Clinicopathological significance of immunohistochemical expression of Filamin A in breast cancer

Lokavarapu Manoj Joshua, Farhanul Huda, Shalinee Rao, Bina Ravi

Abstract:
BACKGROUND: Filamin A is an actin-crosslinking protein expressed in many malignancies, although its prognostic and therapeutic role in breast cancer is not studied. There is enigma regarding its dual role in cancer, the tumor-progressing or tumor-suppressing effects depending on the site to which it localizes in the cell. The current study aimed to detect Filamin A expression in breast cancer and its association with other biomarkers and other clinicopathological parameters and established risk factors in breast cancer so that it can be a potential site for targeted therapy.

MATERIALS AND METHODS: One hundred female patients of histologically proven breast cancer who presented to our hospital over a 2-year period were included in the study. None of the patients received prior radiotherapy, chemotherapy, or immunotherapy. Patients with recurrent breast cancer are not included in the study. All study cases are subjected to immunohistochemistry for estrogen receptor, progesterone receptor, Her2 neu, and ki-67 from core biopsy tissue of cases diagnosed as breast carcinoma. Tissue sections were subjected to immunohistochemistry with anti-Filamin A.

RESULTS: Filamin A is expressed in 69% of cases of invasive breast cancer in our study. There was no statistically significant relationship of Filamin A immunoexpression with histological grade, age, parity, oral contraceptive use, smokeless tobacco use, TNM staging, clinical staging, clinical prognostic staging, and also ER, PR, Her2 neu, and ki-67 status (P > 0.05). Thus, it appears to be an independent biomarker in breast carcinoma. Filamin A was expressed only in the cytoplasm in all our study cases. Filamin A expression can be observed in adjacent normal breast tissue and benign fibroadenoma tissues also, but the pattern of expression is mainly membranous with cytoplasmic positivity. The cytoplasmic expression is seen in malignant cells as well as normal breast and benign tumor sections implicating the dual role of Filamin A in breast cancer.

CONCLUSION: No significant correlation could be found between Filamin A expression and clinicopathological parameters in our study. The cytoplasmic expression is seen in malignant cells as well as normal breast and benign tumor sections implicating the dual role of Filamin A in breast cancer. Filamin A immunoexpression should be further correlated with metastasis-free survival period of breast cancer patients.

Keywords: Breast cancer, Filamin A, immunohistochemistry, risk factors

Introduction
Breast cancer is the second most common malignancy accounting for 11.6% of all new cases diagnosed and 6.6% of all cancer-related deaths, next only to lung cancer worldwide.[1] Investigation of novel biomarkers leads to better understanding of tumor biology and the clinical utility of existing biomarkers. Filamin A is a cytoskeletal protein which crosslinks actin into orthogonal networks. The actin cytoskeleton plays a role in cell division, cell shape, motility, and signal transduction.[2,3] FLNa expression may
Filamin A in Breast Cancer

Filamin A (FLNa) is a cytoskeletal protein that plays a role in various cellular processes, including cell adhesion, migration, and signaling. Its overexpression is associated with tumor metastasis in lung cancer.[1] In melanoma, FLNa expression is linked to tumor-invasive properties.[2] FLNa overexpression is associated with metastasis in lung cancer.[3]

In breast cancer, FLNa's role is not well understood. FLNa expression is associated with tumor progression in some studies,[4] while in others, FLNa expression is associated with tumor-suppressing effects.[5] The dual role of FLNa in cancer progression depends on the specific site of local expression in the cell. The current study aimed to detect Filamin A expression in breast cancer and to investigate its association with other markers and clinicopathological parameters to identify potential risk factors in breast cancer.

Materials and Methods

One hundred female patients with histologically proven breast cancer who presented to our hospital over a 2-year period were included in the study. None of the patients had previous cancer therapy, or immunotherapy. Patients with recurrent breast cancer were excluded from the study. Clinical staging was done according to the AJCC 8th edition 2017. All study cases were subjected to immunohistochemistry for estrogen receptor, progesterone receptor, Her2 neu, and ki-67 from core biopsy tissue of cases diagnosed as breast carcinoma. Four-micron thick sections were cut from paraffin-embedded tissue blocks and were subjected to immunohistochemistry with anti-Filamin A/FLNa polyclonal antibody (Boster Biological Technology, Pleasanton, CA, USA) in lyophilized form at dilution of 0.5 µg/ml with antibody diluent. Sections were examined under low-power (×100) and high-power (×200 and ×400) magnification to observe immunoreactivity. Sections of benign skin from the chest wall of female patients, distant normal breast tissue, and sections from benign fibroadenomas were used as negative controls for anti-Filamin A antibody. Criteria for immunopositivity were membrane and cytoplasmic positivity of malignant cells. Immunopositivity is scored from 0 to 3+ (Filamin score); the intensity of immunopositivity was scored as zero if no staining, 1+ for weak cytoplasmic staining in <10% of cells, 2+ for moderate cytoplasmic staining in >10% of cells, and 3+ for marked cytoplasmic staining in >10% of the cells. A score of 0 or 1 was considered as negative result for FLNa expression (low), whereas scores of 2+ or 3+ were considered as positive (high) FLNa expression. Immunohistochemical analysis of FLNa expression was correlated with clinical stage, clinical diagnostic stage, and histological grade of tumor. Immunohistochemical analysis of FLNa expression will be correlated with parameters such as age, age at first child, menstruation status, use of oral contraceptive pills and hormone replacement therapy, nulliparity, tobacco use, alcohol use, and family history of breast cancer. Statistical analysis was done using SPSS version 23. T-test, Chi-squared test, Fisher’s exact test, Wilcoxon test, and Kruskal–Wallis test were used to test significance.

