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Whole-Exome Analysis of Metaplastic Breast Carcinomas with Extensive Osseous Differentiation

Francisco Beca[1,*], **Ana Paula Martins Sebastiao**[2,3,*], **Fresia Pareja**[2], **Kimberly Dessources**[4], **John R. Lozada**[2], **Felipe Geyer**[2], **Pier Selenica**[2], **Nebras Zeizafoun**[5], **Hannah Y. Wen**[2], **Larry Norton**[6], **Edi Brogi**[2], **Britta Weigelt**[2], **Jorge S Reis-Filho**[2]

[1]Department of Pathology, Stanford University School of Medicine, Stanford, CA, USA

[2]Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, NY, USA

[3]Positivo University Medical School, Curitiba, Paraná, Brazil

[4]Department of Surgery, Memorial Sloan Kettering Cancer Center, New York, NY, USA

[5]Department of Pathology and Laboratory Medicine, Mount Sinai Healthcare System, New York, NY, USA

[6]Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA

Abstract

Aims: Metaplastic breast carcinoma (MBC) is a rare type of triple-negative breast cancer that displays vast histologic and genetic heterogeneity. Osseous differentiation can be found in different subtypes of MBC. Whether MBCs with osseous differentiation are underpinned by specific genetic alterations has yet to be defined. Here we investigate the repertoire of somatic mutations and copy number alterations (CNAs) in three MBCs with extensive osseous differentiation.

Methods and Results: Tumor and normal DNA samples from three MBCs with extensive osseous differentiation were subjected to whole-exome sequencing. Somatic mutations, CNAs and mutational signatures were determined using a validated bioinformatics pipeline. Our analyses revealed clonal *TP53* hotspot mutations associated with loss of heterozygosity of the wild-type allele coupled with mutations affecting genes related to the WNT and/or the PI3K/AKT/mTOR pathways in all cases analyzed. All cases displayed a dominant mutational signature 1 with two cases showing a secondary signature 3 in addition to other features of homologous recombination DNA repair defects (HRD). The Oncostatin M Receptor gene (*OSMR*), which plays a role in

Correspondence to Jorge S. Reis-Filho, MD PhD FRCP, Department of Pathology, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, New York, NY 10065, USA. Phone: +1 212 639 8054. reisfilj@mskcc.org.

AUTHOR CONTRIBUTIONS

HYW, BW and JSR-F conceived the study. EB, HYW, FP, EB, FP, NZ and APMS obtained samples and performed the pathology review. FG, JRL, SSKL, PS performed experiments and the bioinformatic analysis. FB, APMS, KD, FP, LN, BW and JSR-F analyzed and interpreted the data. FB, APMS, FP and JSR-F wrote the first manuscript, which was reviewed by all coauthors.

[*]These authors contributed equally to this work.

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mesenchymal differentiation and bone formation, was found to be mutated in two MBCs with extensive osseous differentiation and in none of 35 previously published 35 MBCs.

Conclusion: Our findings suggest that MBCs with osseous differentiation have somatic mutations similar to those of other forms of MBC.

Keywords

breast cancer; massively parallel sequencing; metaplastic breast carcinoma; PI3K pathway

INTRODUCTION

Metaplastic breast carcinoma (MBC) is a rare histologic special type of breast cancer, characterized by the differentiation of neoplastic epithelium into squamous or mesenchymal-like elements, which are frequently spindle, but can also include chondroid, osseous or rhabdoid elements.[1] This phenotypic diversity is also observed at the transcriptomic level, given that MBCs can display varying intrinsic subtypes, integrative clustering and triple-negative breast cancer (TNBC) subtypes.[2,3]

