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Characterisation of MTA1 expression in canine mammary neoplasms: A comparative study with emphasis on triple-negative phenotypes#

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Abstract

Metastasis-associated protein 1 (MTA1), a key component of the nucleosome remodelling and histone deacetylase (NuRD) complex promotes cancer progression by regulating key pathways involved in oncogenesis, angiogenesis, metastasis and apoptosis. This study examined the immunohistochemical (IHC) expression of MTA1 in comparison to histological subtype, grade and hormone receptor expression in malignant canine mammary tumours (CMTs). Histopathological analysis confirmed that all tumours were malignant and carcinosarcoma was identified as the most prevalent subtype. All tumours showed moderate to strong MTA1 expression, of which four cases showed negative estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) immunostaining and were identified as triple-negative breast cancers (TNBCs). Two cases each of HER2-positive subtype and luminal tumours with HER2-positivity were identified. One case each of luminal tumour with ER negativity and PR negativity was identified. This preliminary investigation on MTA1 expression in CMTs shed light on hormone receptor signalling and MTA 1 in the pathogenesis and the molecular mechanisms involved in the disease, potentially benefiting both canine and human patients.

Keywords: *Metastasis-associated protein 1, triple-negative breast cancer, Estrogen receptor, progesterone receptor, Human epidermal growth factor receptor 2*

Human breast cancer manifests as a diverse disease that varies both in its genetic makeup and clinical presentation, encompassing several distinct subtypes. The most widely recognised classification system is from an immunohistochemical perspective based on the expression patterns of three key hormone receptors: oestrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2). Based on this receptor profile analysis, breast cancer (BC) is primarily classified into four main categories: luminal A, luminal B, HER2-positive and triple-negative subtypes (Orrantia-Borunda *et al*., 2022).

Of these, Triple negative breast cancer (TNBC) is a particularly aggressive subtype of breast cancer characterised

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by the absence of ER, PR and HER2 expression, making it challenging to treat with conventional hormone therapies (Sharaf *et al*., 2024).

Sex steroid hormones play a vital role in the development and advancement of breast cancer (Lanning *et al*., 2011). Estrogens play a pivotal role in the stimulation of growth in both the mammary gland and breast cancer tissues, predominantly through the activation of estrogen receptor α (ERα). This receptor is well-documented for its ability to induce the expression of genes that promote cell cycle progression and inhibit apoptotic pathways. Importantly, in ERα positive breast cancer, there is a marked overexpression of ERα compared to the levels observed in the normal mammary gland, thereby contributing to enhanced tumour proliferation and growth (Huber *et al*., 2024).

The discovery of PR splice variants, PR-A and PR-B, which have distinct roles in breast cancer progression, has added complexity to PR signalling pathways. In luminal BC characterized by ERα expression and high PR levels, PR-A has been associated with increased invasiveness and metastatic potential, whereas PR-B supports metastasis only when progesterone levels are elevated. (Rosati *et al*., 2020).

The protein HER2 is a member of the epidermal growth factor receptor family, characterised by tyrosine kinase activity. Upon receptor dimerisation, several signalling pathways are activated, promoting cell growth and cancer progression (Iqbal and Iqbal, 2014). A significant proportion of human breast cancers with poor prognosis overexpress HER2, making it a potential biomarker for predicting prognosis in these cases.

Metastasis-associated protein 1 (MTA1) is a transcriptional co-regulator that has been found to be aberrantly expressed in various cancers, including TNBCs. This protein is a component of the nucleosome remodelling and deacetylase (NuRD) complex which is involved in various cellular processes including transcriptional regulation, DNA repair and cell cycle progression (Sen *et al*., 2014).

Recent studies have highlighted the significant role of MTA1 in the progression and metastasis of aggressive breast tumours including TNBCs. However, no such studies have been reported in canine mammary tumours (CMTs). In the present study, immunohistochemical expression of MTA1 in comparison to histological subtype, grade and hormone receptor expression was evaluated in CMTs.

Materials and methods

Sample collection

The present study analysed ten excisional biopsy samples from suspected CMT cases submitted to the University Veterinary Hospital, CVAS, Mannuthy. Tissue samples were collected in 10 per cent neutral buffered formalin for histopathology and immunohistochemistry (IHC).