Statistical analysis

Filamin A was expressed only incytoplasm in all our study cases. Filamin A expression can be observed in adjacent normal breast tissue and benign fibroadenomas. Immunohistochemical analysis of FLNa expression was correlated with clinical stage, clinical diagnostic stage, and histological grade of tumor. Immunohistochemical analysis of FLNa expression will be correlated with parameters such as age, age at first child, menstruation status, use of oral contraceptive pills and hormone replacement therapy, nulliparity, tobacco use, alcohol use, and family history of breast cancer. Statistical analysis was done using SPSS version 23. T-test, Chi-squared test, Fisher’s exact test, Wilcoxon test, and Kruskal–Wallis test were used to test significance.

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Discussion

Filamin A has been studied in a number of cancers such as prostate and lung cancer. The possible role of its expression and localization in the malignant cell has not been established in breast cancer. In one study, Filamin A, it is shown to suppress breast cancer cell migration and invasion.[2,6] In another study, it was shown that the silencing of Filamin A inhibited migration and invasion by cancer cells[7]. We studied its expression and possible correlation with other established biomarkers and risk factors of breast cancer in this study. In our study, age majority of the patients are between 40 and 60 years [Figure 1]. Thirty-six percent of the patients are premenopausal and 64% of the patients are postmenopausal. The median age at the first child in our study is 20 years, which is similar to current demographic trends in India.[8] Only 2% of the patients have a history of oral contraceptive usage and none of the patients has a history of hormone replacement therapy. In our present study, none of the patients had a history of smoking or alcohol consumption. About 2% of the patients had smokeless tobacco use (tobacco chewing). The role of smokeless tobacco in breast cancer is investigated in very few studies.

Pattern of expression

Filamin A is expressed in 69% of cases of invasive breast cancer in our study. Tian et al. reported 63.54% and Guo et al. reported 52.6% positivity in their studies; a slightly different scoring system was used in the study done by Guo et al.[9,10] Studies have shown that when Filamin A localizes to the cytoplasm, it has a tumor-promoting effect and when it localizes to the nucleus, it has tumor-suppressing effect.[11]

Filamin A was expressed only in the cytoplasm in all our study cases. Filamin A expression can be observed in adjacent normal breast tissue and benign fibroadenoma tissues also, but the pattern of expression is mainly membranous with cytoplasmic positivity [Figure 2]. The intensity of immunopositivity is scored from 0 - 3+ [Figures 3-6]. Tian et al. have observed cytoplasmic observation mainly at the edge of cells and intercellular substance in malignant cells and no immunoreactivity for Filamin A in normal breast tissue and benign tumor sections,[9] In the study done by Guo et al., immunoreactivity was observed in normal breast tissue.[10] In a study done by Jiang et al., Filamin A expression was noted in the cytoplasm.[12] In another study, Filamin A was observed to be expressed within the cytoplasm and cell membrane.[13] Previous studies failed to find an association between localization of Filamin A and breast cancer.

Association between Filamin A expression and clinicopathological parameters

No significant association was identified between the age of patient and Filamin A expression ($P = 0.574$), which was consistent with the other studies [Figure 1].[9,10] High Filamin A expression was associated with higher histological grade, but there was no statistically significant relationship between Filamin A expression and histological grade of tumor ($P = 0.072$). The ER, PR, and Her2 neu status was not shown to have a significant association with Filamin A expression.
In a study done by Guo et al., positive association has been observed between PR status and Filamin A expression, whereas in the study done by Tian et al., there was no significant relationship between hormone receptor status and Filamin A expression. In our study, there was no significant relationship between the TNM stage, clinical stage, clinical prognostic stage, lymph node metastases, and Filamin A expression (P > 0.05) [Figure 1]. In the study done by Tian et al., Filamin A expression was shown to have a significant association with the TNM stage, lymph node metastasis, and vascular and perineural invasion, whereas in the study done by Guo et al., there was no association with lymph node metastasis.\[9\]

In our study, we studied the association of ki-67, a proliferation marker with Filamin A score and Filamin A expression which was not previously studied and found to be statistically insignificant. Previous studies have shown that Filamin A modulates chemosensitivity in triple-negative breast cancer.\[14\] There was no significant relationship between Filamin A expression and age at menarche, menopausal status, usage of oral contraceptive pills, and parity. Menstruation status has shown to be significantly associated with Filamin A expression (P = 0.038) in other studies.\[9\]

**Conclusion**

No significant correlation could be found between Filamin A expression and clinicopathological parameters in our study. The cytoplasmic expression is seen in malignant cells as well as normal breast and benign tumor sections implicating the dual role of Filamin A in breast cancer. Filamin A immunoexpression should be further correlated with metastasis-free survival period of breast cancer patients.

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**Conflicts of interest**
There are no conflicts of interest.

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