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ASSOCIATION OF "CEP8 AND CEP17 WITH TUMOR MITOTIC INDEX IN BREAST CARCINOMA" STUDIED IN 45 BREAST CARCINOMA TISSUES

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Abstract

Background: Breast cancer is modern day burden in women, in breast cancer prognosis and treatment always chromosomal instability is associated. Breast cancer persists as one of the most widespread malignancies globally, presenting substantial hurdles in diagnosis and treatment. On chromosome 17, HER2 oncogene is located. It is always associated with breast cancers in 20% of cases. This study conducted to find the correlation between molecular subtypes and histologic classifications of breast cancer, shedding light on how these subtypes influence tumor appearances in imaging studies.

Methods: This was a both retrospective and prospective study. The representative tumor area was identified and marked by diamond pencil over the under surface of the slide. A further section is taken again from the same blocks on Poly L lysin coated slides for the Fluorescence in situ hybridization (FISH). Signals were observed and and quantified. Signals were counted by two investigators independently. The amplification index was calculated as the ratio of c-MYC, HER2/neu signals to chromosome 8,17 centromere signals. A 2.2-fold increase served as the criterion to determine the presence of the amplification of c-Myc, HER2/neu polysomy of CEP8, CEP17.

Results: CEP8 polysomy was found 26.6%, CEP17 polysomy was found in 20% of the IDC cases and CEP8 polysomy was found in 26.6%, CEP17 polysomy found in 33.3% of the DCIS with microinvasion cases respectively. whereas polysomy was significantly more frequent in IDC compared with DCIS cases samples (p=0.00000]. DCIS cells showed 93.3% disomy. 6.6% of the cells showed monosomy.

Conclusion: These findings suggest that polysomy of CEP8, CEP17 more frequently observed in IDC and that disomy is more common in tissue of DCIS. Mitotic activity corresponding with the polysomy of CEP8, CEP17.

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Keywords: CEP17, CEP8, DCIS, IDC, Breast Cancer.

Introduction

Breast cancer is a complex ailment influenced by both genetic makeup and environmental triggers. At the forefront of its aggressiveness are breast cancer stem cells (BCSCs), posing a significant hurdle in treatment strategies. Overcoming therapy resistance remains a paramount challenge. Fortunately, a spectrum of treatments exists for breast cancer patients, offering hope and options in their fight against the disease. (1). Histological response to preoperative therapy is a reliable predictor of results for BC patients. Many chemotherapy sessions were conducted in the preoperative context. Identifying patients who benefit from a specific treatment is crucial for effective management. Certain markers can aid in predicting prognosis and therapy response. (2)

Traditional morphologic assessment remains valuable in providing prognostic insights into breast carcinomas. However, recent strides in molecular technologies have revolutionized tumor classification, delineating four distinct subtypes based on intrinsic molecular profiles. These advancements offer both predictive and prognostic value. This article explores the correlation between molecular subtypes and histologic classifications of breast cancer, shedding light on how these subtypes influence tumor appearances in imaging studies. (3)

Methods:

This was a both retrospective and prospective study done in the Department of Division of Cytogenetics, Pondicherry Institute of Medical Sciences, Puducherry between the period from 2020 to 2023. The Institutional Ethics Committee of our institute approved the study (RC /2020/67) and consent from participants taken. A total of 45 processed blocks of carcinoma breast was obtained from the Department of Pathology of our institution according to standard procedure. Histological tumor grade, mitotic activity, tumor invasion and tumor type were taken from records. Sections of 3µ thickness were cut by Leica RM 2245 semiautomated microtome and sections were mounted on glass slides and processed for Hematoxylin and Eosin (H&E). The representative tumor area was identified and marked by diamond pencil over the under surface of the slide. A further section is taken again from the same blocks on Poly L lysin coated slides for the Fluorescence in situ hybridization (FISH).

FLUORESCENCE IN SITU HYBRIDIZATION (FISH):
DEPARAFFINIZATION: Overnight at temperature of 60°C.

CLEARING: Clearing is accomplished for 10 min in two changes of xylene.

DEHYDRATION: Dehydration done by two changes of absolute alcohol for 5 min.

