

Genomic profiling of histological special types of breast cancer

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Abstract Histological special types of breast cancer have distinctive morphological features and account for up to 25 % of all invasive breast cancers. We sought to determine whether at the genomic level, histological special types of breast cancer are distinct from grade- and estrogen receptor (ER)-matched invasive carcinomas of no special type (IC-NSTs), and to define genes whose expression correlates with gene copy number in histological special types of breast cancer. We characterized 59 breast cancers of ten histological special types using array-based com-

parative genomic hybridization (aCGH). Hierarchical clustering revealed that the patterns of gene copy number aberrations segregated with ER-status and histological grade, and that samples from each of the breast cancer histological special types preferentially clustered together. We confirmed the patterns of gene copy number aberrations previously reported for lobular, micropapillary, metaplastic, and mucinous carcinomas. On the other hand, metaplastic and medullary carcinomas were found to have genomic profiles similar to those of grade- and ER-matched IC-NSTs. The genomic aberrations observed in invasive carcinomas with osteoclast-like stromal giant cells support its classification as IC-NST variant. Integrative aCGH and gene expression analysis led to the identification of 145 transcripts that were significantly overexpressed when amplified in histological special types of breast cancer. Our results illustrate that together with histological grade and ER-status, histological type is also associated with the patterns and complexity of gene copy number aberrations in breast cancer, with adenoid cystic and mucinous carcinomas being examples of ER-negative and ER-positive breast cancers with distinctive repertoires of gene copy number aberrations.

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Array CGH data, the analysis history, script and code are available at <http://rock.icr.ac.uk/collaborations/Mackay/Archetypes>.

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Introduction

Invasive breast cancer is a heterogeneous disease, encompassing multiple entities with distinct biological and clinical features. The latest edition of the World Health Organization (WHO) classification recognizes the existence of at least 21

histological types of invasive breast carcinoma [1]. The most common type is invasive carcinoma of no special type (IC-NST; formerly known as invasive ductal carcinoma), which accounts for 50–80 % of all breast cancers [2, 3]. The remaining tumor types are referred to as histological “special types” of breast cancer, which collectively account for approximately 25 % of invasive breast carcinomas [1–4].

Previous studies have provided evidence that some histological special types of breast cancer, including mucinous, neuroendocrine, and invasive lobular carcinomas (ILCs), may constitute entities distinct from histological grade- and/or estrogen receptor (ER)-matched IC-NSTs at the transcriptomic level [5–8], whereas tubular and medullary carcinomas harbored gene expression profiles similar to those of molecular subtype-matched IC-NSTs [9, 10]. Conflicting results on the molecular features of metaplastic cancers have been reported, as transcriptomic profiles were found to be similar to those of basal-like IC-NSTs [11], as well as to be distinct and related to those of claudin-low breast cancers [12, 13].

From a genetic standpoint, there is also evidence that some histological special types of breast cancer are underpinned by distinct repertoires of genetic aberrations. For instance, secretory carcinomas and adenoid cystic carcinomas have been shown to harbor the recurrent fusion genes *ETV6-NTRK3* [14] and *MYB-NFIB* [15, 16], respectively. At the genomic level, secretory, mucinous, metaplastic, micropapillary, lobular, and adenoid cystic, but not papillary breast carcinomas have been shown to be distinct from IC-NSTs [3, 12, 16–21]. It remains to be investigated whether the remaining histological special types would constitute genomically distinct entities from grade- and ER-matched IC-NSTs. In addition, it is unknown whether the specific transcriptomic profiles of special type breast cancers are underpinned by distinct patterns of genetic aberrations.

The aims of this study were (i) to characterize the constellation of gene copy number aberrations in histological special types of breast cancer, (ii) to determine whether these special types of breast cancer are distinct from ER- and grade-matched IC-NSTs at the genomic level, and (iii) to define in each histological special type those genes whose expression correlates with gene copy number. We therefore investigated the pattern and type of gene copy number aberrations in 59 breast cancers of ten histological special types and in a cohort of grade- and ER-matched IC-NSTs using microarray-based comparative genomic hybridization (aCGH). In addition, we performed an integrative analysis of the aCGH and the microarray-based gene expression profiles of the 59 histological special types of breast cancer included in this study.

Materials and methods

Cases

Histological special types

One-hundred-and-thirteen invasive breast carcinomas from eleven histological special types with a tumor cell content of ≥ 50 % were selected from the frozen tissue bank of the Netherlands Cancer Institute/Antoni van Leeuwenhoek hospital (NKI/AVL) and subjected to gene expression profiling as previously described [8]. Of these 113 cases, those with ≥ 70 % neoplastic cells were selected for DNA extraction, and DNA of sufficient yield and quality to perform aCGH was obtained from 59 samples from ten special types (Supplementary Table 1). Cases were reviewed centrally by two pathologists (FCG and JSR-F) and were typed according to the World Health Organization criteria [1]. Immunohistochemistry results for ER, progesterone receptor (PR), and HER2 are described elsewhere [8]. Histological grade was determined by two pathologists (FCG and JSR-F) using the modified Scarff–Bloom–Richardson system [22]. Ethical approval was obtained from local ethical committees.

Control group (grade- and ER-matched IC-NSTs)

To compare, the genomic profiles obtained by aCGH of histological special types with those of IC-NSTs, special types with ≥ 5 cases were matched 1:2 with IC-NSTs, retrieved from the frozen tissue bank of the NKI/AVL and centrally reviewed and graded by two pathologists (FCG and JSR-F), according to histological grade and ER-status. The matching between histological special types and IC-NST was performed on this basis given the evidence to demonstrate that in breast cancer, histological grade and ER are strongly associated with the pattern of genomic changes [23–27].

DNA extraction

DNA was extracted from frozen tumor sections, using a standard proteinase K digestion, followed by phenol/chloroform extraction as previously described [28]. DNA concentration was measured using Picogreen (Invitrogen, Paisley, UK) according to the manufacturer’s instructions [29].

Microarray-based comparative genomic hybridization (aCGH)

aCGH was performed using a previously described 32 K bacterial artificial chromosome (BAC) array platform [21,

30] which has been shown to be as robust as, and to have comparable resolution with, high-density oligonucleotide arrays [31–33]. DNA labeling, array hybridization and image acquisition, and hierarchical clustering analysis were performed as previously described [20, 21, 30, 34] (Supplementary Information). aCGH data, analysis history, script, and code are available at <http://rock.icr.ac.uk/collaborations/Mackay/Archetypes>.