Although MBCs are unlikely to be underpinned by a highly recurrent or pathognomonic somatic mutation or fusion gene,[4–9] the histologic diversity of MBCs may be underpinned by distinct genetic alterations.[4–9] Chondroid, spindle and squamous MBCs have been shown to display distinct genomic and transcriptomic profiles.[4,6,7] In particular, spindle cell MBCs less frequently harbor gains of 7q11.22–23[7] and show a significantly higher prevalence of *PIK3CA* mutations, whereas chondroid MBCs lack *PIK3CA* mutations, but harbor *CHERP* mutations and more frequently a dominant mutational signature 3, a signature associated with homologous recombination deficiency (HRD)[6]. Although the genomic features of spindle cell, chondroid and squamous MBCs have been characterized by microarrays and massively parallel sequencing, [4,6–9] MBCs with osseous differentiation have been less well studied and whether MBCs with extensive osseous differentiation would be characterized by specific genetic alterations has yet to be investigated. Here we sought to determine whether MCBs with osseous differentiation resemble MBCs with other types of differentiation at the genetic level.

MATERIALS AND METHODS

Subjects and samples

Following approval by the Institutional Review Board of Memorial Sloan Kettering Cancer Center (MSKCC), representative formalin-fixed paraffin-embedded tissue blocks of metaplastic carcinomas of the breast with osseous differentiation were retrieved from the archives of the Department of Pathology of MSKCC or the consultation files of one of the authors (E.B.). Patient consent was obtained according to the research protocol approved by the Memorial Sloan Kettering Cancer Institutional Review Board. Samples were anonymized, reviewed by four pathologists (E.B., F.P., A.P.M.S. and J.S.R-F) and classified according to the latest World Health Organization (WHO) criteria.[1] All cases subjected to whole-exome sequencing (WES) in this study (Supplementary Table 1) have not been

previously reported. For the comparative analysis, we used the data from a previously published series of MBCs with mesenchymal differentiation.[6]

Whole Exome Sequencing analysis

DNA was extracted from microdissected representative tumor and normal breast tissue, as previously described[4] and subjected to WES at the Integrated Genomics Operations (IGO) of Memorial Sloan Kettering Cancer Center (MSKCC), as also previously described.[6] Details of the bioinformatics analysis employed for somatic mutation detection, gene copy number alteration (CAN) analysis, mutational signature decomposition and genomics features of homologous recombination DNA repair defect (HRD) are described in the Supplementary Methods.

Statistical analysis

Statistical analyses were conducted using R v3.1.2. Fisher's exact tests were employed for comparisons between categorical variables, and Mann-Whitney U test were used for continuous variables. All tests were two-sided and P-values <0.05 were considered statistically significant.

RESULTS

The three cases of MBCs included in this study were found to display extensive osseous differentiation, given that the osseous component was the predominant metaplastic element, ranging from 40 to 60% (Supplementary Table 1). Two of the cases showed additional spindle cell components (cases MTC27 and MTC26) with markedly atypical spindle cells arranged in diverse architectural patterns including herringbone and storiform patterns. with areas of osseous differentiation intermingled (Figures 1A and 1B). MCT 27 and MTC28 also displayed minor chrondroid components in addition to the marked osseous differentiation (Figure 1C). Minor epithelial components were observed in all cases and were classified as of Nottingham histologic grade 3. Additional clinico-pathologic information is available in the supplementary materials (Supplementary Table 1). The carcinomas were either totally submitted or extensively sampled for histologic evaluation (Supplementary Table 1).

To determine whether MBC with osseous differentiation would be underpinned by a pathognomonic genetic alteration, these cases were subjected to WES (Supplementary Tables 2 and 3). This analysis revealed a median of 88 (range, 37–178) non-synonymous somatic mutations (Supplementary Table 2 and 3), 33 of which pathogenic or likely pathogenic, and an median of 9 (range, 3–11) mutations affecting cancer related genes[10] per case (Figure 2A). Clonal *TP53* hotspot mutations (R175H, P151S and R248Q) associated with loss of heterozygosity (LOH) of the wild-type allele were detected in all three cases analyzed. Other mutations in cancer related genes observed included a clonal *APC* (R1607T) missense mutation with LOH of the wild-type allele (Figure 2A and Supplementary Figure 1) in case (MTC26), as well as missense mutations in *CSF3R* (P549S), *HNF1A* (V448A), *ARHGEF12* (G865A), *DNMT3A* (R167W) and *SET* (R77H). MTC27 harbored a *PTEN* clonal frameshift mutation (H61Tfs*36) associated with LOH of the wild-type allele and a clonal *PIK3R1* in-frame deletion (D440_E443del). Case MTC28