PRE-TREATMENT: Kept the sections in freshly prepared citrate buffer (2.52gms of citric acid dissolved in 30ml of distilled water brought PH to 6.0 by adding NAOH) for 30 min at 98°C in waterbath. Then kept the sections in 2X SSC (5ml of 20XSSC mix with 45 ml of distilled water) for 5 min. Section were then digested with Proteinase K buffer (0.5gms of Sodium lauryl sulphate dissolve

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in 50 ml of 2 XSSC, taken 40ml of buffer added 80 µl of proteinase K) for 2-5 min until sections were cleared. Then sections kept in 2X SSC for 5min, and two changes of absolute alcohol for 2 min air dried the sections for 5-10 min in room temperature.

PROBE APPLICATION: Metasystems' DNA XL MYC Amplification probe kept on slides were covered with coverslip and used rubber cement as adhesive.

DENATURATION & HYBRIDIZATION: Denaturation was perforformed at for 94°C for 3 minutes on a thermos Brite followed by hybridization for 20hrs at 37° C.

POST WASH: Removed rubber cement and coverslip the slides were washed with 0.4x SSC (add 1ml of 20x SSC in 49ml of distilled water) at 72°C in water bath for 2 min. Wash the slides with NP40 buffer (add 10µl of NP40 in 50ml of 2x SSC) for 30 sec then slides were washed and air dried.
COUNTER STAIN: Counterstained with DAPI covered with the coverslip margins of the coverslip sealed with the nail polish.

ENUMERATION OF SIGNALS: Signals were observed and quantified with microscope, which was equipped with a Leica fluorescence and this microscope was with suitable filters was utilized for this method. In this procedure, the c-MYC amplification probe manifested as an orange signal, while a green signal was emitted by the control probe for the chromosome 8, HER2/neu gene manifested orange signals, centromere of chromosome 17 emitted a green signal. This method ensured exclusive counting of tumor cells while excluding normal cells.

Up to 100 tumor cell nuclei were evaluated from each Fluorescence in Situ Hybridization (FISH). Signals were counted by two investigators independently. The amplification index was calculated by as the ratio of c-MYC, HER2/neu signals to chromosome 8,17 centromere signals. A 2.2-fold increase served as the criterion to determine the presence of the amplification of c-Myc, HER2/neu polysomy of CEP8, CEP17.

Results:
To assess the chromosome 8, 17 copy number, the current study includes 45 cases of breast carcinoma tissues, 15 cases of Ductal Carcinoma In Situ (DCIS), 15 cases of DCIS with microinvasion, and 15 cases of invasive Ductal carcinoma (IDC). The patient presented with a breast mass. Post-operative tissues revealed encapsulated and well-circumscribed tumors with local invasion, necrosis, and a high-grade DCIS with microinvasion (Figure 1A), demonstrating mitotic activity. The current study used double color-fluorescence in situ hybridization to detect CEP8 and CEP17. High histologic grade was associated with polysomy of CEP8, CEP17 ($P < 0.05$).

There was a strong association ($P < 0.05$) between lymph node metastasis and polysomy of all two chromosomes (CEP8, CEP17). Increase in the number of polysomic chromosomes indicated the probability of lymph node metastasis. FISH scoring was done by counting the fluorescence signals in

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100 tumor cells. All samples were analyzed double-blindly. Cytogenetic technicians scored independently without clinical information or knowledge of other histological and pathological data. The number of signals per nucleus was categorised as 1, 2, or ≤3, suggesting monosomy, disomy, and polysomy. FISH images were taken with camera fitted fluorescence microscope equipped multiple fluorescence filter sets with three colour filter set. The centromere 8 (CEP8) signals were counted as green whereas the MYC signals were viewed as orange, chromosome 17 (CEP17) signals were counted as green and HER2 signals viewed as orange with the TRITC filter (Figure1: B & C). We have considered amplified, when it was as increased ratio more than 2:2 and considered as normal control when it was 2:2.

The results were expressed in the ration of MYC: CEP8. Table 1: CEP8 polysomy was found 26.6%, CEP17 polysomy was found in 20% of the IDC cases and CEP8 polysomy was found in 26.6%, CEP17 polysomy found in 33.3% of the DCIS with microinvasion cases (Fig1: B & C) respectively. whereas polysomy was significantly more frequent in IDC compared with DCIS cases samples (p=0.00000]. DCIS cells showed 93.3% disomy. 6.6% of the cells showed monosomy. These findings suggest that polysomy of CEP8, CEP17 more frequently observed in IDC and that disomy is more common in tissue of DCIS. Mitotic activity corresponding with the polysomy of CEP8, CEP17. Future studies should be performed to increase the amount of breast tissue DCIS with microinvasion and invasive breast carcinoma cases to support the results of this study.