Integration aCGH and gene expression analysis

Detailed information on the gene expression microarray platform, RNA extraction, amplification, labeling, hybridization, and analysis, and the gene expression profiles are described elsewhere (ArrayExpress; E-NCMF-3) [8, 10]. The methods for the identification of genes whose expression levels correlate with copy number changes are essentially as previously described [35–37] (Supplementary Information). *p* values were corrected using the Benjamini and Hochberg adjustment to minimize type I or alpha errors, and adjusted *p* values <0.05 were considered significant.

Results

Genomic profiling of breast cancer histological special types

Of the 59 breast cancers from ten histological special types included in this study, 27 had a triple-negative phenotype (i.e., ER-, progesterone receptor (PR)-, HER2-negative), 31 were ER-positive/HER2-negative, and one case was ER-negative/HER2-positive as determined by centrally performed immunohistochemistry. In addition, 13, 20, and 26 were of histological grades 1, 2, and 3, respectively (Supplementary Table 1).

As a group, the entire cohort of histological special types profiled here displayed an overall pattern of copy

number aberrations (Supplementary Table 2) similar to that previously reported for IC-NSTs [23, 26, 38]. Using the definition of Hicks et al. [39], 20 cases (33.9 %) showed a complex firestorm genomic architectural pattern, 16 (27.1 %) a complex sawtooth, and 23 a simplex pattern (39 %; Table 1; Supplementary Table 1).

The analysis of each histological special type separately revealed that while the proportion of the genome with copy number changes varied widely among the entire cohort (0.8–76.9 %), samples of the same histological special type tended to display similar levels of gene copy number changes. Adenoid cystic (4.4 ± 5.6 %), mucinous (8.2 ± 19.6 %), and carcinomas with neuroendocrine features (10.7 ± 10.4 %) showed the lowest levels of gene copy number aberrations, whereas ILCs (22.7 ± 9.2 %), carcinomas with osteoclast-like stromal giant cells (25.5 ± 4.2 %), carcinomas with apocrine differentiation (30.0 ± 18.4 %), invasive micropapillary (32.3 ± 18.3 %), medullary (43.9 ± 8.4 %), and metaplastic carcinomas (48.7 ± 19.1 %) showed higher proportions of aberrations (Fig. 1a). Of note, a correlation between histological grade and levels of genetic instability was observed, given that special types typically of low histological grade, such as adenoid cystic, tubular, and mucinous carcinomas displayed low levels of genetic instability, whereas, metaplastic and medullary carcinomas that are typically of high histological grade displayed the highest levels of genetic instability (grade I: 8.8 ± 12.1 %; grade II: 18.0 ± 18.8 %; grade III: 40.1 ± 14.9 %; heteroscedastic *t* test, grade I vs II, $p = 0.0192$, grade I vs III, $p < 0.001$, grade II vs III $p = 0.003$; Fig. 1a). Consistent with these observations, a correlation between histological special types and genomic architectural patterns was found. The majority of adenoid cystic, mucinous, tubular, and carcinomas with neuroendocrine features analyzed here displayed genomic profiles of simplex architecture, whereas invasive micropapillary, carcinomas with medullary features, and metaplastic carcinomas displayed complex (either firestorm or sawtooth) profiles (Table 1). Analysis of the genome-wide patterns of

Table 1 Genomic architectural pattern of 59 histological special type breast cancers

| Histological special type | Firestorm <i>n</i> (%) | Sawtooth <i>n</i> (%) | Simplex <i>n</i> (%) |
|--|---------------------------|--------------------------|-------------------------|
| Adenoid cystic carcinoma (<i>n</i> = 4) | 0 (0 %) | 0 (%) | 4 (100 %) |
| Carcinoma with apocrine differentiation (<i>n</i> = 4) | 2 (50 %) | 0 (0 %) | 2 (50 %) |
| Carcinoma with osteoclast-like stromal giant cells (<i>n</i> = 4) | 1 (25 %) | 1 (25 %) | 2 (50 %) |
| Invasive lobular carcinoma (<i>n</i> = 7) | 3 (42.9 %) | 1 (14.3 %) | 3 (42.9 %) |
| Carcinoma with medullary features (<i>n</i> = 10) | 4 (40 %) | 6 (60 %) | 0 (0 %) |
| Metaplastic carcinoma (<i>n</i> = 11) | 5 (45.5 %) | 5 (45.5 %) | 1 (9.1 %) |
| Invasive micropapillary carcinoma (<i>n</i> = 6) | 4 (66.7 %) | 2 (33.3 %) | 0 (0 %) |
| Mucinous carcinoma (<i>n</i> = 9) | 1 (11.1 %) | 1 (11.1 %) | 7 (77.8 %) |
| Carcinoma with neuroendocrine features (<i>n</i> = 3) | 0 (0 %) | 0 (0 %) | 3 (100 %) |
| Tubular carcinoma (<i>n</i> = 1) | 0 (0 %) | 0 (0 %) | 1 (100 %) |

According to the definition by Hicks et al. [39]

amplification/high level gains revealed that 1q (15 %) and 8q (22 %) amplifications/high level gains were relatively frequent across the entire cohort (Fig. 1).

In agreement with our previous observations [8], unsupervised hierarchical clustering using the gene expression data, the 59 special type tumors clustered mainly according to ER-status, and samples of the same histological type tended to cluster together in the same branches (Supplementary Fig. 1). By contrast, unsupervised hierarchical clustering using DNA copy number states (i.e., gains, amplifications, losses) revealed two major clusters (Fig. 2). Cluster 1 was significantly enriched for low histological grade special type tumors (Fisher's exact test two-tailed, $p < 0.0001$) and simplex genomic architectural patterns ($p < 0.0001$), including mucinous and adenoid cystic carcinomas. Cluster 2 was significantly enriched for high-grade special type tumors with a complex firestorm or sawtooth pattern (Fig. 2). Within cluster 2, two secondary clusters were identified: one enriched for ER-positive

tumors (Fisher's exact test two-tailed, $p < 0.0001$), including invasive micropapillary carcinomas, and the other composed of mainly ER-negative tumors including medullary and metaplastic carcinomas. Although samples from each of the histological special types preferentially clustered together, the overall pattern of low level gains and losses varied notably by subtype. Taken together, these observations suggest that different histological types of breast cancer have distinct levels and/or types of gene copy number aberrations. Our findings demonstrate that although the patterns of gene copy number aberrations segregate with ER-status and histological grade, they are also associated with the histological type of breast cancers. Finally, we also confirm previous observations that, at variance with IC-NSTs of ER-negative and basal-like subtype [40], adenoid cystic carcinomas, which are of ER-negative and basal-like subtype but have an indolent clinical behavior, have low levels of copy number aberrations [16] and cluster together with ER-positive breast cancer special types.

