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Expression of cancer stem cell marker CD44 and axillary lymph node metastasis in invasive ductal carcinoma of breast

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Abstract: *Introduction:* CD44 is a cell surface transmembrane glycoprotein is a member of cell adhesion molecules responsible for mediating communication and adhesion between adjacent cells and extracellular matrix. In recent years, CD44 has garnered a significant attention because of its utility as a stem cell marker and has surfaced as a potential therapeutic target, necessitating a greater understand of CD44 in breast cancer. *Aim:* The aim of this study is to determine the correlation between CD44 expression of tumour cells in breast cancer and presence of axillary lymph node metastasis. *Materials and Methods:* A retrospective study was conducted by the department of Pathology at Kamineni Academy of Medical Sciences and Research Centre, Hyderabad, India from August 2022 to March 2023. Female patients who underwent modified radical mastectomy for invasive ductal carcinoma were taken in this study. Tumour and axillary lymph node histologically examined and histological grading was given. Immunohistochemistry of CD44 marker and its expression by the tumour cells was done by standard immunoperoxidase method. *Results:* The study included 35 invasive ductal carcinoma of breast. Out of 35 cases, 19 cases showed lymph nodal metastasis. Among these 19 cases, only 13 showed CD44 expression in tumour cells with P value of 0.108 which is not statistically significant. A positive trend is noted with CD44 expression with higher tumour grade. *Conclusion:* The above results do not show any significant association between CD44 expression in tumour cells and lymph nodal metastasis in invasive breast carcinoma.

Keywords: CD44, Metastasis, Invasive Breast Cancer, Carcinoma.

Introduction

The breast carcinoma is most commonly diagnosed cancer in females (accounting for 24% of all female cancers) and also the leading cause of female cancer death worldwide. This carcinoma has distinct morphologies, with biomarker - defined subtypes, varied metastatic behaviour, distinct outcomes and responses to the therapy [1]. It is actually known that variation in transcriptional programmes is the major reason for biological diversity among human breast cancers [2].

The sub typing of breast carcinoma according to molecular and genetic subtypes has an impact n the prognostication of disease and treatment modalities [3]. In recent times, many clinical trials are being carried out based on breast cancer molecular profiles [4]. However, the most promising targeted therapies came with the evolution of identification of cancer cells with stem cell like properties. Cancer stem cells are defined by their ability to generate more self renewal and to produce cells that differentiate. Asymmetric cell division achieves both tasks, as one progeny retains self renewal identity and the other undergoes rounds of cell division and subsequent post mitotic differentiation. Initially, cancer stem cells were believed to represent a small fraction of total cell population in a tumour tissue however, it has been claimed that as many as 25% of cancer cells may have the properties of cancer stem cells [5].

A well established fact that a tumour cell to metastasise requires cooperative activities between tumour and surrounding tissues in which several molecules are involved and most important being the adhesion molecules. CD44, one of the adhesion molecule is a

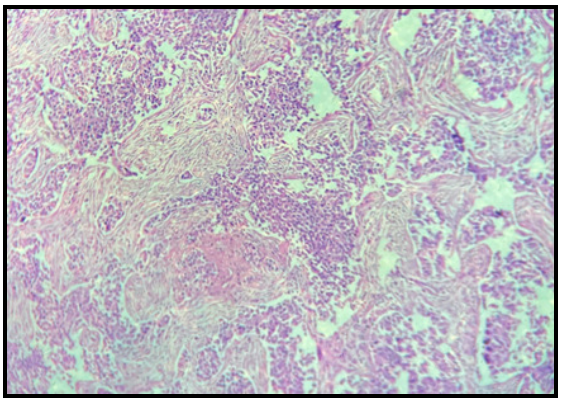
transmembrane glycoprotein encoded by a single gene on chromosome locus 11p13 and is ubiquitously expressed throughout the body and has a molecular weight of 85-200kDa [6].

It initiates cascade of events like signal transducing molecules activation, growth factors, matrix degrading enzymes activation etc [7]. In an attempt to determine its role in breast carcinoma, we have investigated the correlation between CD44 expression of tumour cells in breast cancer and presence of axillary lymph node metastasis along with histological grade of the tumour [8].