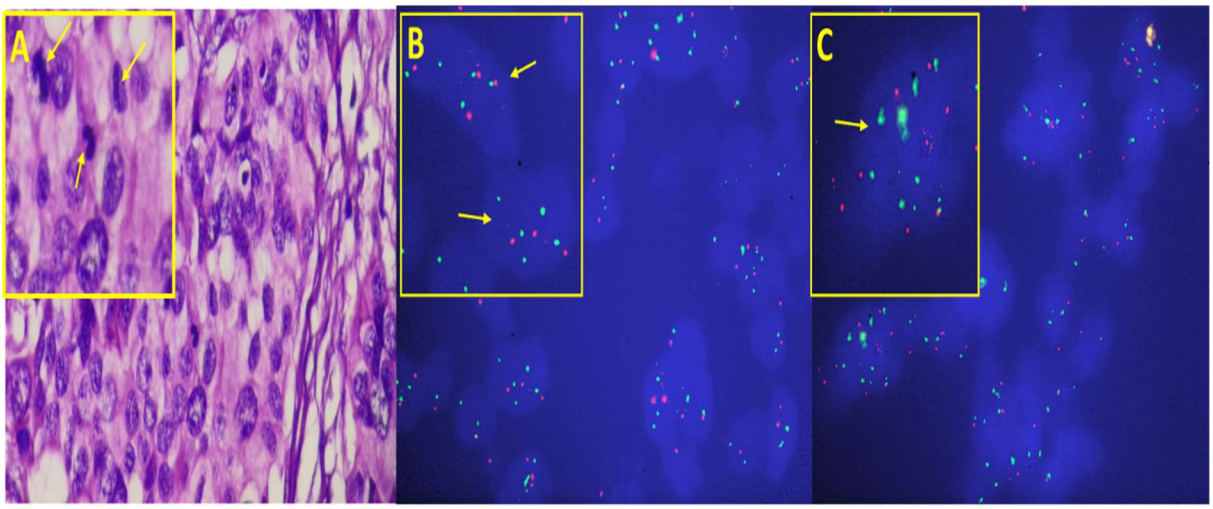

Figure 1: A High-grade DCIS with microinvasion showing mitotic activity, B showing CEP8 polysomy DCIS with microinvasion, C showing CEP17 polysomy IDC.

Table1

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Distribution of the CEP8 / CEP17 signals in DCIS, DCIS with microinvasion and Invasive carcinoma of the breast n=45

Cases	DCIS (n=15)	DCIS with Microinvasion (n=15)	Invasive carcinoma (n=15)
Cells	1500	1500	1500
Monosomy	CEP8/CEP17 (6.6%)/ -	CEP8/CEP17–Absent	CEP8/CEP17–Absent
Disomy	CEP8/CEP17 (93.3%)/ -	CEP8/CEP17– Present 73.3%/66.6%	CEP8/CEP17 - Present 73.3%/80%
Polysomy		CEP8/CEP17- Present 26.6%/33.3%	CEP8/CEP17 - Present 26.6%/20%

Discussion

When cell having abnormal number of chromosomes called as polysomy, usually with additional copies of specific chromosomes. Chromosomes 8 (CEP8) and 17 (CEP17) are particularly interesting in breast cancer because they are involved in polysomy. polysomy of chromosomes 8 and 17 in breast cancer has significant clinical implications, influencing prognosis and treatment strategies. While CEP8 polysomy may indicate poor prognosis and resistance to therapies, CEP17 polysomy complicates HER2 testing, impacting the determination of HER2-positive status and subsequent treatment decisions. The more advanced research to understanding breast cancer with polysomy, forunderstanding minute level of mechanisms, which help in treating better.

Perez et al (4) noted that tumors exhibiting MYC gain or polysomy 8 seemed to experience greater advantages from trastuzumab compared to tumors lacking these genetic alterations. Additionally, the study highlighted a significant benefit of trastuzumab in patients with both MYC and HER2 amplification, surpassing those with HER2 amplification alone.