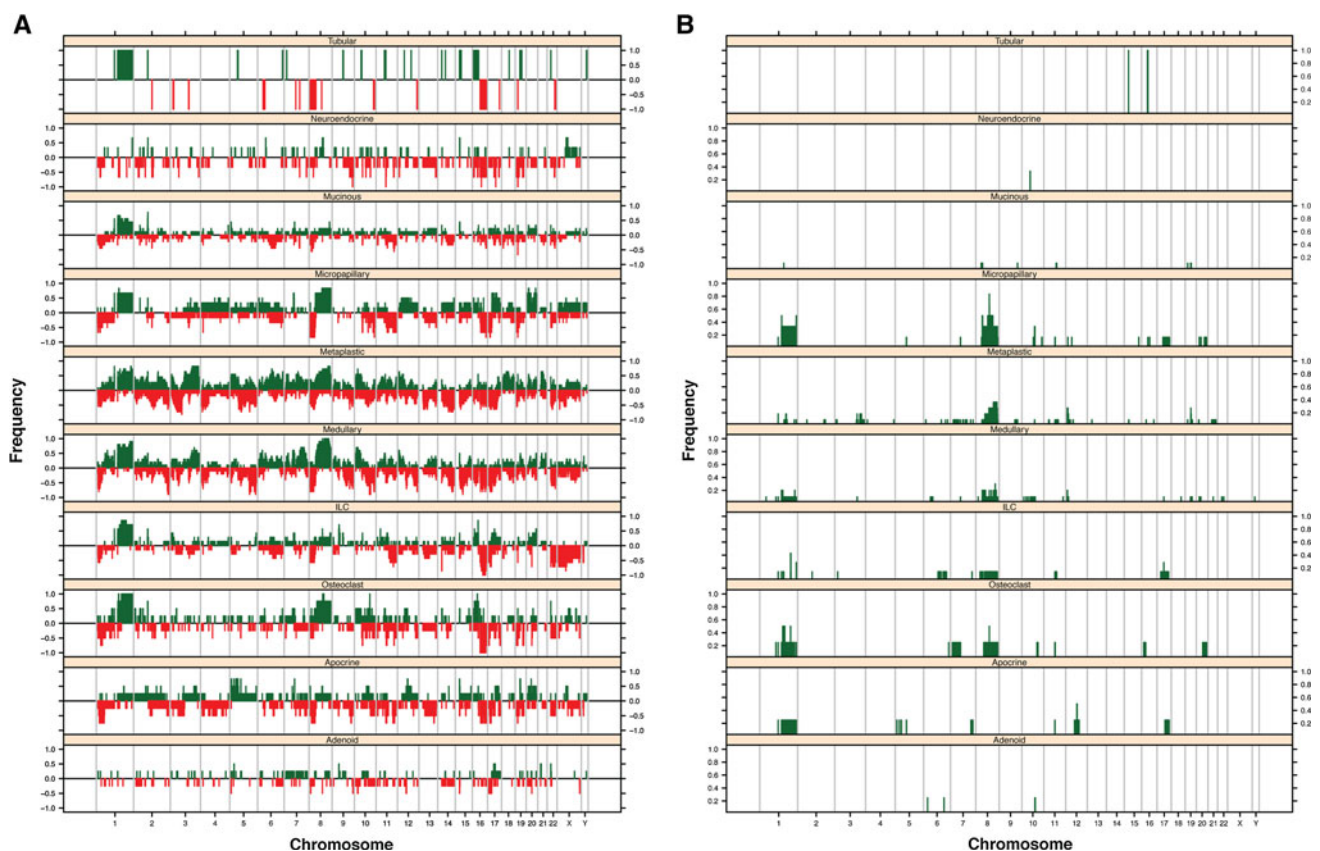
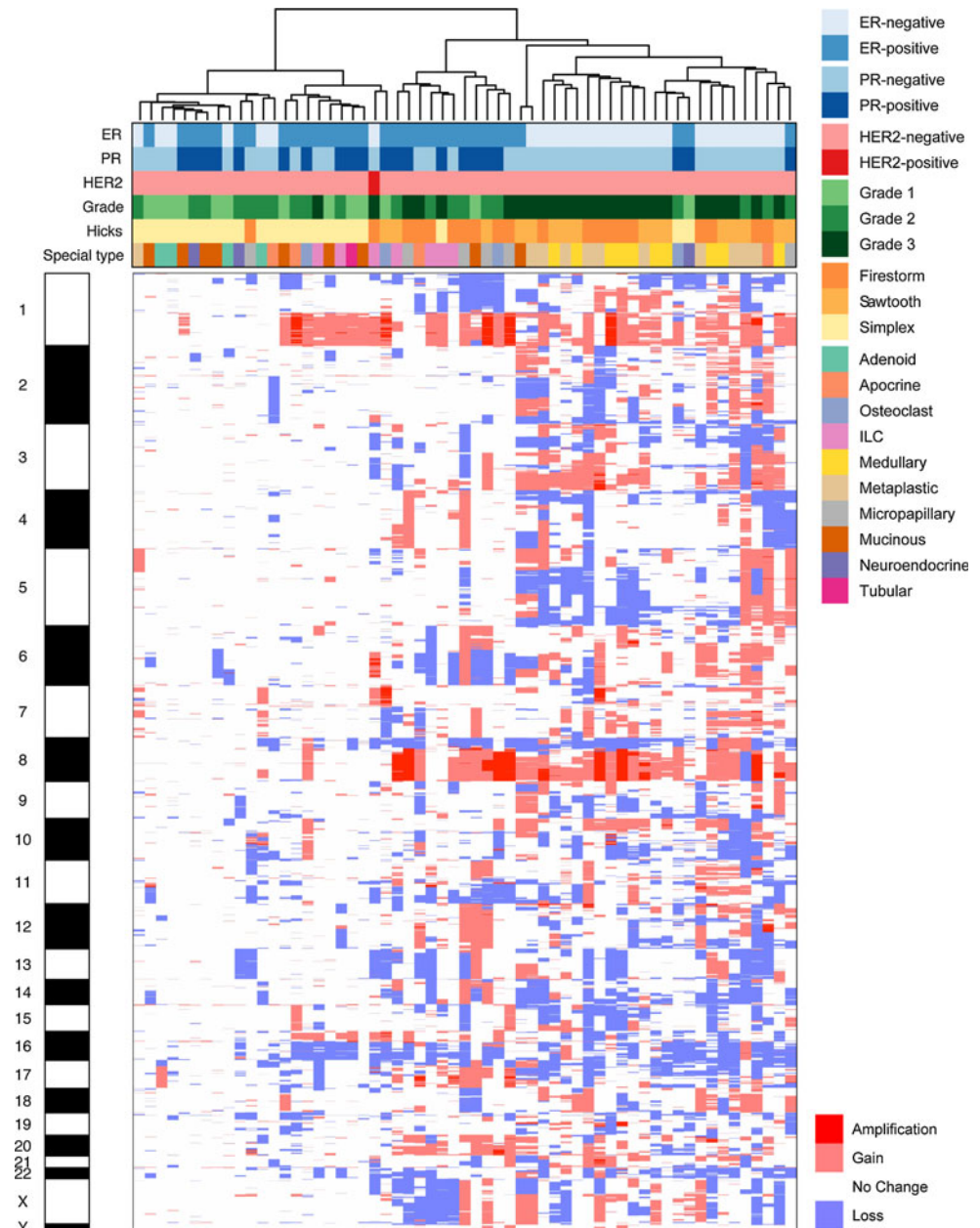


