of GATA3 as determined by immunohistochemistry in immature and mature placental tissues (Figures 1 and 4).

## Discussion

GATA3 is a well-known regulator of trophoblast-specific gene expression and placental function. Small studies have suggested that trophoblast express GATA3; however, a comprehensive evaluation of trophoblastic tissues and neoplasia has only recently been performed.[11,13] Our data, similar to the previously reported studies, show that all trophoblast lineages (cytotrophoblast, intermediate trophoblast, and syncytiotrophoblast) in placental tissue can express GATA3, but that this expression varies with trophoblast type and gestational age. Whereas the cytotrophoblast and intermediate trophoblast show consistent diffuse and strong nuclear expression of GATA3, staining in the syncytiotrophoblast, a more differentiated form of the trophoblast, is variable, ranging from diffuse expression to a lack of expression. The extent of GATA3 immunoreactivity in first-trimester immature placentas was significantly increased as compared with that in third-trimester

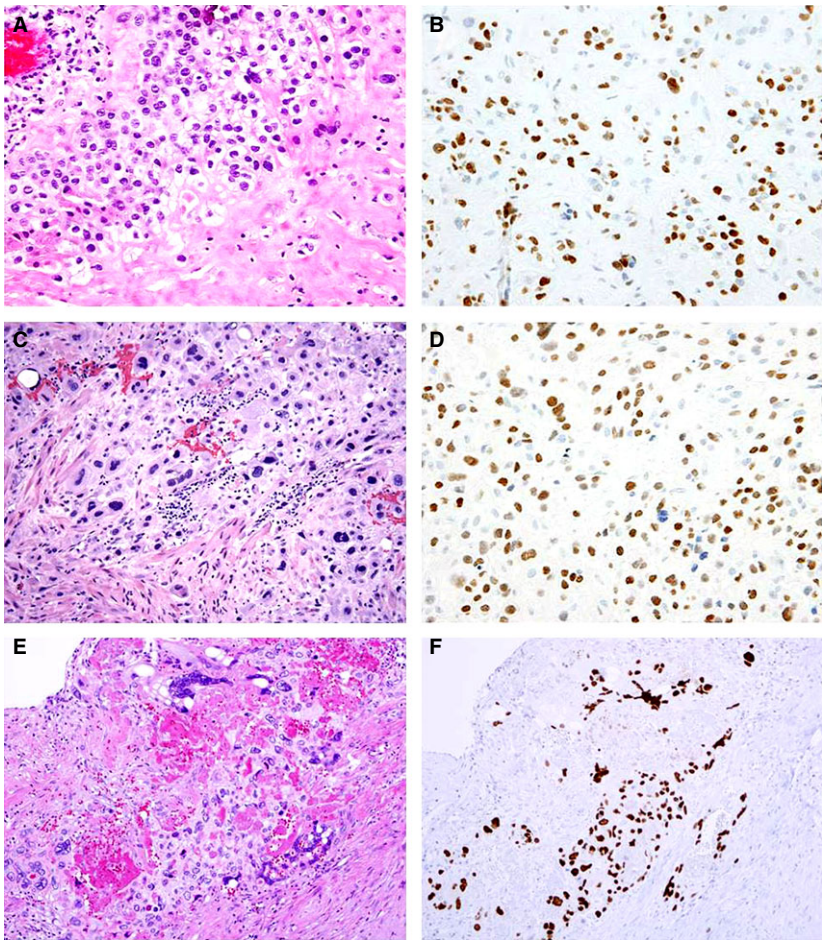


**Figure 3.** Immunohistochemical expression of GATA3 in gestational trophoblastic tumours. Epithelioid trophoblastic tumours (**A**,**B**), placental site trophoblastic tumours (**C**,**D**) and choriocarcinomas (**E**,**F**) were all positive for GATA3, with a significant majority of choriocarcinomas showing strong and diffuse expression in both the mononuclear and multinucleated neoplastic cells.

mature placentas, which typically showed scattered positive cells in the villous cytotrophoblast, and little to no expression in syncytial knots, suggesting a role in trophoblast maturation. Similarly, choriocarcinoma cell lines, which are derived from immature trophoblasts, have the highest GATA3 expression among the different tumour types analysed by western blotting (Figure 4), whereas GATA3 expression appeared to be absent in a mature normal trophoblast cell line. Nevertheless, the mechanism by which GATA3 expression decreases with trophoblast maturation is not completely understood. However, one explanation may include the fact that GATA3 can increase differentiation by overriding pluripotency and directing the expression of multiple trophoblast genes in embryonic and trophoblast stem cells.[3,4] Regardless of the actual explanation, our western blot results obtained with cell line cultures representing various trophoblastic tissues provide an experimental platform with which to address this question in future studies. More specifically, manipulation of GATA3 expression in these cell lines could be used to study the role of GATA3 in trophoblast transformation and development.