Tsukamoto et al (5) discovered a significant association between lymph node metastasis and polysomy of chromosomes 1, 11, and 17. Moreover, they observed a notably elevated prevalence of progesterone receptor negativity among patients with chromosomes 11 & 17 polysomy. Torrisi et al. also reported similar findings (6). Takehisa et al study demonstrated with histological evidence, that there was statistical corelation in cases associated with CEP17 (7). Lu et al identified an association between polysomy of chromosome 17, elevated nuclear atypia as well as lymphatic metastases (8). Salido and co-authors revealed that while nodal involvement exhibited a significant association with CEP17 and cases displaying CEP17 demonstrated a non-significant movement(9). Krishnamurti U et al stated that, cases with with unamplified CEP17 are linked to numerous adverse prognostic factors. These include higher nuclear grade, increased mitotic activity, elevated Nottingham score, more tumor stage, advanced histologic grade and greater negativity for estrogen receptors, with a tendency towards the amplified group, as opposed to patients lacking both amplification and polysomy (10). The study of Kim A et al [11] conducted with 594 invasive breast cancer samples. In this study in 22.8% cases noted HER 2 amplifications, in 11.1 % cases found TOP2A deletion and they reported 8.3% cases as TOP2A amplification. In 33.2% cases CEP17 mortification was observed. In their study they identified CEP17

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as a trusting prognostic marker.

Lee K et al (12) study was reported, adverse clinic pathological parameters of breast cancer are associated with CIN. Out all independent predictors of high CIN, CEP17 copy number gain important marker. High CIN score always found significantly with higher CEP17 Copy number. In study of Smid *et al* (13) reported in subtype with HER2-positive was associated the increased CIN-score. Bartlett et al (14) also reported that duplication of CEP17 was importance biomarker and it is associated with OS and relapse.

Polysomy of chromosome 8 in breast cancer has been associated with various clinical implications. Huang et al highlighted the importance of CEP8 polysomy, suggesting it can be used a marker for poor prediction and resistance in treatment. Increased gene dosage resulting from CEP8 polysomy can lead to overexpression of oncogenes, potentially contributing to tumor aggressiveness (15). Perou et al research has indicated that CEP8 polysomy might influence the response to targeted therapies. For example, in HER2-positive breast cancer, which often involves CEP17 polysomy as well, CEP8 polysomy has been linked to resistance to HER2-targeted therapies like trastuzumab (16). Dowsett et al (17) studies revealed that CEP17 polysomy is particularly relevant in breast cancer due to its impact on HER2 status determination. HER2-positive breast cancer is characterized by overexpression of the HER2 gene, typically because of gene amplification on chromosome 17q12-21. CEP17 polysomy can lead to an overestimation of HER2 gene copies and HER2 protein expression, potentially affecting treatment decisions.

Vanden Bempt et al studied the presence of CEP17 polysomy complicates the interpretation of HER2 testing results, as it can occur independently of HER2 gene amplification. Therefore, strategies to accurately assess HER2 status in the context of CEP17 polysomy are crucial for determining appropriate treatment regimens in breast cancer patients (18).

In the present study, we observed a significant correlation between polysomy of chromosome 8 (CEP8) in breast cancer and adverse clinical outcomes, consistent with previous findings. This reinforces the notion that CEP8 polysomy may serve as a prognostic indicator for disease progression and resistance to treatment modalities. Additionally, our investigation corroborates existing literature on the impact of CEP17 in HER2-positive cancer of breast. We found that CEP17 polysomy complicates HER2 status determination, echoing previous reports of its influence on HER2 testing accuracy and treatment decision-making. These results found the importance of chromosome polysomy in cancer of breast, which will help in co relate clinical features of patients, highlighting the need for standardized testing protocols and tailored therapeutic approaches based on comprehensive genetic profiling. More advanced studies required to elevate micro level mechanisms, which will help in validate these findings in larger patient cohorts.

Conclusion

The present study shows that polysomy of CEP8 and CEP17 are more frequently observed in IDC and disomy is more common in DCIS. Mitotic activity is usually associated with polysomy of CEP8 and CEP17. Future studies should be performed in larger sample sizes and in DCIS with microinvasion and invasive breast carcinoma to further confirm the results of this study.

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