Fig. 1 Gene copy number aberrations in histological special types of breast cancer. **a** Frequency plots of copy number gains and losses or **b** amplifications in tubular carcinoma ($n = 1$), carcinoma with neuroendocrine features ($n = 3$), mucinous carcinoma ($n = 9$), invasive micropapillary carcinoma ($n = 6$), metaplastic carcinoma ($n = 11$), carcinoma with medullary features ($n = 10$), ILCs ($n = 7$), carcinoma with osteoclast-like stromal giant cells ($n = 4$), carcinoma

with apocrine differentiation ($n = 4$) and adenoid cystic breast carcinomas ($n = 4$). The proportion of tumors in which each bacterial artificial chromosome (BAC) clone is gained (green bars) or lost (red bars) (**a**) or amplified (green bars) (**b**) is plotted (Y axis) for each BAC clone according to its genomic position (X axis). ILC invasive lobular carcinoma, osteoclast carcinoma with osteoclast-like stromal giant cells

Fig. 2 Hierarchical clustering of the genomic profiles of histological special type tumors. Hierarchical cluster analysis performed with microarray genomic hybridization (aCGH) categorical states (i.e. gains, losses, and amplifications) using Euclidean distance metric and the Wards algorithm. Special type breast cancers of low histological grade with a simplex genomic architectural pattern cluster distinct from high-grade tumors with a firestorm or sawtooth genomic architectural pattern as defined by Hicks et al. [39]. *ER* estrogen receptor, *ILC* invasive lobular carcinoma, *osteoclast* carcinoma with osteoclast-like stromal giant cells, *PR* progesterone receptor



Comparison of genomic profiles between histological special types and grade- and ER-matched IC-NSTs

Metaplastic and medullary breast carcinomas are consistently of triple-negative phenotype (i.e., ER-, PR-, HER2-negative) and associated with distinct prognosis [2, 3]. In the comparison of the genomic profiles of metaplastic carcinomas with those of carcinomas with medullary features (Fig. 3a), metaplastic breast cancers with those of grade- and ER-matched IC-NSTs (Fig. 3b) and medullary carcinomas and those of grade- and ER-matched IC-NSTs (Fig. 3c) (Supplementary Table 3), no significant differences were observed other than the regions affecting

8q24.12–q24.3 and 3p13 and 3q11.2, 16q12.1 and Xp22.2, respectively. It should be noted, however, that these loci map to regions of frequent germline copy number polymorphisms (<http://projects.tcag.ca>), and should be interpreted with caution. These results indicate that metaplastic, medullary, and IC-NST matched according to grade and ER-status display similar patterns of gene copy number aberrations.

We next focused on preferentially ER-positive histological subtypes. The comparison between the genomic profiles of invasive micropapillary carcinomas and histological grade- and ER-matched IC-NSTs revealed that both had similar levels of gene copy number aberrations (Fig. 4a;

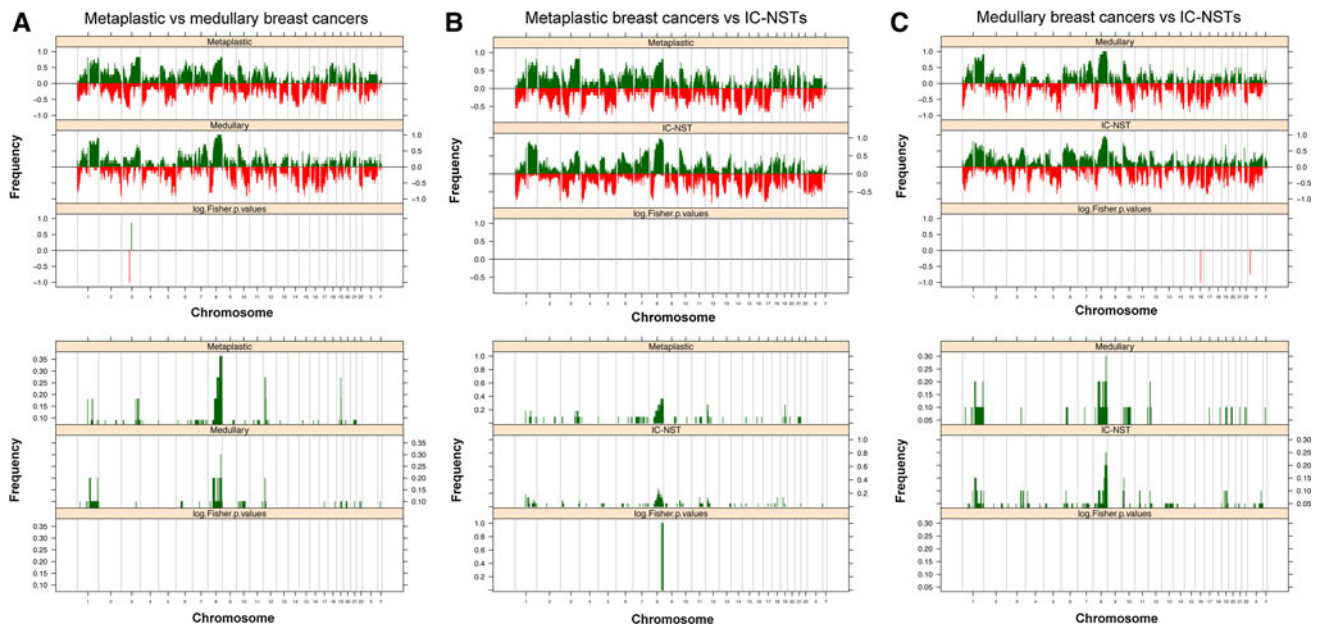


Fig. 3 Metaplastic breast carcinomas, carcinomas with medullary features and histological grade- and ER-matched IC-NSTs have similar patterns of gene copy number aberrations. Frequency plots of copy number gains and losses (*top*) or amplifications (*bottom*) in **a** medullary and metaplastic breast cancers, **b** metaplastic breast cancers and matched IC-NSTs, and **c** medullary breast cancers and matched IC-NSTs. The proportion of tumors in which each bacterial