Tissue processing

Tumour tissue samples fixed in 10 percent neutral buffered formalin were processed by routine paraffin embedding method. Serial sections were cut at 5µm thickness using rotary microtome and sections were stained using the routine haematoxylin and eosin procedure (Suvarna *et al*., 2019). Histopathological changes in tissue sections were recorded by observing under a light microscope.

Classification and histologic malignancy grading of CMTs

The histological classification of tumours followed the criteria established by Goldschmidt *et al*. (2011), while malignancy grading was conducted based on the approach outlined by Clemente *et al*. (2010), which evaluates tubule formation, nuclear pleomorphism and mitotic count (Christy *et al*., 2021). For soft tissue sarcomas, grading was performed in accordance with Augsburger *et al*. (2017) taking into account tumour differentiation, mitotic index and the extent of tumour necrosis.

Immunohistochemistry

The IHC staining of the tumour sections was carried out using commercially available secondary antibody kit and primary antibodies for ER (1:50 dilution, ORIGIN diagnostics & research, Kerala), PR (1:50 dilution, ORIGIN diagnostics & research, Kerala), HER2 (1:100 dilution, ORIGIN diagnostics & research, Kerala) and MTA1 (1: 50 dilution, ORIGIN diagnostics & research, Kerala) proteins. Briefly 5 µm tumour sections were deparaffinised and hydrated through graded alcohols to water. Heat induced antigen retrieval was done by immersing slides in 0.01M citrate buffer at 95^{0}C for 20 min. After cooling and washing in 1 per cent Tris-buffered saline with tween 20, endogenous peroxidase blocking was done by incubating with 3% hydrogen peroxide for 20 min. After washing, power blocking was done to minimise non-specific binding of antibodies to highly charged sites on the tissue sections. The slides were incubated with primary antibodies overnight. Following washing, slides were incubated with primary antibody enhancer for 20 minutes. The slides were then incubated with secondary antibody (PolyQ stain 2 step detection kit, AJ Scientific, Kerala) for 30 minutes at room temperature. Immunostaining was visualized using diaminobenzidine chromogen, with Mayer's haematoxylin used as the counterstain.

The IHC staining was evaluated semi quantitatively as the percentage of positive stained cells and the intensity of the immunostaining for MTA1 (Vakkala

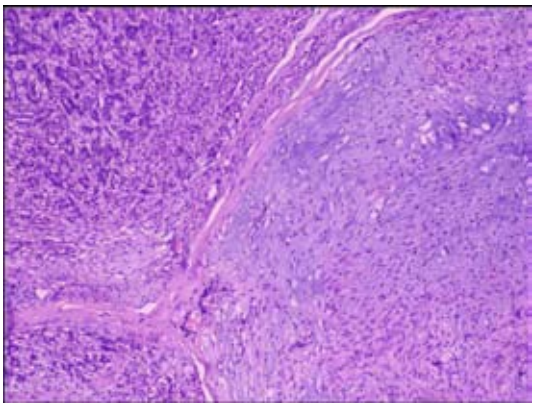

Fig. 1. Carcinosarcoma- Malignant basophilic chondroid matrix and an uneven arrangement of neoplastic epithelial cells (H&E x 100)

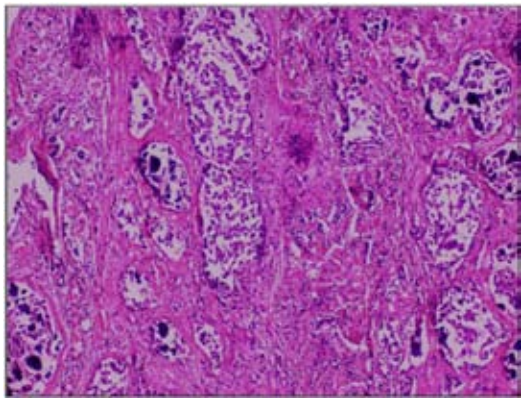

Fig. 2. Anaplastic carcinoma – Pleomorphic neoplastic cells with prominent desmoplasia (H&E x 100)

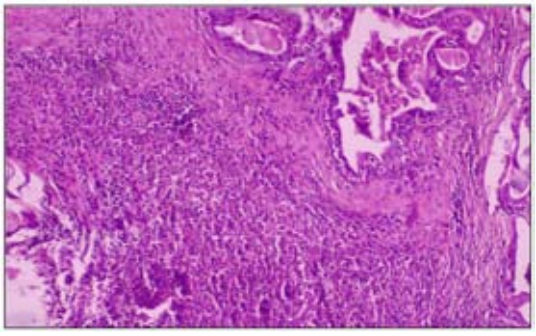

Fig. 3. Inflammatory carcinoma – Severe inflammatory cell infiltration(H&E x 100)