Also consistent with recent studies,[11,13] all choriocarcinomas (6/6) in this study were positive for GATA3. In addition, all PSTTs (4/4) and ETTs (3/3), as well as one placental site nodule, were positive for GATA3. Therefore, GATA3 does not help distinguish different GTTs from normal immature placental tissue, nor does it distinguish different types of trophoblastic tumours. Instead, the combination of other immunohistochemical markers is needed to differentiate subtypes of GTT. As is the case for GATA3, all trophoblastic lesions are characterized by cytokeratin and inhibin-α expression, and therefore these cannot be used to differentiate subtypes.[14,15] However, human placental lactogen (hPL) and Mel-CAM

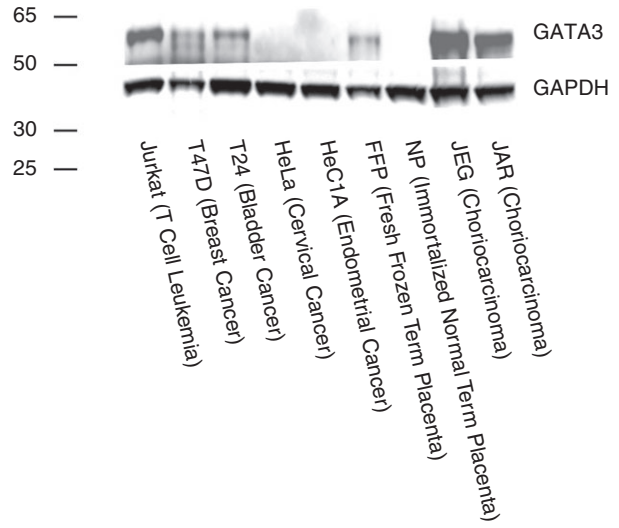


**Figure 4.** GATA3 specificity as determined by western blot analysis. GATA3 protein expression was seen in T-cell leukaemia, breast cancer, bladder cancer and choriocarcinoma cell lines, whereas cervical and endometrial cancer cell lines did not express GATA3. Placental tissues also expressed GATA3 to a lesser extent (GATA3 expression in immortalized normal placenta was only seen with overexpression of the blot; not shown). Jurkat: T-cell leukaemia cell line. T47D: breast cancer cell line. T24: bladder cancer cell line. HeLa: cervical cancer cell line. HeC1A: endometrial cancer cell line. JEG3: choriocarcinoma cell line. JAR: choriocarcinoma cell line. FFP, fresh frozen term placenta; NP, immortalized normal placenta.

(CD146) are typically positive in lesions of the implantation site intermediate trophoblast, including those in exaggerated placental implantation sites and PSTTs.[15,16] In contrast, lesions of the so-called chorionic-type intermediate trophoblast, including placental site nodules and ETTs, generally express hPL and Mel-CAM only focally.[17] Furthermore, ETTs are characterized by p63 immunohistochemical expression, wheras PSTTs are typically negative.[17–19]

In addition to the malignant trophoblastic neoplasms, our data indicate that complete and partial HMs are characterized by diffuse GATA3 expression in the cytotrophoblast and implantation site, and heterogeneous expression in extravillous trophoblastic hyperplasia. Therefore, GATA3 is not helpful for distinguishing molar gestations from normal immature placental tissue. As has been previously shown, morphological features in combination with p57 immunohistochemical staining (loss of p57 expression in complete moles) and molecular genotyping are more useful for differentiating normal immature placental tissue from molar gestations, and to differentiate between partial and complete molar gestations.[20]