artificial chromosome (BAC) clone is gained/amplified (*green bars*) or lost (*red bars*) is plotted (*Y axis*) for each BAC clone according to its genomic position (*X axis*). Inverse log 10 values of the Fisher's exact *p* values are plotted according to genomic location (*X axis*) at the *bottom of each graph*. Only few significant differences were identified between the genomic profiles of these three histological types of breast cancer. *IC-NST* invasive carcinoma of no special type

32.3 ± 18.3 %, micropapillary vs 35.5 ± 16.7 %, IC-NSTs), and only focal regions of the genome were differentially altered (8q21.2, 19p13.2; Fig. 4a, Supplementary Table 3). When compared to histological grade- and ER-matched IC-NSTs, ILCs harbored similar levels of copy number changes (22.7 ± 9.2 %, ILCs vs 27.7 ± 17.0 %, IC-NSTs). Fisher's exact test analysis revealed that ILCs displayed higher frequency of gains and losses in focal regions than IC-NSTs of the same grade and ER-status, including 1p32.3, 5p14.3, and Xp21.1–21.3 [17, 19] (Fig. 4b, Supplementary Table 3). In agreement with our previous findings [20], mucinous carcinomas displayed fewer copy number changes than grade- and ER-matched IC-NSTs (8.2 ± 19.6 %, mucinous vs 21.8 ± 15.9 %, IC-NSTs). Notably, mucinous carcinomas significantly less frequently displayed gains of 16p and losses of 16q than grade- and ER-matched IC-NSTs (Fig. 4c, Supplementary Table 3) [41]. In fact, only one of the mucinous carcinomas harbored gain of 1q and loss of 16q (11 %), which were present in 63 % of grade- and ER-matched IC-NSTs analyzed in this study (Fisher's exact test, $p < 0.05$).

Genes whose expression is associated with gene copy number

By overlaying the aCGH and gene expression data of the 59 histological special type tumors, we identified 4,536

transcripts (3,797 unique gene symbols) whose mRNA expression levels significantly correlated with copy number (Pearson's correlation adjusted $p < 0.05$, Supplementary Table 4). Of these, 2,352 and 600 unique gene symbols have been found previously to have mRNA expression levels significantly correlated with gene copy numbers in 48 grade 3 IC-NSTs [36] and 101 primary breast cancers [42], respectively, including *PPM1D*, *STARD3*, *ERBB2*, *GRB7*, *FAD*, *CTTN*, *CCND1*, and *NME1*. In this study, we restricted these integrative analyses to individual histological special types with ≥ 5 cases. ILCs harbored three genes whose mRNA expression levels significantly correlated with copy number, including the transcription factor *SPI* (Supplementary Table 5). Mucinous carcinomas displayed one gene, the transcriptional repressor *BCL6*, and medullary carcinomas 40 genes whose expression significantly correlated with copy number (Supplementary Table 5). The latter included the member *RAS* oncogene family *RAB7A*, the histone demethylase *KDM5A*, and the ubiquitin protein ligase *UBE3C*. In the other two histological types with ≥ 5 cases, the metaplastic and invasive micropapillary breast carcinomas, no genes, whose expression is driven by gene copy number, were found after correction for multiple testing.

Next, we sought to identify potential amplicon drivers and interrogated the genes significantly overexpressed when amplified. The analysis of all 59 special type cancers

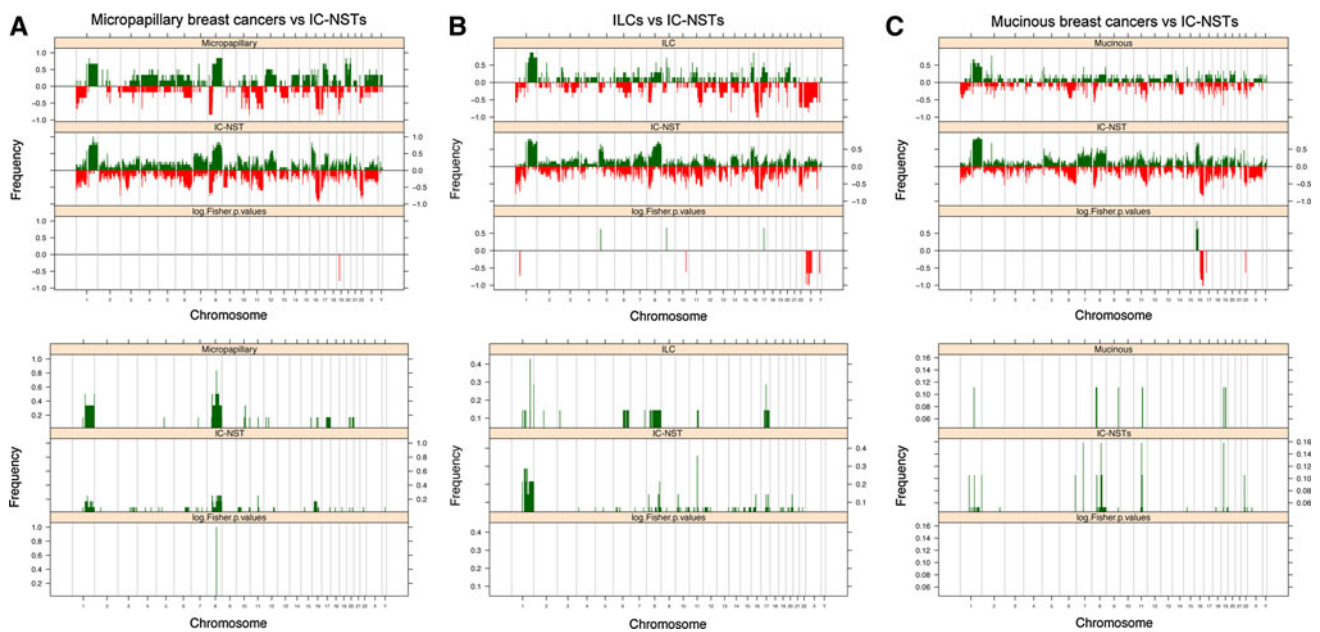


Fig. 4 Comparison of genomic profiles of ER-positive histological special types and histological grade- and ER-matched IC-NSTs. Frequency plots of copy number gains and losses (*top*) or amplifications (*bottom*) in **a** invasive micropapillary breast carcinomas, **b** ILCs, and **c** mucinous breast carcinomas, each compared to matched IC-NSTs. The proportion of tumors in which each bacterial artificial chromosome (BAC) clone is gained/amplified (*green bars*), or lost (*red bars*) is plotted (*Y axis*) for each BAC clone according to

