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Micropapillary Variant of Mucinous Carcinomas of the Breast Display Genetic Alterations Intermediate Between Mucinous Carcinomas and Micropapillary Carcinomas

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Abstract

Aims: Micropapillary variant of mucinous carcinoma of the breast (MPMC) is a rare histologic form of estrogen receptor (ER)-positive invasive carcinoma, characterized by micropapillary clusters of tumor cells in lakes of extracellular mucin. We sought to determine the genetic alterations underpinning MPMCs, and to define whether they overlap with those from mucinous carcinomas and/or invasive micropapillary carcinomas.

Methods and Results: DNA from five MPMCs was subjected to whole-exome sequencing. Somatic mutations, copy number alterations and mutational signatures were determined using state-of-the-art bioinformatics methods. No mutations in genes significantly mutated in breast cancer, including *TP53*, *PIK3CA*, *GATA3* or *MAP3K1* were detected. We identified copy number alterations reported in invasive micropapillary carcinomas, such as recurrent gains in 1q, 6p, 8q and 10q, and recurrent losses in 16q, 11q and 13q, as well as a recurrent 8p12-8p11.2 amplification encompassing *FGFR1*. Akin to mucinous carcinomas, three of the five MPMCs analyzed lacked *PIK3CA* mutations, 1q gains and 16q losses, the hallmark genetic alterations of ER-positive breast cancers, whereas two MPMCs harbored 16q losses and/or a complex pattern of copy number alterations similar to those found in breast invasive micropapillary carcinomas.

Conclusions: MPMCs are heterogeneous at the genetic level; some tumors display a pattern of somatic genetic alterations similar to those of mucinous carcinomas, whereas others resemble

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AUTHOR CONTRIBUTIONS

EB, JSR-F and YHW conceived the study. YHW provided tissue samples. AD coordinated the retrieval of samples. FP, APMS, EB and YHW performed the pathology review. FP, APMS, ADCP and performed experiments. PS and DNB performed the bioinformatic analysis. FP, PS, DNB, APMS, ADCP, LF and BW analyzed and interpreted the data. FP, YHW, BW and JSR-F wrote the first manuscript, which was reviewed by all coauthors.

CONFLICTS OF INTEREST

JSR-F reports personal/consultancy fees from VolitionRx, Page.AI, Goldman Sachs, Grail, Ventana Medical Systems, Invivo and Genentech, outside the scope of the submitted work. All other authors declare no conflicts of interest.

invasive micropapillary carcinomas at the genetic level. These findings suggest that MPMCs may not constitute one histologic subtype, but a convergent phenotype that can stem from mucinous carcinomas or invasive micropapillary carcinomas.

Keywords

breast cancer; massively parallel sequencing; micropapillary variant of mucinous carcinoma

INTRODUCTION

The micropapillary variant of mucinous carcinoma of the breast (MPMC) is a rare histologic form of estrogen receptor (ER)-positive invasive carcinoma, accounting for less than 1% of breast cancer.¹ MPMCs display hybrid mucinous and micropapillary features, consisting of micropapillary clusters of tumor cells with scalloped edges floating in stromal mucin, which display reverse polarity and outward apical snouts. Psammomatous calcifications are not infrequent.^{1, 2} In contrast to mucinous carcinomas of the breast, which generally have an indolent behavior, MPMCs are associated with a higher rate of nodal metastasis,³ and outcomes intermediate between those of patients with mucinous carcinomas and with invasive micropapillary carcinomas of the breast.^{3, 4}

The genomic characterization of rare breast cancer types has demonstrated that their histologic heterogeneity is paralleled by vast genetic heterogeneity.^{5, 6} Work by our group and others has led to the identification of pathognomonic genetic alterations in rare breast cancers, such as the *MYB-NFIB* fusion gene or *MYBL1* rearrangements in adenoid cystic carcinoma,⁶⁻¹⁰ the *IDH2* R172 hotspot or *TET2* mutations concurrently with *PIK3CA* or *PIK3R1* mutations in solid papillary carcinomas with reverse polarity,^{11, 12} and *HRAS* Q61 hotspot mutations concurrently with PI3K pathway gene mutations in ER-negative adenomyoepitheliomas.¹³ The analyses of mucinous carcinomas,¹⁴⁻¹⁶ however, have not resulted in the identification of a pathognomonic genetic alteration; rather, these studies have demonstrated that mucinous carcinomas lack *PIK3CA* mutations, concurrent 16q losses and 1q gains, characteristic features of common forms of ER-positive breast cancers. Likewise, breast micropapillary carcinomas have been shown to display genomics features similar to those of luminal B ER-positive invasive ductal carcinomas of no special type.^{17, 18}

Whilst the genomic landscape of breast mucinous carcinomas and micropapillary carcinomas has been well characterized,^{13, 15-17, 19, 20} little is known about the genetic alterations of MPMCs. Here, we sought to determine the genetic alterations underpinning MPMCs, and to define whether they would overlap with those from mucinous carcinomas and/or micropapillary carcinomas.