Material and Methods

This is a retrospective descriptive study done of female patients who underwent modified radical mastectomy with axillary lymph node clearance for primary infiltrating breast carcinoma at Kamineni Academy of Medical Sciences and Research Centre, Hyderabad, India for a period of eight months from August 2022 to March 2023 were included. Patients with prior neoadjuvant chemotherapy and or radiation therapy, biopsies and male patients with carcinoma of breast are excluded from this study. Formalin fixed, paraffin embedded tissues were taken from the primary tumour and processed for histopathology examination (Figure 1).

Fig-1: Shows tumour cells with marked desmoplasia reaction of stroma (H&E; 200x)

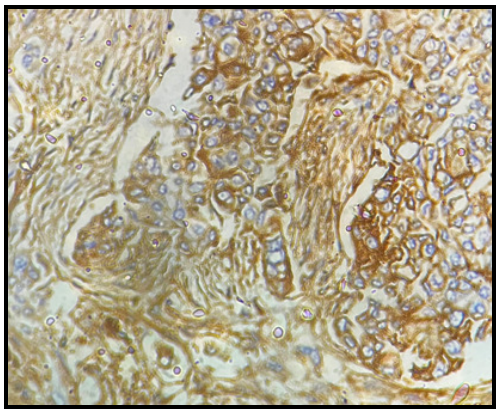


All sections made were reviewed for confirmation of diagnosis, histological typing and grading (Nottingham histological grading score) according to the College of American Pathology (CAP) reporting protocol. All axillary lymph nodes were examined histologically for presence of metastasis.

Immunohistochemistry: Cases diagnosed with infiltrating ductal carcinoma were subjected to immunohistochemical analysis for CD44 (cancer stem cell marker). About 4-5 um were deparaffinised and rehydrated. To block the endogenous peroxide activity, the sections were placed in 3% hydrogen peroxide diluted in nine parts of methanol for 30 minutes. Target antigen retrieval was achieved using citrate buffer (pH- 6.0) in the microwave. The sections were incubated overnight in a moist chamber with primary antibody CD44.

The next day after bringing them to room temperature and washing, the secondary antibody (biotinylated anti-mouse monoclonal by Pathnsitu) was applied and sections were re-incubated for 1 hour. Chromogen was added for 1-3 minutes and checked under microscope. Counterstaining was done with haematoxylin for 1 minute. Assessment of positivity or negativity for CD44 was performed semi- quantitatively from immunohistochemically stained sections of tumour samples from patients. Tumour cells were localized using 10x objective of microscope and the level of positivity was graded for CD44 staining as 0(negative), 1 (mild), 2 (moderate) and 3 (strong positive) (Figure 2).

Fig-2: Shows CD44 membrane immunopositivity in tumour cells (IHC CD44, 400x).



Positive staining for CD44 was defined as minimum 2+ circumferential membranous staining in at least 10% of tumour cells. Only membranous staining in tumour cells is scored and included in our study; stromal or non membranous staining in tumour cells is not included. The statistical data analysis was

done using chi-square test to correlate the positive CD44 expression in tumour cells and axillary lymph nodes metastasis from infiltrating breast carcinoma. A *P* value of <0.05 was considered significant.

Results

About 35 cases of invasive ductal carcinoma of breast in females treated by modified radical mastectomy and axillary lymph node clearance were taken. The patient ages ranged from 35 to 76 years. All the tumour sections and axillary lymph node sections were examined histologically. The tumours were graded based on Nottingham-Bloom-Richardson system. Eight (22.8%) tumours were categorised as grade I, while fifteen (42.8%) were grade II and twelve (34.2%) were grade III. Out of 35 cases, twenty four cases (68.5%) had histologically proven axillary lymph node metastasis. Nineteen (54.2%) invasive breast cancer cases exhibited cytoplasmic membrane immunopositivity for CD44.