its genomic position (*X axis*). Inverse log 10 values of the Fisher's exact *p* values are plotted according to genomic location (*X axis*) at the bottom of each graph. Several significant differences were identified between the genomic profiles of these three ER-positive histological special type breast cancers and matched IC-NSTs. *IC-NST* invasive carcinoma of no special type, *ILC* invasive lobular carcinoma

revealed 115 unique genes (145 transcripts; Mann–Whitney *U* adjusted *p* < 0.05) with significant overexpression–amplification correlation (Supplementary Table 6). This approach identified known amplicon drivers including *ERBB2*, *STARD3*, and *GRB7* on 17q12 (Fig. 5a), genes whose expression has been shown to be required for the proliferation and/or survival of cancer cells harboring amplification of this locus [43, 44]. Genes identified to be significantly overexpressed when amplified included *FADD*, *CCND1*, *RAD21*, *STK3*, and *ADAM9* among others (Fig. 5b–d; Supplementary Table 6).

Analysis of each histological subtype with ≥ 5 cases revealed 23 transcripts (21 unique genes) significantly overexpressed when amplified in medullary breast cancers, and 30 transcripts (24 unique genes) in metaplastic carcinomas (Mann–Whitney *U* test *p* < 0.05; Fig. 6; Supplementary Table 7). Of note, in metaplastic breast cancers, the 12p13.33–13.32 amplicon contains several overexpressed when amplified genes, including *FOXMI*, *FGF6*, *RAD51AP1*, and *TULP3* (Fig. 6a). In addition, *HEY1*, mapping to 8q21.11–21.13, was found to be overexpressed when amplified in this triple-negative breast cancer special type (Fig. 6b). Due to the small sample size, when corrected for multiple testing, however, these genes failed to reach statistical significance (Supplementary Table 7). Likewise, in invasive micropapillary carcinomas, mucinous

carcinomas, and ILCs, the other histological types with ≥ 5 cases, no genes significantly overexpressed when amplified could be found (data not shown), probably due to the limited sample size.

Discussion

Here we demonstrated that together with histological grade and ER-status, histological type is also associated with the patterns, type, and complexity of gene copy number aberrations in breast cancer. In fact, unsupervised analysis of the ten histological special types of breast cancer based on the categorical aCGH states revealed that in general samples from each of the histological special types of breast cancer preferentially segregated to one dendrogram branch (e.g., all adenoid cystic carcinomas were allocated to the same cluster, all carcinomas with medullary features mapped to the same cluster, all but one micropapillary carcinoma were also allocated to the same cluster; Fig. 2). It should be noted, however, that the correlations between gene copy number aberrations and ER-status and histological grade in histological special types of breast cancer were not as strong as those observed between the gene expression profiles and ER-status and histological grade of these cancers (Supplementary Fig. 1) [8]. In fact, the

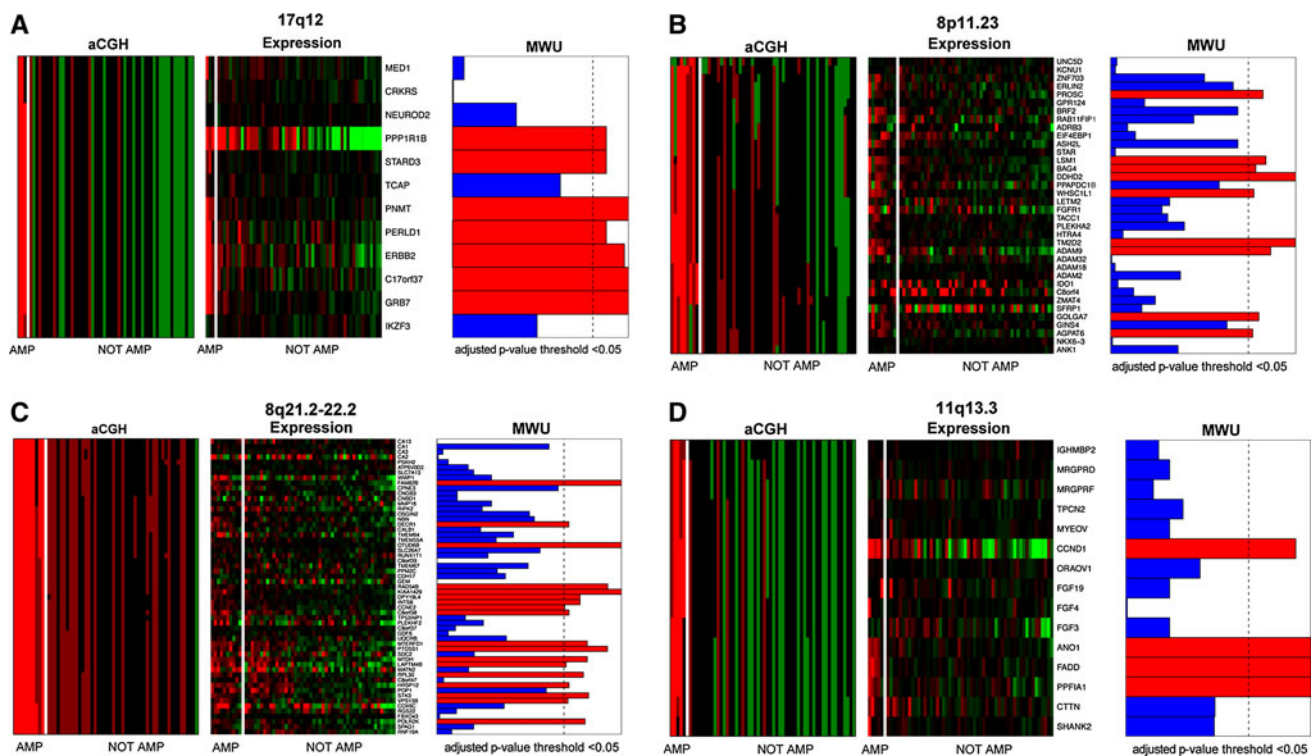


Fig. 5 Matched heatmaps of gene expression and gene copy number aberrations of genes significantly overexpressed when amplified in histological special types of breast cancer. Amplifications on **a** 17q12, **b** 8p11.23, **c** 8q21.2–22.2, and **d** 11q13.3 are shown. Expression and aCGH values are depicted in two matching heatmaps, aCGH states on the *left* and expression values on the *right*, in which the genes are ordered according to their chromosomal position. *Bar plots* on the