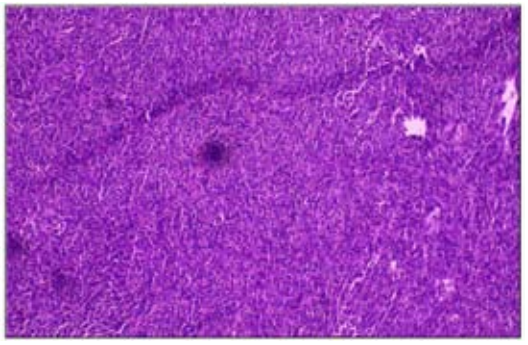

Fig. 4. Solid carcinoma- Sheets of pleomorphic epithelial cells without lumen (H&E x 100)

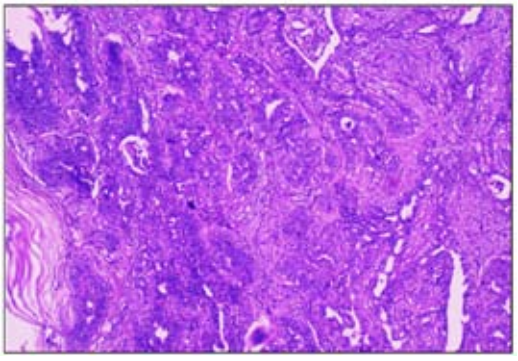

Fig. 5. Tubular adenocarcinoma - Adenomatous cells in tubular arrangement (H&E x 100)

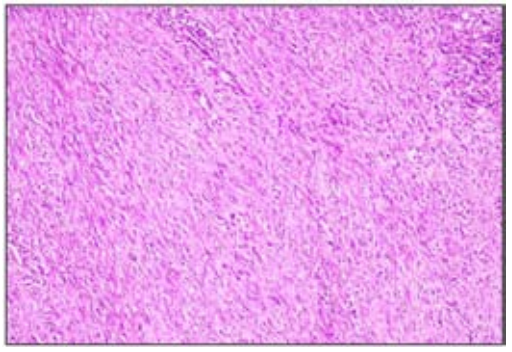

Fig. 6. Fibrosarcoma – Proliferation of spindle shaped cells in interwoven pattern (H&E x 100)

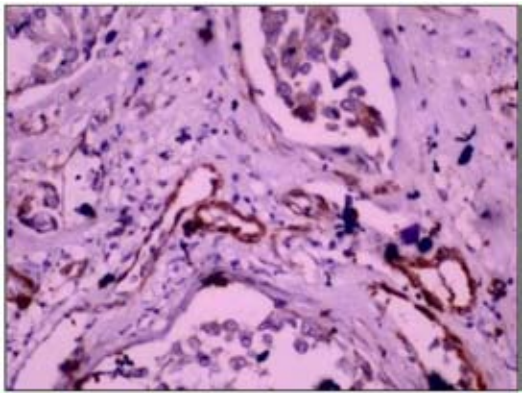

Fig. 7. Anaplastic carcinoma – strong MTA1 immunopositivity (IHC x 400)

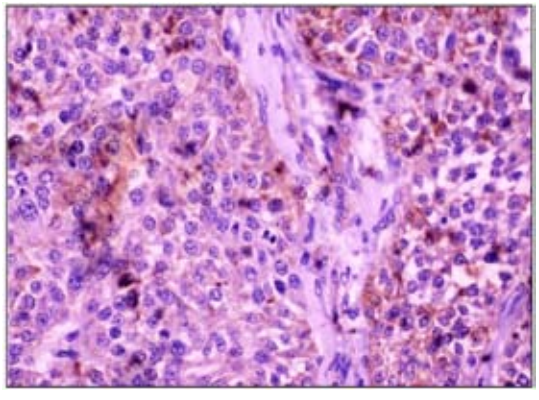

Fig. 8. Solid carcinoma – strong MTA1 immunopositivity (IHC x 400)

