ORIGINAL RESEARCH PAPER

Pathology

STUDY OF EPITHELIAL TO MESENCHYMAL TRANSITION PROCESS BY VIMENTIN EXPRESSION IN DUCTAL CARCINOMA OF BREAST

KEY WORDS: EMT, Ki67, Vimentin.

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ABSTRACT

Introduction - Epithelial - mesenchymal transition (EMT) is defined by loss of epithelial characteristics and acquisition of mesenchymal phenotype. Vimentin is a type III intermediate filament highly expressed in high grade ductal breast carcinoma.. **Objectives -** The objective is to study epithelial to mesenchymal transition process by IHC expression of vimentin in ductal carcinoma breast. **Materials and Methods -** This prospective study conducted on 100 cases of breast carcinoma at Department of Pathology over a period of 18 months. Sections from specimen used for IHC staining of Vimentin. **Results -**There is significant Vimentin expression in early age group as compared to older age group in present study. There is inverse relationship between ER and PR positivity and Vimentin status. Triple negative breast carcinoma have highest Vimentin positivity . **Discussion & Conclusion -** In present study Vimentin expression is significantly associated with early age breast carcinoma, high proliferative index Ki67 and triple negative type of breast carcinoma.

INTRODUCTION

EMT is characterized by loss of intercellular adhesion (E-cadherin and occludins); down-regulation of epithelial makers (cytokeratins); up-regulation of mesenchymal markers [vimentin and smooth muscle actin (SMA)]; acquisition of fibroblast-like (spindle) morphology with cytoskeleton reorganization; increase in motility, invasiveness, and metastatic capabilities.[1]

In basal-like stage, cancer cells increase level of cancer malignancy by reversible epithelial-mesenchymal transition (EMT) process, which can be found in tissue repair, in tissue fibrosis and cancer progression. EMT plays vital role in various pathological processes, including wound healing, tissue fibrosis, and cancer progression.[2]

Vimentin:

Vimentin is a 57 KD, type III intermediate filament found in the mesenchymal cells of tissue during their developmental stages, maintains cell and tissue integrity. Vimentin is significant in supporting and anchoring position of organelles in cytosol. Intermediate filaments share common structures: central rod domain, head domain at N-terminal, and tail domain at C-terminal.[3] In terms of organ function, vimentin is involved in development of the mammary gland, nervous system, and angiogenesis.[4,5,6.] Studies have confirmed that vimentin can regulate epithelialmesenchymal transition (EMT), affecting diverse physiological and pathological processes.[7]

Aims And Objectives

To study epithelial to mesenchymal transition process by IHC expression of Vimentin in ductal carcinoma breast, neoplastic dissected lesions received for routine Histopathological evaluation at histopathology section of Shree M .P. Shah Medical College.

Co-relation of various IHC markers,clinical and pathological findings with Vimentin expression in ductal carcinoma breast.

MATERIALS AND METHODS

Type of Study: Prospective study.

Duration of study: July 2021 to December 2022.

Place of study: Department of pathology, Shri M.P.Shah Medical College,Jamnagar.

METHOD

Sections from specimen containing lesion of interest were used for IHC staining of Vimentin.

IHC staining performed using Peroxidase antiperoxidase method using paraffin embedded blocks cut into 3-4um thick sections. Sections taken on poly L lysine coated glass slides. Secondary antibody kit of Biogenex Ltd was used for study. Primary monoclonal ready to use antibodies were of Biogenex Ltd. Tris-EDTA solution of high pH (8.4) used for antigen retrieval, i.e Heat Induced Epitope Retrieval (HIER), in pressure cooker for up to one whistle.

Thereafter slides were brought down to room temperature and taken through steps of immunostaining protocol (Annexure IV) using Tris buffered saline (Annexure III) as the wash buffer. The peroxide block was freshly prepared for each use and protein block was used as per company protocol from Biogenex Ltd. for use of primary antibodies. Sections were covered with one to two drops of primary antibody for incubation for 30 mins as required. Incubation Vimentin (clone V9) done in humidity chamber. Slides thoroughly washed with tris buffered saline in between each step. Thereafter polymer HRP provided in kit was used. Finally DAB chromogen used in specific concentration as specified . Slides counter stained by haematoxylin and mounted by DPX. After drying test slides examined under light microscopy simultaneously with positive controls.

RESULTS

Immunohistochemistry Vimentin marker status (n=100)

Various clinopathological parametres and Vimentin association (n=100)

Parameter	Vimentin positive No (%)	Vimentin negative No (%)	P value (<0.05 significant)