Table-1: Correlation between CD44 immunopositivity, histological grade and axillary lymph node metastasis

	CD44 Positive	CD44 negative	Total
Grade I tumour	2 (25%)	6 (75%)	8
Grade II tumour	9 (60%)	6 (40%)	15
Grade III tumour	8 (66.6%)	4 (33.3%)	12
Nodal metastasis present	13 (54.1%)	11 (45.8%)	24
Nodal metastasis absent	6 (54.5%)	5 (45.4%)	11

Table 1 compares CD44 immunopositivity against histological tumour grade and presence of axillary lymph node metastasis. There was a progressive increase in percentage of CD44 positive tumours in concordance with increase in histological grade of tumour. Although, a large proportion of tumours about 24 cases (68.5%) metastasise to lymph nodes in which 13 cases (54.1%) show CD44 positive tumour expression. The above result of CD44 expression in tumour cells and metastasis to axillary lymph nodes did not reach statistical significance (p=0.108).

Discussion

CD44 is a multistructural, multifunctional cell adhesion molecule encoded by 20 exons and at least 10 of which are subjected to alternative splicing, resulting in many variants of CD44 [9]. The variants or isoforms modulate the many reported roles of CD44 in cell biology and Pathology, including activation and homing of lymphocytes, cell to cell interactions, matrix degrading enzymes activation & T cell activation [9-10].

CD44 normally binds to its primary ligand hyaluronic acid (HA). This binding is thought to be responsible for cellular signalling, and regulating other biological process within cells. Cells within a tissue interact either through the intracellular matrix (ICM) or through cellular junctions. CD44 as an adhesion molecule is enables cell communication by cell-cell signal transduction [11]. In addition, CD44 also mediates the signal transduction of human epidermal growth factor receptor (HER) and common cell signalling pathways regulates cell division (MET) receptor tyrosine kinases [12]. Based on the above features, CD44 appears promising as a modulator of wound healing, angiogenesis and cancer metastasis process [13].

In view of isoforms and complexity of the molecule due to splicing and subsequent modification by glycosylation and proteases it has lead to conflicting findings that are present in the scientific literature [14-17]. In a study conducted by Fillmore and Kuperwasser suggested that CD44 is a basal line cell that could be used as a marker for cases with poor breast cancer prognosis [18]. Our results indicate an increasing trend in CD44 expression with increasing histological grade of invasive breast carcinoma suggesting that CD44 have a role to play linked to increasing aggressiveness and tumour differentiation. CD44 was also expressed in higher proportion of tumours which had undergone lymph node metastasis by the time of surgery compared to those which did not. But, the above finding did not reach statistical significance.

In a study conducted by M.O. Idowu et al. [19], a total of 45 invasive breast carcinomas were taken with age ranging from 38 to 76 years. Tumours with Grade III features were about 46.6% but our study showed majority of grade II features. Axillary lymph node metastasis was seen only in 8.8% cases which far low seen in our study. CD44 positivity was seen in 60% of cases in triple negative breast cancers with most of them are of histological grade II and III, which is concordance to our study analysis.

In another study conducted by Iris Rabinovich et al [20], a total of 144 cases were taken with age ranging from 27 to 88 years. Tumours with histological grade II constituted about 50.7% which was in concordance to our study. Axillary lymph node metastasis was seen in 43.05% of cases which is little less to metastasis cases in present study. CD44 positivity was seen only in 12.9% of cases with most of the cases were of higher histological grade which is in concordance to our study.

Conclusion

CD44 expression in tumour cells and metastasis to axillary lymph nodes showed P value 0.621 which is statistically not significant and is in consonance result to our present study analysis.

Our study, being based on immunohistochemistry detectable expression of unspliced CD44 standard, it completely doesn't determine expression patterns of various isoforms of CD44 and hence the exact true status of its expression by tumours studied. In spite of the limitations, a trend suggestive of positive relationship between CD44 expression and tumour aggressiveness (grade) has been observed. We emphasise the need of further investigate the interaction of CD44 including its various isoforms and other cellular mechanisms which may contribute to cancer advancement in prognosis and as well as therapeutic purposes.

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