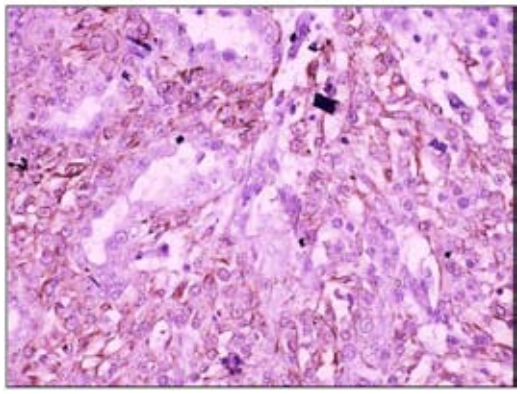

Fig. 9. Cribriform carcinoma – strong MTA1 immunopositivity (IHC x 400)

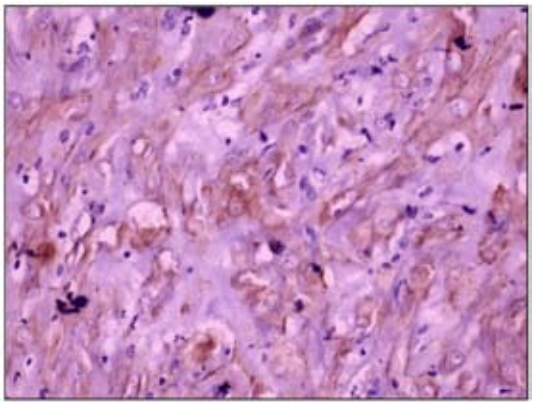

Fig. 10. Carcinosarcoma – moderate MTA1 immunopositivity (IHC x 400)

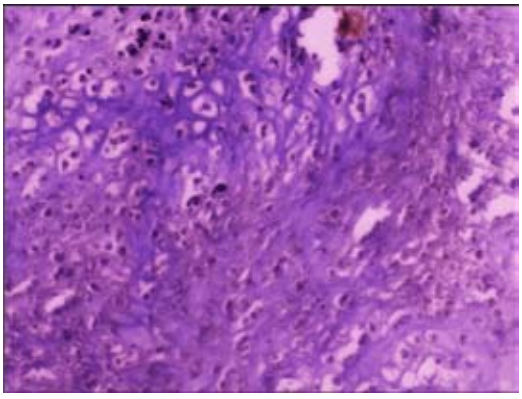

Fig. 11. Carcinosarcoma – strong MTA1 immunopositivity (IHC x 400)

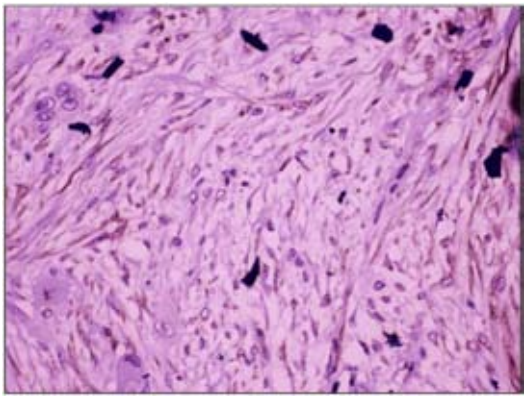

Fig. 12. Inflammatory carcinoma – moderate MTA1 immunopositivity (IHC x 400)

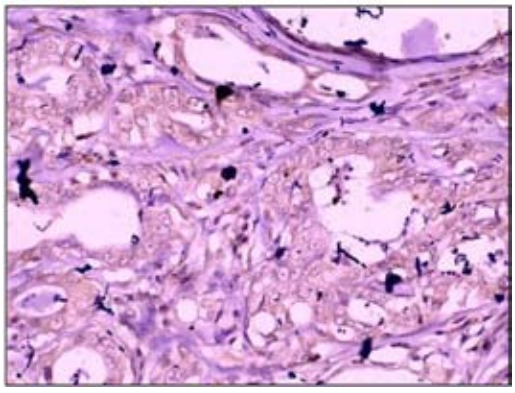

Fig. 13. Tubular adenocarcinoma – moderate MTA1 immunopositivity (IHC x 400)