displayed the highest mutational burden and a missense *ARID2* mutation with LOH of the wild-type allele, as well as a missense mutation in *NRG1* (S107T) (Figure 2A and Supplementary Figure 1). MTC26 and MTC28 harbored mutations affecting the *Oncostatin M Receptor* (*OSMR*) gene (Supplementary Table 3), genes whose protein product has been associated with bone homeostasis and development.[11,12] In addition, in MTC26, a clonal and likely pathogenic affecting the HECT Domain E3 Ubiquitin Protein Ligase (*HECTD1*) gene (W1360L), another gene whose protein product plays a role in mesenchymal differentiation and bone homeostasis and development,[13] was observed. An exploratory, hypothesis generating comparison between MBCs with osseous differentiation and 35 spindle cell, chondroid and squamous MBCs[6] revealed *OSMR* was significantly more frequently mutated in MBCs with osseous differentiation (67% vs 0%, p=0.004), however this analysis ought to be interpreted with caution owing to the small sample size of this study.

Copy number alteration analysis revealed a profile consistent with the previously reported in other MBCs and similar to TNBCs of no special type (IDC-NSTs), such as recurrent losses in 17p (3/3; 100%), and in 1p, 3p and 8p in 67% each (2/3), and recurrent gains of 1q and 8q in 67% of cases, each (2/3) (Figure 2B and 2D, Supplementary Figure 2). Amplification of *AKT1* and *TERT* (MTC28) and *CDKN2C* (MTC27) were detected. Of note, *BRCA1* showed heterozygous deletion in two cases (MTC26 and MTC28). An exploratory, hypothesis generating comparison of the CNA profiles of MBCs with osseous differentiation vs other previously reported MBCs[6] did not reveal any differences in amplifications or homozygous deletions (Figure 2C).

We also sought to determine whether MBC with osseous differentiation would display genomic features suggestive of HRD. The three MBCs analyzed here displayed a dominant mutational signature 1 (MTC16: 30.3%, MTC27: 59.4% and MTC28: 27.9%). Cases MTC26 and MTC28 displayed a secondary signature 3 (26.7% and 20.3% of the mutational profiles of each case, respectively; Supplementary Figure S3). These two MBCs also displayed other features consistent with HRD, including a high LOH scores (17 and 8, respectively, vs 3 in MTC27), high large-scale state transitions (LST) scores[14] (37 and 22, respectively, vs 4 in MTC27) and high telomeric allelic imbalance (NtAI) scores[15] (26 and 24, respectively, vs 5 in MTC27; Supplementary Table 4). No bi-allelic mutations affecting HRD-related genes[16] were detected in these two cases.

DISCUSSION

Here we demonstrate that in a way akin to other forms of MBC, MBCs with extensive osseous differentiation are also underpinned by clonal *TP53* bi-allelic alterations and harbor mutations affecting genes known to be altered in MBCs,[5–7] including those related to the Wnt and PI3K pathway family of genes. Despite our limited statistical power due to the small sample size, our study suggests that osseous differentiation in MBCs may not be underpinned by a pathognomonic mutation or copy number alteration.