Although GATA3 is not a useful marker for differentiating trophoblast subtype, it can be used clinically for other purposes. For example, the differential diagnosis for a malignant epithelioid neoplasm in a uterine biopsy specimen can include an endometrial carcinoma, as well as a trophoblastic malignancy in a small subset of cases. As this study demonstrated that all 160 endometrial carcinomas were negative for GATA3, the presence of GATA3, especially in the absence of PAX8 (a marker of Mullerian carcinomas),[21] would argue against an endometrial primary and support a trophoblastic proliferation. Similarly, if there is a question of a trophoblastic tumour versus an endocervical carcinoma, the presence of GATA3 would support the former over the latter, as endocervical carcinomas appear to be negative for this marker. In contrast, GATA3 must be used with caution when the differential diagnosis includes a squamous cell carcinoma of the cervix, as cervical squamous cell carcinomas have been shown to be positive for GATA3 in approximately one-third of cases.[10] However, in the study by Chang *et al.*,[10] most of the squamous cell carcinomas of the cervix showed weak, patchy staining patterns, whereas most trophoblastic tumours, as seen in this study, are diffusely positive for GATA3 (the latter are also negative for human papilloma virus).

A second clinical use for GATA3 includes confirmation of implantation site. In small biopsy specimens obtained to exclude or support an ectopic pregnancy, the presence of trophoblastic elements may be subtle and/or focal. Our data indicate that, whereas implantation site intermediate trophoblasts are always diffusely and strongly positive for GATA3, decidualized endometrium does not express this marker. Therefore, GATA3 could be used as a reliable marker of implantation site, confirming an intrauterine pregnancy. GATA3 may also be able to identify a focus of choriocarcinoma within a term placenta, as the former would be diffusely positive for GATA3 and the latter is only positive in scattered cells.

Finally, GATA3 may be helpful in subtyping metastatic tumours of unknown origin when a trophoblastic neoplasm is in the differential diagnosis. A recent systematic analysis of 2500 epithelial and non-epithelial tumours showed that there are a number of tumours apart from breast and urothelial carcinomas that frequently express GATA3, including basal cell carcinomas, cutaneous squamous cell carcinomas, skin adnexal tumours, and choriocarcinomas; malignant mesotheliomas and ductal carcinomas from the salivary gland and pancreas expressed GATA3 in a smaller subset of cases.[11] Additional studies have also shown that GATA3 is expressed in the majority of ovarian Brenner tumours[22] and in tumours from the

autonomic nervous system, including paragangliomas, pheochromocytomas, neuroblastomas, ganglioneuroblastomas, and ganglioneuromas.[23] On the basis of these findings and the general low specificity GATA3, this antibody should only be used with a directed differential diagnosis in mind and/or in combination with a panel of biomarkers when a tumour of unknown origin is evaluated. This is especially important when breast carcinomas (mammaglobin and GCDFP) and urothelial carcinomas (p63 and CK20), as well as some renal cell carcinomas (PAX8), paragangliomas, and mesonephric lesions (CD10 and calretinin), are in the differential diagnosis. Additionally, given the low specificity of GATA3, morphological features should always be taken into consideration.

In summary, this study confirms that all trophoblast lineages in normal placenta express GATA3, but that this expression varies significantly with trophoblast type and gestational age. GATA3 does not help in distinguishing molar gestations from normal immature placental tissue, nor does it distinguish the various subtypes of GTT. Nevertheless, GATA3 can help in distinguishing trophoblast (neoplastic or non-neoplastic) from Mullerian epithelial malignancies, and this could be especially useful in small endocervical and endometrial biopsy specimens. Finally, GATA3 may be helpful in subtyping metastatic tumours of unknown origin when a trophoblastic neoplasm is in the differential diagnosis; however, distinction from other neoplasms based on the histological impression and additional markers would be required.

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## Conflicts of interest

The authors have no conflicts of interest or funding to disclose.

## Author contributions

M. S. Hirsch, J. Mirkovic, and K. Elias: performed the research. M. S. Hirsch and J. Mirkovic: designed the research study. R. Drapkin and J. A. Barletta: contributed essential reagents or tools. M. S. Hirsch, J. Mirkovic, and K. Elias: analysed the data. M. S. Hirsch and J. Mirkovic: wrote the paper. M. S. Hirsch, J. Mirkovic, K. Elias, R. Drapkin, J. A. Barletta, and B. Quade: provided discussion and feedback, and edited the manuscript.

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