MATERIALS AND METHODS

Subjects and samples

Following Institutional Review Boards approval, frozen sections or formalin-fixed paraffin embedded tissue blocks of MPMCs were retrieved from the archives of the Department of Pathology of Memorial Sloan Kettering Cancer Center (MSKCC, NY, USA). For power

calculations, assuming that MPMCs would be driven by a pathognomonic or highly recurrent genetic alteration, present in 70% of cases, the analysis of five samples would be sufficient to identify it with >80% statistical power. Samples were anonymized and reviewed by four pathologists (FP, APMS, EB and HW) as detailed in the Supplementary Methods.

Whole-exome sequencing analysis

DNA samples from microdissected tumor and normal tissue from pure/ mixed MPMCs were subjected to whole-exome sequencing (WES) at MSKCC's Integrated Genomics Operations using validated protocols, as previously described (Supplementary Methods).^{13,21}

RESULTS

Clinicopathological characteristics of MPMCs

Our series included five MPMCs from female patients with a median age of 57 years (range 35-68). The median tumor size was 1.7 cm (range 1.3-1.8 cm; Supplementary Table 1). All MPMCs were centrally reviewed by four pathologists and featured clusters of tumor cells arranged in micropapillae floating in lakes of mucin (Figures 1A-1F). Four MPMCs, which were composed of tumor cells floating in lakes of mucin in at least 90% of their area were classified as pure (Figures 1A-1D), whereas MPMC22 which displayed MPMC areas (Figure 1E) admixed with a ductal component (Figure 1F) was classified as mixed. Three pure MPMCs were of histologic grade 2, and one pure MPMC and both components of the mixed MPMC were of histologic grade 3. All MPMCs were ER-positive and one pure MPMC (MPMC15; 1/5; 20%), although equivocal (2+) for HER2 expression by immunohistochemistry (Supplementary Figure 1A-B), was found to be *HER2*-amplified by fluorescence *in situ* hybridization (Supplementary Table 1).

MPMCs display somatic genetic alterations intermediate between mucinous carcinomas and invasive micropapillary carcinomas of the breast.

MPMCs displayed a median of 13 (range 10-58) non-synonymous somatic mutations as defined by WES (Supplementary Tables 2 and 3). Our analysis did not reveal any recurrent somatic mutation in the MPMCs analyzed. We did not identify mutations in genes significantly mutated in breast cancer, including *TP53*, *PIK3CA*, *GATA3*, *MAP3K1* and *CDH1*.²² Our analyses revealed non-synonymous mutations in cancer genes of uncertain biological significance, such as subclonal missense mutations in *PTPRC* (T62S) and *MYO5A* (R862C), and frameshift mutations in *STAT5B* (Q368Pfs*9) and *CUX1* (F1193Sfs*8) without loss of heterozygosity of the wild-type allele (Figure 2A, Supplementary Figure 2, Supplementary Tables 2 and 3). A comparative analysis of the mutational repertoire of MPMCs with that of mucinous carcinomas¹⁶ and of micropapillary carcinomas of the breast¹⁷ revealed no statistically significant differences (Supplementary Tables 4 and 5). Akin to common forms of breast cancer and to mucinous carcinomas,¹⁶ all MPMCs analyzed displayed a dominant mutational signature 1, ascribed to aging (Supplementary Figure 3).

Our analyses revealed that MPMCs are heterogeneous at the copy number level. We observed copy number alterations reported in micropapillary carcinomas, such as recurrent

gains in 1q (2/5; 40%), 6p (2/5; 40%), and 8q (3/5, 60%), and recurrent losses in 16q (2/5; 40%), 11q (2/5; 40%), 13q (2/5; 40%) and 8p (3/5, 60%; Figure 2B-2F, Supplementary Figure 4). We have also observed a recurrent 8p12-8p11.2 amplification encompassing *FGFR1* in two cases (2/5; 40%; Figure 2B and 2E, Supplementary Figure 4, Supplementary Table 1). Two pure MPMCs harbored 16q losses and one of them displayed a complex pattern of copy number alterations, akin to breast micropapillary carcinomas (Figure 2B-2C, Supplementary Figure 4). In contrast, akin to mucinous carcinomas, three cases (3/5; 60%) including two pure MPMCs (MPMC15 and MPMC16) and the mucinous micropapillary component of a mixed MPMC (MPMC22) lacked coexisting 1q gains and 16q losses, hallmark genetic alterations of ER-positive breast cancers (Figure 2B-2F, Supplementary Figure 4). Taken together, despite the limited size of our cohort, these findings suggest that MPMCs may not harbor a pathognomonic or highly recurrent genetic alteration. Importantly, however, whilst some MPMCs resemble breast mucinous carcinomas at the genetic level, others display genetic alterations similar to those of micropapillary carcinomas and other common forms of ER-positive breast cancers.²³