An exploratory comparative analysis of MBCs with extensive osseous differentiation vs previously published MBCs of other histologic appearances revealed that mutations

affecting *OSMR* were only numerically more frequently found in MBCs displaying osseous differentiation. Presently we have no data with statistical significance to support a difference in the prevalence of mutations affecting these genes in MBCs with extensive osseous differentiation, but we recognize the role of *OSMR* in bone development and homeostasis.[11–13] In addition, we have also detected a clonal and likely pathogenic mutation affecting *HECTD1*, a gene whose silencing has been implicated in EMT, development of metastasis and reduced survival in breast cancer.[17] Further studies to ascertain the role of *OSMR* and *HECTD1* in the biology of MBCs are warranted.

This study has several limitations, including its small sample size, due to the rarity of MBCs with extensive osseous differentiation and the requirements for decalcification, which often result in nucleic acids of suboptimal quality being extracted from formalin-fixed paraffin embedded tissue samples. In addition, we were unable to extract RNA of sufficient quality to perform RNA sequencing analysis of the samples included in this study. Finally, we cannot rule out the presence of a pathognomonic mutation affecting non-coding regions of the genome or non-protein coding genes, or a pathognomonic fusion gene in MBCs with extensive osseous differentiation.

Despite the limitations, our study provides the characterization of the repertoire of somatic mutations, CNAs and mutational signatures in MBCs with extensive osseous differentiation, and demonstrates that these tumors share many of the genomics features of other forms of MBCs including frequent Wnt and PI3K pathways alterations. In addition, we provide evidence that osseous differentiation in MBCs is unlikely to be underpinned by a highly recurrently mutated protein coding gene or CNA.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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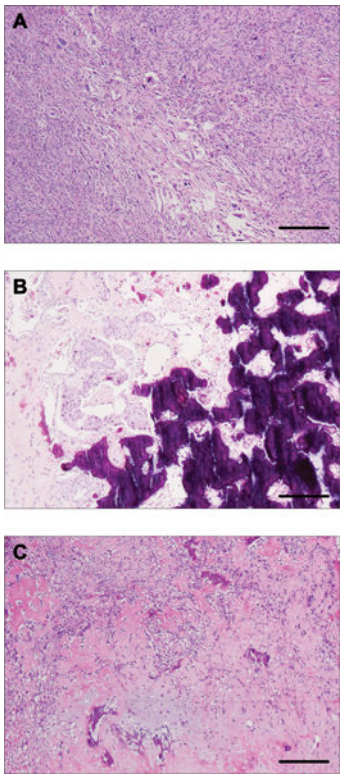

Figure 1 - Histologic characteristics of metaplastic breast carcinomas (MBCs) with osseous differentiation.

Representative photomicrographs of hematoxylin and eosin (H&E)-stained MBCs included in this study. **(A)** MTC26 displaying areas of spindle cell morphology with cells arranged in a storiform pattern. **(B)** MTC27 also displays the intervening epithelial differentiation around osseous areas. **(C)** MTC28 classified showing osseous and chondroid differentiation in a myxoid background. Scale bar, 500 μm (A, C), 200 μm (B).