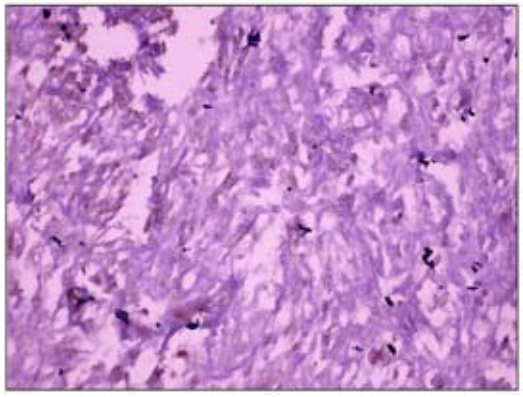

Fig. 14. Fibrosarcoma – moderate MTA1 immunopositivity (IHC x 400)

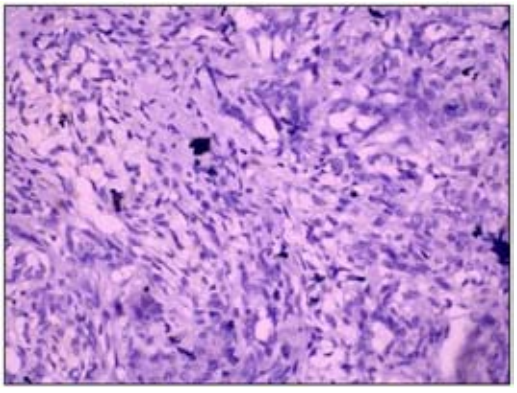

Fig. 15. Negative immunostaining - ER (IHC x 400)

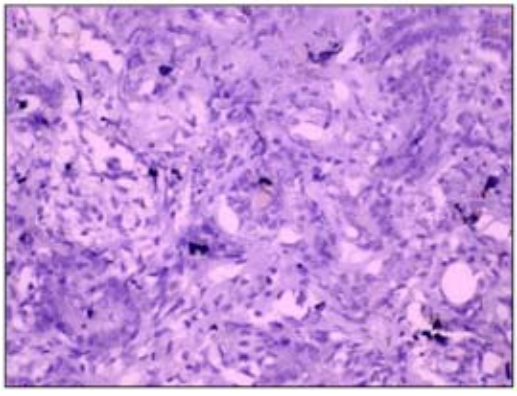

Fig. 16. Negative immunostaining - PR (IHC x 400)

et al., 1999).

The Allred scoring system evaluated ER and PR expression by combining proportion and intensity scores, resulting in a total score from 0 to 8. Scores of 0 to 1 were considered ER/PR negative, while scores of 2 to 8 were positive. The HercepTest system interprets HER2 expression using immunohistochemistry (IHC) scores: 0 indicates no membrane staining, 1+ for partial or < 10% light/moderate staining, 2+ for > 10% light/moderate or < 30% strong staining, and 3+ for > 30% strong membrane staining (Niyas *et al*., 2024). Comparative evaluation of MTA1 expression with respect to the histological subtype, grade and status of hormone receptor expression was done.

Results and discussion

Classification and Histologic malignancy grading of CMTs

All the tumours (n=10) were diagnosed as malignant. Histological classification of the tumours as depicted in the Fig. 1 to 6 revealed four cases of carcinosarcoma and one case each of anaplastic carcinoma, cribriform carcinoma, solid carcinoma, inflammatory carcinoma, tubular adenocarcinoma and fibrosarcoma. This aligns with the findings of Tavasoly *et al*. (2013), who reported that the majority of mammary tumours were carcinomas, with only a small percentage being sarcomas.

Table 1. MTA1 expression in different histological subtypes and grades of tumours

Histological sub-type	Histologic malignancy grade	MTA1 expression
Carcinosarcoma	Grade III	Strong
Carcinosarcoma	Grade III	Moderate
Carcinosarcoma	Grade II	Moderate
Carcinosarcoma	Grade II	Strong
Anaplastic carcinoma	Grade III	Strong
Inflammatory carcinoma	Grade III	Moderate
Solid carcinoma	Grade III	Strong
Cribriform carcinoma	Grade III	Strong
Tubular adenocarcinoma	Grade I	Moderate
Fibrosarcoma	Grade II	Moderate

All the tumours were lymph node negative. The cases of carcinosarcoma, anaplastic carcinoma, cribriform carcinoma, solid carcinoma, inflammatory carcinoma and fibrosarcoma were classified as high grade tumours while the tubular adenocarcinoma case was classified as low grade tumour. This is in line with the study of Mathew *et al*. (2019) who reported that simple carcinomas tend to present with higher grades.

