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Original contribution

MAST2 and *NOTCH1* translocations in breast carcinoma and associated pre-invasive lesions☆,☆☆

Michael R. Clay MD[a,*], Sushama Varma[b], Robert B. West MD, PhD[b]

[a]*Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305-5324, USA*
[b]*Department of Pathology, Room L235, Stanford University Medical Center, Stanford, CA 94305-5324, USA*

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Summary There are several mutations and structural variations common to breast cancer. Many of these genomic changes are thought to represent driver mutations in oncogenesis. Less well understood is how and when these changes take place in breast cancer development. Previous studies have identified gene rearrangements in the microtubule-associated serine-threonine kinase (*MAST*) and *NOTCH* gene families in 5% to 7% of invasive breast cancers. Some of these translocations can be detected by fluorescence in situ hybridization (FISH) allowing for examination of the correlation between these genomic changes and concurrent morphologic changes in early breast neoplasia. *NOTCH* and *MAST* gene rearrangements were identified by FISH in a large series of breast cancer cases organized on tissue microarrays (TMA). When translocations were identified by TMA, we performed full cross-section FISH to evaluate concurrent pre-invasive lesions. FISH break-apart assays were designed for *NOTCH1* and *MAST2* gene rearrangements. Translocations were identified in 16 cases of invasive carcinoma; 10 with *MAST2* translocations (2.0%) and 6 cases with *NOTCH1* translocations (1.2%). Whole section FISH analysis of these cases demonstrated that the translocations are present in the majority of concurrent ductal carcinoma in situ (DCIS) (6/8). When DCIS wasn't associated with an invasive component, it was never translocated (0/170, $P = .0048$). We have confirmed the presence of *MAST* and *NOTCH* family gene rearrangements in invasive breast carcinoma, and show that FISH studies can effectively be used with TMAs to screen normal, pre-invasive, and coexisting invasive disease. Our findings suggest that these translocations occur during the transition to DCIS and/or invasive carcinoma.

1. Introduction

Large genomic studies have identified numerous recurrent mutations and aneuploidies that can be found in the vast majority of breast cancers [1–3]. In contrast to invasive carcinomas, little is understood about the genomic changes associated with progression to breast cancer, from normal tissue to early neoplasias to carcinoma in situ to invasive carcinoma. However, it is likely that these

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* Corresponding author. Department of Pathology, Room L235, Stanford University Medical Center, 300 Pasteur Drive, Stanford, CA 94305-5324.

E-mail addresses: mrclay@stanford.edu (M. R. Clay), svarma@stanford.edu (S. Varma), rbwest@stanford.edu (R. B. West).

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genomic changes are important contributors to the process of carcinogenesis.

Prior research on pre-invasive breast neoplasia has largely focused on DNA copy number changes [4–6]; however, these studies did not have the current understanding of recurrent aneuploidies and did not focus on matched progression to invasive carcinoma. Very little work has been done on recurrent mutations in pre-invasive neoplasia. Some work has focused on general cancer hotspots, but other recurrent breast cancer–specific mutations have not been examined. With the exception of human epidermal growth factor receptor–2 (*HER2*) amplification, DNA copy number changes and the single nucleotide variations can be difficult to observe in situ. This is important as early breast neoplasia lesions are typically microscopic and can only be routinely observed in formalin-fixed paraffin-embedded material once a slide has been prepared [7].

A recent study found recurrent *MAST* (microtubule-associated serine-threonine kinase) and *NOTCH* family fusions in 5% to 7% of breast cancers [8]. Rearrangements were identified in the *NOTCH1*, *NOTCH2*, *MAST1*, and *MAST2* genes, and these rearrangements were associated with increased oncogenic functional activity. Notch signaling is an evolutionarily conserved pathway that is essential for embryonic development, organogenesis, and tissue homeostasis. Aberrant Notch signaling is associated with several inherited developmental diseases and various types of cancer [6]. Clinical evidence supports a pro-oncogenic function for Notch signaling in several solid tumors, especially in breast cancer. Notch inhibitory agents, such as gamma-secretase inhibitors, are being investigated as candidate cancer therapeutic agents in a number of organ systems, and are becoming more and more relevant in the clinical setting [6,9,10].

Less is known about the *MAST* family of genes. They are thought to play a role in normal cell division, and alterations have reportedly produced a number of mitotic abnormalities, including spindle malformation, chromosome missegregation, centrosome amplification, and failure of cytokinesis [11]. This sort of chromosomal instability is common to carcinoma, and may help explain the role of *MAST* genes in carcinogenesis.

We sought to identify the previously described rearrangements in the *MAST* and *NOTCH* genes in a large number of breast cancer cases and to determine the extent, if any, to which concurrent pre-invasive lesions harbor these translocations. For this purpose we utilized fluorescence in situ hybridization (FISH), which allows for the identification of translocations with morphologic correlation. When rearrangements were identified in carcinoma, we went back to the primary tissue and analyzed whole section FISH preparations to evaluate for the absence or presence of involvement of earlier lesions such as ductal carcinoma in situ (DCIS), atypical ductal hyperplasia (ADH), flat epithelial atypia (FEA), columnar cell change (CCC), and finally normal breast epithelium where available.

2. Materials and methods

Tumor and normal samples were collected in compliance with the Health Insurance Portability and Accountability Act and approved by the Stanford University Medical Center Institutional Review Board.

2.1. Tissue microarrays

Interchromosomal translocations and large intrachromosomal translocations are easily identified by FISH in archival material. This approach allows for the correlation of morphologic features with these genomic changes. This enables the large scale screening of breast cancer tissue microarrays (TMAs) for these events and the examination of other types of breast neoplasia that are not easily obtained as fresh frozen material. We evaluated *NOTCH1* and *MAST2* gene rearrangements in 3 separate TMAs containing breast neoplasia. These included 283 cases of invasive carcinoma (TA-221), 285 cases of DCIS (TA-239), and 115 cases of invasive carcinoma (TA-241) that are from patients of the DCIS array. TA-239 contains 170 cases of pure DCIS without associated invasive disease, and 115 cases of DCIS that was associated with invasive disease, represented by TA-241. These cores represent material from 568 patients, with 20% of patients represented by two cores, and the remaining patients represented by a single core. The TMA was produced manually using the Manual Tissue Arrayer MTA-1 by Estigen Tissue Science (Tartu, Estonia). TMAs also included several tissue types for control purposes. The control tissues utilized included normal breast, placenta, appendix, adrenal, bladder, epididymis, liver, muscle, gallbladder, kidney, esophagus, lung, skin, seminal vesicle, pancreas, salivary gland, and prostate. The TMAs were divided into quadrants, with each quadrant having control tissues placed at the 4 corners, with additional controls placed diagonally through the center of each quadrants' long axis. Using 2-color break-apart probes with FISH, we evaluated 1656 tissue cores (0.6 mm in diameter each).

2.2. Fluorescence in situ hybridization

Sections (4 μm thick) of the TMA slides were pretreated as described [12].

Locus-specific FISH analysis was performed by using the following bacterial artificial chromosomes (BACs) from the Human BAC Library RPCI-11 (BACPAC Resources Centre, Children's Hospital Oakland Research Institute, Oakland, CA) and CTD (Caltech-D BAC library) clones from Invitrogen (Grand Island, NY) listed centromeric to telomeric: RP11-112P19, CTD-2310H10 (*MAST2*), and RP11-83N9, CTD-3213A21 (*NOTCH1*), and CTD-2182G14,RP11-713O7, RP11-11I5, RP11-153P24 (*MAST1*), and RP11-671M21, CTD-2574B15, CTD-