Age	≤40 years	6/18 (33.34%)	12/18(66.66 %)	0.041
	>40 years	11/82 (13.41%)	71/82(86.59 %)	
Sex	Male	00/02(00%)	02/02(100%)	0.517
	Female	17/98(17.34 %)	81/98(82.66 %)	
Histological grade	Grade I	00/4 (00%)	04/04(100%)	0.410
	Grade II	17/92 (18.48%)	75/92(81.52 %)	
	Grade III	00/04(00%)	04/04(100%)	
Lymph node status	Negative	09/34(26.47 %)	25/34(73.53 %)	0.024
	Positive	03/41(7.31 %)	38/41(92.69 %)	
ER status	Negative	08/21(38.09 %)	13/21(61.91 %)	0.0037
	Positive	09/79(11.40 %)	70/79(88.60 %)	
PR status	Negative	09/29(31.04 %)	20/29(68.96 %)	0.016
	Positive	8/71(11.26 %)	63/71(88.74 %)	
Her 2 Neu status	Negative	16/87(18.40 %)	71/87(81.60 %)	0.338
	Positive	01/13(7.70 %)	12/13(92.30 %)	
Ki-67 status	Low (≤14%)	4/65(6.15%)	61/65(93.85 %)	0.00008
	High (>14%)	13/35(37.14 %)	22/35(62.86 %)	
Molecular subtypes	Luminal A	03/61(4.92 %)	58/61(95.08 %)	<0.00001
	Luminal B	06/18(33.34 %)	12/18(66.66 %)	
	Her 2 enriched	01/12(8.34 %)	11/12(91.66 %)	

In present study immunohistochemistry marker Vimentin is positive in 33.34% cases of early age breast carcinoma (≤40 years) which is higher than > 40 years age group. (13.41% cases are Vimentin positive). All Vimentin positive cases are seen in female gender.

All Vimentin positive cases are seen in modified bloom Richardson grade II. 18.48% cases of histological grade II are positive for Vimentin. All grade I and grade III cases are negative for Vimentin.

Lymph node metastasis negative cases have 26.47% positive ratio of Vimentin whereas positive lymph node metastasis have 7.31% positive cases of Vimentin.

ER positive cases include 38.09% positive cases of Vimentin whereas ER negative cases have 11.40% positive Vimentin rate. As PR positive cases include 31.04% positive cases of Vimentin whereas ER negative cases have 11.26% positive Vimentin rate.

Her 2 neu negative cases have 18.40% positive rate of Vimentin whereas her 2 neu positive cases have 7.70% cases with positive Vimentin. Cases with high proliferative index (Ki 67 >14%) have high Vimentin positivity 37.14% whereas low ki 67 index (≤14%) have only 6.15% Vimentin positivity.

In molecular subtypes triple negative breast carcinoma have highest vimentin positivity (77.78%), followed by luminal B (33.34),her 2 enriched (8.34%) and luminal A (4.92%).

DISCUSSION

In present study we have studied 100 cases of invasive ductal carcinoma-NST type and presence of EMT by expression Vimentin.

Positive	Negative	Total
17(17%)	83(83%)	100 (100%)

The following details were observed :

- Most specimens are modified radical mastectomy (75%).Other specimens include tru cut biopsy, incisional and excisional biopsy, simple mastectomy and toilet mastectomy comprising total 25% of all specimens.
- Out of 100 case 98 cases are of female breast carcinoma and 2 cases are of male breast carcinoma.
- Out of 100, 55 cases are of left side breast carcinoma and 45 cases are of right breast carcinoma.
- Age of patient ranged from 25 years to 100 years. Age group between 41 to 50 years and 51 to 60 years was mostly affected. The 5th and 6th decade comprises total of 61 cases out of 100 cases. Early age group(≤40 years) comprises 18 cases.
- The majority of cases in present study are of modified bloom Richardson grade II comprising 92 cases out of 100 cases. Grade I and grade III comprises 4 of each cases out of 100 cases.
- Out of total 100 cases lymph nodes are submitted or found in 75 cases out of which 41 cases (54.67%) are positive for malignancy. There is no significant correlation between lymph node status and Vimentin positivity.
- In present study epithelial to mesenchymal transition is studied by expression of Vimentin. Out of total 100 cases 17 are positive for Vimentin.
- There is significant Vimentin expression in early age group as compared to older age group in present study.
- There is inverse relationship between ER and PR positivity and Vimentin status
- According to IHC diagnosis triple negative breast carcinoma type have highest Vimentin positivity .

Types of studies taken into consideration:

Nami Yamashita et al [8,9](2013)
Osvaldo Padilla et al [10](2018)
David Sarrio et al [11](2018)
Siddhi Gaurish Sinai Khandeparkar et al [12](2019)
Charlotte Levin Tykjær Jørgensen et al [13](2020)
WHO CLASSIFICATION [14]

CONCLUSION

- Concept of EMT in breast carcinoma is a valuable for morphologic and molecular changes observed in tumor cell invasion and metastasis . In present study to evaluate process of EMT in breast carcinoma Vimentin expression is used as a marker of EMT.
- In present study Vimentin expression is significantly associated with early age breast carcinoma, high proliferative index ki67 and triple negative type of breast carcinoma.
- Triple negative subtype have highest Vimentin positivity suggesting epithelial to mesenchymal transition.
- There is inverse relationship with Vimentin expression and ER and PR expression and there is no significant correlation of Vimentin and Her 2 expression.

Future vision :- Currently, role of Epithelial to mesenchymal transition in breast tumors is unknown. Expression of Vimentin and its correlation with various clinopatholoical parameters acts as a diagnostic and predictive biomarker for early breast cancer metastasis and also prognostic marker to facilitate clinical decisions.Additionally Vimentin also act as a therapeutic target for development of individualized breast cancer therapy and can provide base of research for investigating newer promising treatment options for breast cancer.

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