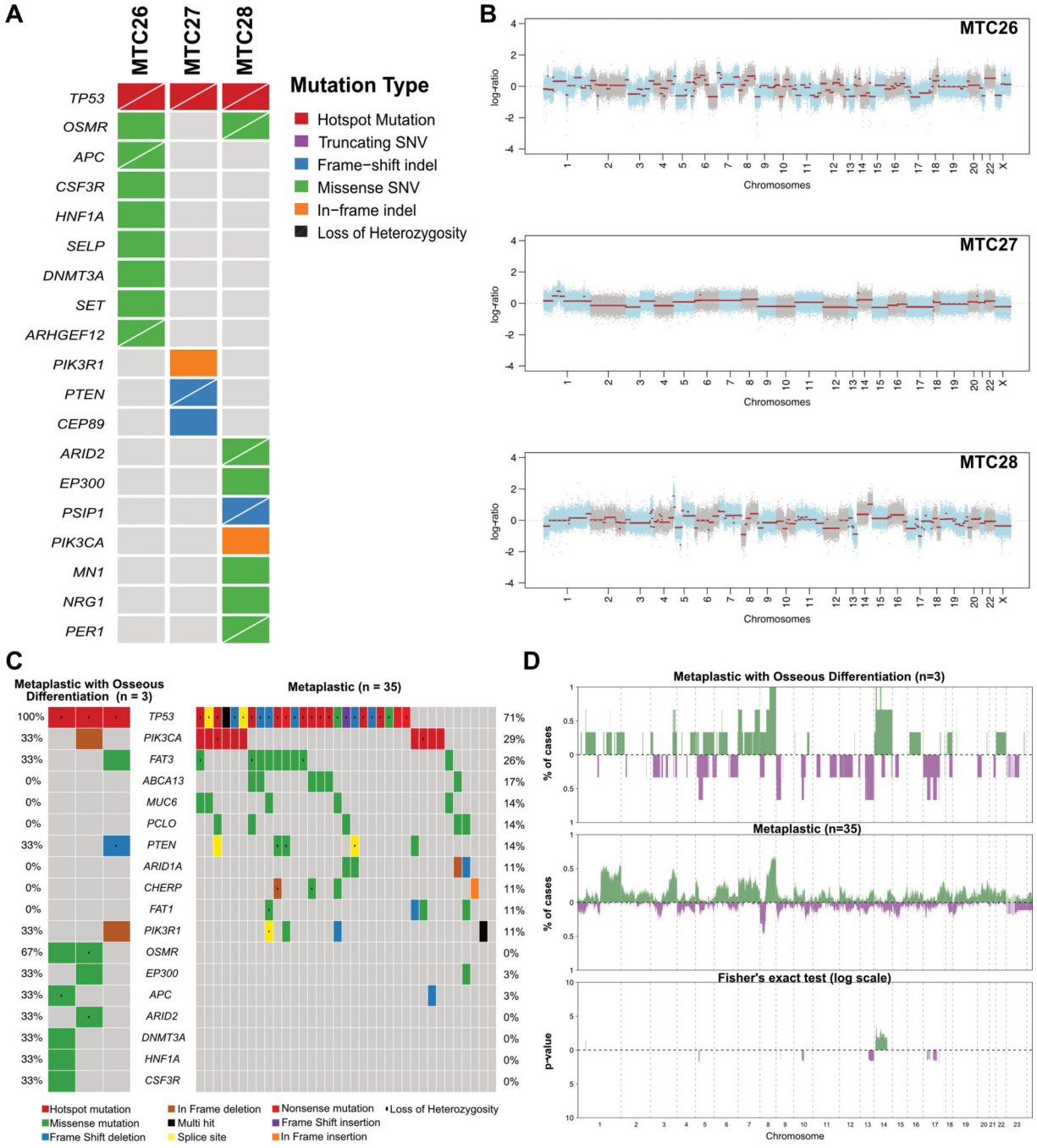

Figure 2. Repertoire and comparison of somatic mutations and copy number alterations (gains/losses) of metaplastic breast carcinomas with osseous differentiation.

(A) Non-synonymous somatic mutations shared among cases and mutations affecting cancer-related genes in the MBC with osseous differentiation (n=3) subjected to whole-exome sequencing (WES). Cases are shown in columns and genes in rows. **(B)** Copy number plots depicting segmented Log_2 ratios (y-axis) plotted according to genomic position (x-axis). Chromosomes are demarcated by alternating blue and gray colors **(C)** Comparison of non-synonymous somatic mutations prevalence in the MBC with osseous differentiation

(n=3) and MBCs with no osseous differentiation (n=35). **(D)** Frequency plots and Fisher's exact test comparison corrected for multiple testing of copy number gains and losses between the MBC with osseous differentiation (n=3) and MBCs with no osseous differentiation (n=35). Frequency (y-axis) of amplifications (green) and homozygous deletions (purple) is shown for each genomic region (x-axis). Inverse Log 10 values of the two-sided Fisher's exact test p-values are plotted according to the genomic region (lower panel).