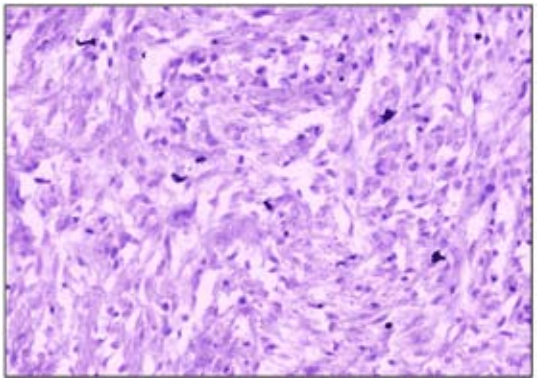

Fig. 17. Negative immunostaining – HER2 (IHC x 400)

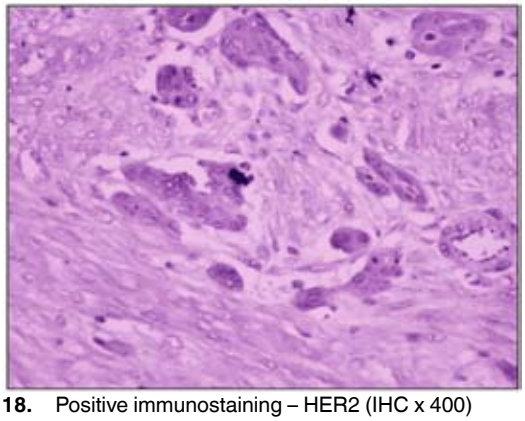

Fig. 18. Positive immunostaining – HER2 (IHC x 400)

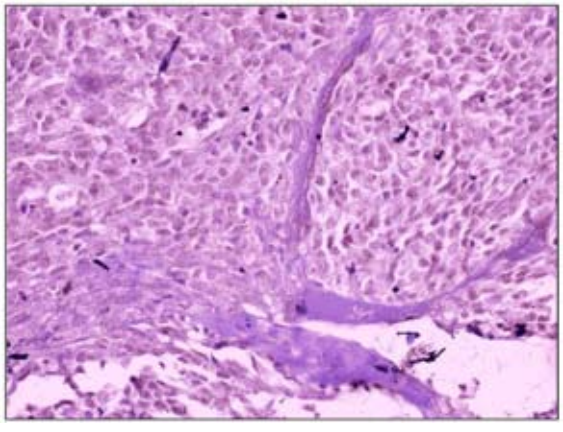

Fig. 19. Positive immunostaining – ER (IHC x 400)

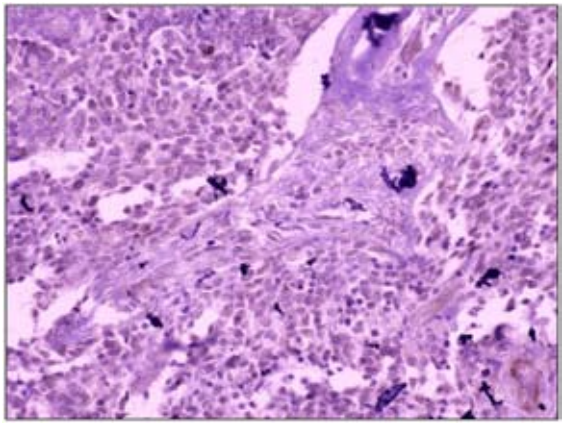

Fig. 20. Positive immunostaining – PR (IHC x 400)

Table 2. Classification of CMTs (n=10) based on hormone receptor expression

S. No.	ER	PR	HER2	Tumour subtype	MTA1
1.	Negative	Negative	Negative	TNBC	Strong
2.	Positive	Positive	Positive	Luminal tumour with HER2 positivity	Strong
3.	Positive	Positive	Positive	Luminal tumour with HER2 positivity	Strong
4.	Negative	Positive	Negative	Luminal tumour with ER negativity	Strong
5.	Positive	Negative	Negative	Luminal tumour with PR negativity	Strong
6.	Negative	Negative	Positive	HER2-positive	Moderate
7.	Negative	Negative	Negative	TNBC	Moderate
8.	Negative	Negative	Positive	HER2-positive	Moderate
9.	Negative	Negative	Negative	TNBC	Moderate
10.	Negative	Negative	Negative	TNBC	Strong

Immunohistochemistry

In this study, all cases exhibited positive MTA1 immunostaining as depicted in Table 1, ranging from moderate to strong. Of the 10 cases, 30% (n=3) were high-expressing carcinomas, aligning with Zhao *et al*. (2016), who reported that 36.7% of breast cancers showed high MTA1 expression.