DISCUSSION

MPMCs feature a hybrid morphology, intermediate between that of mucinous carcinomas and micropapillary carcinomas.^{1, 3} We and others have shown that mucinous carcinomas differ from common forms of ER-positive breast cancer at the molecular level.^{15, 16, 19} Mucinous carcinomas of the breast have a lower rate of mutations affecting *PIK3CA* and *TP53*, genes commonly mutated in ER-positive disease. Moreover, pure mucinous carcinomas have been shown to lack concurrent 1q gains and 16q losses, detected in the majority of common forms of ER-positive breast cancer.^{15, 16, 19} In contrast to mucinous carcinomas, micropapillary carcinomas harbor frequent mutations in *PIK3CA* and *TP53* and display complex copy number profiles, with recurrent gains of 8q.¹⁷

Akin to the morphology and behavior of MPMCs which is intermediate between that of mucinous carcinomas and micropapillary carcinomas,¹⁻³ our analyses revealed that some MPMCs bore similarities with micropapillary carcinomas, such as a complex pattern of a copy number alterations and 16q losses, whilst others displayed simple copy number profiles and lacked concurrent 1q gains and 16q losses, akin to mucinous carcinomas.¹⁶ Moreover, akin to mucinous carcinomas which harbor a lower frequency of *TP53* and *PIK3CA* mutations than common forms of ER-positive breast cancer,¹⁶ we did not identify mutations affecting these genes or any other significantly mutated gene in breast cancer. Nonetheless, we identified a frameshift mutation affecting the haploinsufficient gene *CUX1*²⁴ in a pure MPMC (MPMC14), which also displayed a 16q loss. *CUX1* encodes for a transcription factor with a tumor suppressor role, and its loss of function results in activation of the PI3K pathway.²⁵ Given that *PIK3CA* mutations are common in micropapillary carcinomas, one could posit that MPMC14 displays genetic features of micropapillary carcinomas, harboring a 16q loss and activation of the PI3K pathway via loss of function of *CUX1*. These findings suggest that some MPMCs might represent mucinous carcinomas that acquired micropapillary features, whereas others might correspond to micropapillary carcinomas^{17, 18} that acquired mucin production. In addition, given that micropapillary carcinomas appear not to harbor pathognomonic genetic alterations and have a repertoire of somatic mutations

and gene copy number alterations similar to that of common forms of ER-positive invasive breast cancer,^{22, 23} one could hypothesize that micropapillary carcinomas themselves may constitute a convergent phenotype²⁶ or that the micropapillary phenotype is a histologic pattern, which can be found in cancers from many anatomical sites but lacks a unique disease-defining coding somatic genetic alteration. We cannot rule out the possibility, however, that MPMCs might originate from mucinous carcinomas that acquired 16q losses relatively late in their evolution.

Our study has limitations, such as the small number of cases and the fact that we sequenced the mucinous micropapillary component of only one mixed MPMC. Hence, our findings should be perceived as hypothesis-generating. Nonetheless, our findings support the notion that some MPMCs display a pattern of genetic alterations similar to that of mucinous carcinomas, whereas other bore similarities with micropapillary carcinomas, suggesting that MPMCs may not constitute a histologic subtype, but rather a convergent phenotype that can stem from mucinous or micropapillary carcinomas.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1: Histologic features of the micropapillary variant of breast mucinous carcinomas. (A-F) Representative photomicrographs of hematoxylin and eosin (H&E)-stained micropapillary variant of breast mucinous carcinomas (MPMCs) from this study, including the pure MPMCs MPMC13 (A), MPMC14 (B), MPMC15 (C), and MPMC16 (D), and the mucinous micropapillary (E) and ductal (F) components of MPMC22, a mixed MPMC. Scale bar, 100 μ m

Figure 2. Repertoire of somatic mutations and copy number profiles of the micropapillary variant of breast mucinous carcinomas.

(A) Non-synonymous somatic mutations affecting cancer genes identified in the micropapillary variant of breast mucinous carcinomas (MPMCs; n=5) subjected to whole-exome sequencing (WES). Cases are shown columns and genes in rows. Clinicopathologic characteristics are depicted in phenotype bars (top). Copy number plots of (B) MPMC13, (C) MPMC14, (D) MPMC15, (E) MPMC16 and (F) MPMC22 depicting segmented Log₂ ratios (y-axis) plotted according to their genomic positions (x-axis). Alternating blue and gray demarcate the chromosomes.