right show the results of a Mann–Whitney U test for expression as a continuous variable and gene amplification as the grouping variable. *Bars in red* show adjusted p values < 0.05 . aCGH: *green* copy number loss, *black* no copy number change, *dark red* copy number gain, *bright red* amplification. Gene expression: *green* downregulation, *red* upregulation. aCGH array-based comparative genomic hybridization, AMP amplified, MWU Mann–Whitney U test

aCGH analysis performed here revealed a substantial degree of intertumor genetic heterogeneity within the subgroups of ER-positive and ER-negative disease, and in particular, metaplastic breast carcinomas appear to comprise a diverse group of tumors in terms of their repertoire of gene copy number alterations.

This study provides molecular evidence to demonstrate that the information offered by histological typing should not be disregarded [2, 3]. Our findings corroborate and further expand on those of previous studies [12, 16, 17, 20, 21, 34, 45] by demonstrating that the constellation of gene copy number aberrations found in ER-positive breast cancers appear to differ according to histological subtype, given that all but one mucinous carcinomas and carcinomas with neuroendocrine features lacked the hallmark chromosomal aberrations reported in grade 1 and 2 ER-positive IC-NSTs and ILCs (i.e., gains of 1q and losses of 16q) [41], at variance with IC-NSTs of similar histological grade and ER-status. These observations are consistent with the recent findings that mucinous carcinomas of the breast also lack *PIK3CA* mutations [46], which are found in approximately 40 % of IC-NSTs [23]. Moreover, our findings demonstrate that some of the molecular heterogeneity documented in

ER-negative/HER2-negative breast cancers stems from the differences in the repertoire of genetic aberrations according to histological type. While medullary and all but one metaplastic carcinoma displayed complex genomes, adenoid cystic carcinomas, which have an indolent clinical behavior and have been shown to be driven by a t(6;9) *MYB–NFIB* fusion gene [15, 16], consistently displayed rather simple patterns of gene copy number aberrations. Without histological subtyping, these tumors would be classified as of ER-negative/HER2-negative subtype, despite the important differences in the repertoire of molecular aberrations and clinical behavior. We were unable, however, to validate the reported higher prevalence of gains of 10p, 9p and 16q, loss of 4p, and amplifications of 1q, 8p, 10p, and 12p in medullary carcinomas than in basal-like IC-NSTs [45]. In fact, in the present study, no significant differences in the prevalence of gene copy number aberrations were detected between medullary carcinomas and grade- and ER-matched IC-NSTs or metaplastic breast carcinomas. In agreement with the results of previous studies [12, 47], here we demonstrate that metaplastic breast cancers display a complex pattern of gene copy number aberrations, however, at variance with the results by Hennessy et al. [12], our study