The study found significant statistical correlation between MTA1 expression and histological grade (r_s = 0.7263, p (2-tailed) = 0.01737). This is in line with the findings of Nagaraj *et al.* (2015), who reported higher MTA1 expression in grade III tumours compared to grades I and II, indicating that MTA1 protein expression correlates with tumour grade and progression in HBC.

Strong MTA1 immunostaining was observed in cases of anaplastic carcinoma, solid carcinoma and cribriform carcinoma (Fig. 7 to 9), indicating that aggressive carcinomas are associated with higher MTA1 expression. This supports the established role of MTA1 protein in enhancing the aggressive phenotype of breast cancer cells by repressing oestrogen receptor (ER) trans-activation through the deacetylation of ERE chromatin in ER-responsive genes, as demonstrated by Manavathi *et al*. (2007). Immunostaining in carcinosarcomas varied from moderate to strong expression (Fig. 10 and 11). Inflammatory carcinoma, tubular adenocarcinoma and fibrosarcoma showed moderate immunostaining (Fig. 12 to 14).

The classification of the CMTs based on the IHC expression of ER, PR and HER2 is depicted in Table 2.

In the present study, majority of the cases showed negative ER (n=7), PR (n=6) and HER 2 staining (n=6) as depicted in Fig. 15 to 17. This study infers that high MTA1 was associated with negative ER and PR staining, consistent with the findings of Singh *et al*. (2005) who demonstrated that MTA1 overexpression suppresses ER trans-activation, leading to the development of aggressive ER-negative phenotypes. This is in line with the findings of Geraldes *et al*., 2000, who reported that progesterone receptor-negative malignant tumours exhibited faster proliferation compared to progesterone receptor-positive tumours, suggesting that the progression of malignancy in spontaneous mammary tumours is linked to a reduction in hormonal steroid dependence.

Of the 10 CMTs, four cases were negative for ER, PR and HER2 expression and were diagnosed as TNBCs. In the four TNBCs, the MTA1 expression varied from moderate to strong. This is supported by the findings of Wang *et al*. (2018) who reported that circular ubiquitin-associated protein 2 (circ-UBAP2), an oncogenic circular RNA can bind to microRNA-661 (miR-661), inhibiting its activity, which in turn increases the expression of the oncogene MTA1 and promotes the progression of TNBC.

In this study, two cases were identified as HER2-positive subtypes (Fig. 18), and two were classified as luminal tumours (Fig. 19 and 20) with HER2 positivity. The MTA1 expression ranged from moderate to strong in the HER2-positive tumours, aligning with multiple breast cancer studies that highlight MTA1 expression as being stimulated by HER2 (Martin *et al*., 2006; Sharma *et al*., 2011; Cheng *et al*., 2012).

Further, moderate to strong expression of MTA1 was observed in all the TNBC cases, indicating its possible role in the pathogenesis of this aggressive form of CMT, similar to that of human BC indicating a possible oncogenic role of this protein in TNBC.

Our study found that MTA1 showed higher expression in high grade tumours and aggressive phenotypes of CMTs with no significant correlation to hormone receptor status. This highlights an association between MTA1 overexpression and higher tumour grade, as well as between MTA1 overexpression and tumour aggressiveness rather than hormone receptor status. These findings are similar to that observed in HBC by Jang *et al*. (2006). The study offers valuable insights into the molecular pathogenesis of CMTs, suggesting that targeting MTA1-mediated pathways could provide new

therapeutic strategies, particularly for higher grade and more aggressive tumours.

Conclusion

In conclusion, the association of MTA1 overexpression with high tumour grade and tumour aggressiveness highlight MTA1 as a potential biomarker of higher grade and aggressive phenotype in CMTs, suggesting its use as both a therapeutic target and a prognostic indicator. Future studies with larger sample sizes are recommended to validate these findings and explore their clinical applications.

Acknowledgements

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Conflicts of interest

The authors declare that they have no conflict of interest.

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