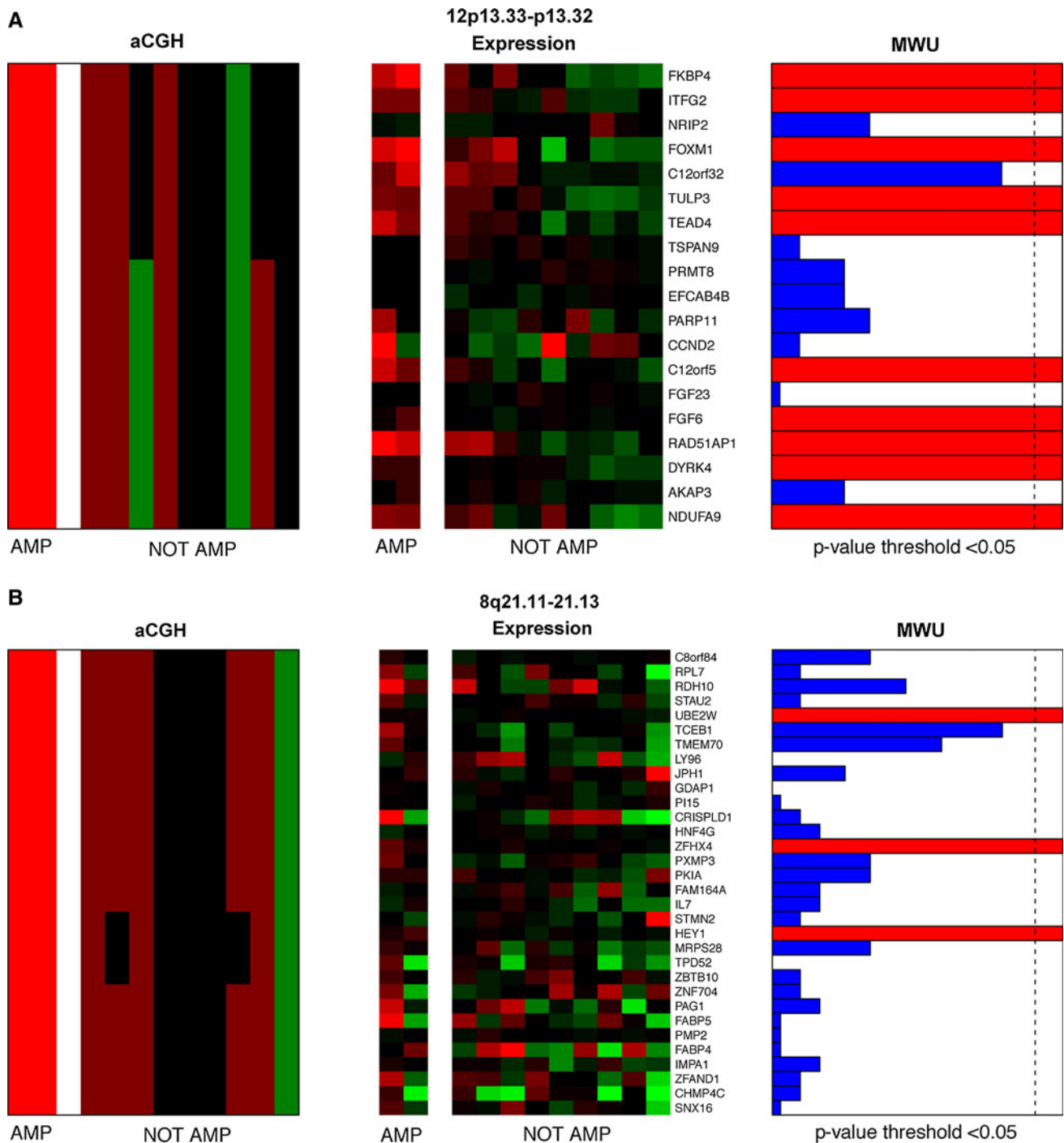


Fig. 6 Matched heatmaps of gene expression and gene copy number aberrations within regions of recurrent amplification in metaplastic breast cancers. Amplifications on **a** 12p13.33–12p13.32 and **b** 8q21.11–q21.13 are shown. For each amplicon, genes within the amplified region are recovered, and median aCGH values and states are assigned. Expression and aCGH values are depicted in two matching heatmaps, aCGH states on the *left* and expression values on the *right*, in which the genes are ordered according to their

chromosomal position. *Bar plots* on the right show the results of a Mann–Whitney *U* test for expression as a continuous variable and gene amplification as the grouping variable. *Bars in red* show *p* values <0.05. aCGH: *green* copy number loss, *black* no copy number change, *dark red* copy number gain, *bright red* amplification. Gene expression: *green* downregulation, *red* upregulation. aCGH array-based comparative genomic hybridization, AMP amplified, MWU Mann–Whitney *U* test

did not reveal significant differences in the prevalence of specific gene copy number aberrations between metaplastic breast cancers and grade- and ER-matched IC-NSTs. In fact,

our findings suggest that metaplastic breast carcinomas constitute a heterogeneous group of tumors in terms of their gene copy number aberrations and transcriptomic profiles.

Invasive breast carcinomas with osteoclast-like stromal giant cells are considered to be a variant of IC-NSTs according to the WHO classification [1]. These cancers are defined by the presence of osteoclast-like giant cells in an often hypervascular stroma; their carcinomatous component is most frequently a grade 1 or grade 2 ER-positive IC-NST. In the present study, all cases of invasive breast carcinomas with osteoclast-like stromal giant cells were preferentially of histological grade 2 (i.e., three out of four cases), ER-positive and HER2-negative. Here we provide the first genomic characterization of invasive breast carcinomas with osteoclast-like stromal giant cells and demonstrate that the constellation of gene copy number aberrations found in these cancers was consistent with that expected in grade 2 ER-positive IC-NSTs, including gains of 1q and losses of 16q, gains of 8q, and 11q13 amplifications coupled with 11q deletions. Therefore, our findings provide a molecular basis for the classification of invasive breast carcinomas with osteoclast-like giant stromal cells as variants of IC-NSTs.

Our integrative aCGH and gene expression analysis led to the identification of 4,536 transcripts whose expression correlates with copy number and 145 transcripts that are significantly overexpressed when amplified in special type breast cancers, which recapitulated those found in IC-NSTs [36, 42]. Although no gene was shown to be significantly overexpressed when amplified in each special histological type of breast cancer after correction for multiple testing, some observations are noteworthy. The recurrent amplification of 12p13.33–13.32 found in two metaplastic carcinomas (Fig. 6a) resulted in overexpression of the transcriptional activator *FOXM1*, which is involved in proliferation, cancer initiation, and progression [48], *FGF6*, which has high homology with *FGF4* and is overexpressed in prostate carcinomas [49], *RAD51API*, which has been shown to play a role in cell proliferation and DNA repair [50], and the *TULP3* gene, which is a critical repressor of the sonic hedgehog pathway and may be involved in tissue differentiation and morphogenesis [51]. *FOXM1*, *RAD51API*, and *TULP3* were found to be amplified in 1.45 % of 482 invasive breast cancers included in The Cancer Genome Atlas and to be overexpressed when amplified [23, 52] (Supplementary Table 7). Furthermore, amplification of 8q21.11–q21.13 in two metaplastic carcinomas (Fig. 6b) resulted in overexpression of *HEY1*, *ZFHX4*, and *UBE2W*; although limited information is available about potential roles of the latter two genes in breast cancer, *HEY1* is downstream effector of Notch signaling and a partner of a recently identified fusion gene in mesenchymal chondrosarcoma (i.e., *HEY1-NCOA2*) [53]. Further studies investigating the potential roles of the overexpressed when amplified genes in metaplastic breast cancers are warranted.

This study has important limitations. First, given the rarity of some of the histological special types of breast

cancer and the requirement of fresh/frozen tissue for gene expression profiling, the sample sizes for each special type were limited. Hence, the statistical power of the analyses performed is limited, and this study should be interpreted as exploratory and hypothesis-generating. Second, previous studies [7, 8, 10] have demonstrated that some of the transcriptomic differences between histological special types of breast cancer are related to the characteristics of their stroma. As we have included samples with up to 50 % of stromal cells in the gene expression analysis, it is possible that the integrative analysis of aCGH and gene expression data may not have identified all copy number regulated genes, given the contribution of stromal cells.

In conclusion, here we demonstrate that in a way akin to IC-NSTs, the patterns of gene copy number changes in histological special types of breast cancer are significantly associated with ER-status. Importantly, however, the patterns of genomic changes found in ER-positive and ER-negative breast cancers correlate with histological type (e.g., adenoid cystic and mucinous carcinomas have patterns of genomic aberrations distinct from those of other grade- and ER-matched breast cancers, respectively). Furthermore, our findings also demonstrate that ER-positive breast cancers are characterized by varying levels of genetic instability, whereas the majority of ER-negative cancers display high levels of genetic instability and rather complex genomic profiles, with the exception of adenoid cystic carcinomas. Given that (i) mucinous and neuroendocrine carcinomas, albeit of grades 1 and 2 and ER-positive, lack deletions of 16q and gains of 1q, the hallmark gene copy number aberrations of low-grade ER-positive IC-NSTs, (ii) adenoid cystic carcinomas, contrary to IC-NSTs of triple-negative phenotype, have remarkably simple genomes and an indolent clinical course, and (iii) as a group, tumors from each of the histological special types display less molecular heterogeneity than IC-NSTs [2, 3, 8], our results highlight the importance of histological subtyping in studies aiming to characterize the genetic underpinning and the molecular drivers of breast cancer.

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Conflict of interest The authors have no conflicts of interest to declare.

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