Immunohistochemical and molecular profiling of histologically defined apocrine carcinomas of the breast

UNIVERSITÀ DEGLI STUDI DI TORINO

Immunohistochemical and molecular profiling of histologically defined apocrine carcinomas of the breast

Semir Vranic, MD, PhD[1,2], Caterina Marchiò, MD, PhD[1], Isabella Castellano, MD[1], Cristina Botta, BSc[1], Maria Stella Scalzo, BSc[1], Ryan P. Bender, PhD[3], Cesar Payan-Gomez, MD, PhD[4, 5], Ludovica Verdun di Cantogno, BSc[1], Patrizia Gugliotta, MSc[1], Fabrizio Tondat, PhD[6], Paola Francia di Celle, PhD[6], Sara Mariani, MSc[6], Zoran Gatalica, MD, DSc[3], Anna Sapino, MD[1]

a Department of Medical Sciences, University of Turin, Turin 10126, Italy

b Department of Pathology, Clinical Center, University of Sarajevo, Sarajevo 71000, Bosnia and Herzegovina

c Caris Life Sciences, Phoenix, AZ 85040, USA

d Medical Genetics Center, Department of Cell Biology and Genetics, Center of Biomedical Genetics, Erasmus MC, Rotterdam 3015, the Netherlands

e Facultad de Ciencias Naturales y Matemáticas, Universidad del Rosario, Bogotá 110010, Colombia

f Center for Experimental Research and Medical Studies, San Giovanni Battista Hospital, Turin 10100, Italy

Summary

Despite the marked improvement in the understanding of molecular mechanisms and classification of apocrine carcinoma, little is known about its specific molecular genetic alterations and potentially targetable biomarkers. In this study, we explored immunohistochemical and molecular genetic characteristics of 37 invasive apocrine carcinomas using immunohistochemistry (IHC), fluorescent in situ hybridization (FISH), multiplex ligation-dependent probe amplification (MLPA), and next-generation sequencing (NGS) assays. IHC revealed frequent E-cadherin expression (89%), moderate (16%) proliferation activity [Ki-67, phosphohistone H3], infrequent (~10%) expression of basal cell markers [CK5/6, CK14, p63, caveolin-1], loss of PTEN (83%), and overexpression of HER2 (32%), EGFR (41%), cyclin D1 (50%), and MUC-1 (88%). MLPA assay revealed gene copy gains of MYC, CCND1, ZNF703, CDH1, and TRAF4 in 50% or greater of the apocrine carcinomas, whereas gene copy losses frequently affected BRCA2 (75%), ADAM9 (54%), and BRCA1 (46%). HER2 gain, detected by MLPA in 38% of the cases, was in excellent concordance with HER2 results obtained by IHC/FISH ($\kappa = 0.915$, $P < .001$). TOP2A gain was observed in one case, while five cases (21%) exhibited TOP2A loss. Unsupervised hierarchical cluster analysis revealed two distinct clusters: HER2-positive and HER2-negative ($P = .03$ and .04, respectively). NGS assay revealed mutations of the TP53 (2 of 7, 29%), BRAF/KRAS (2 of 7, 29%), and PI3KCA/PTEN genes (7 of 7, 100%). We conclude that morphologically defined apocrine carcinomas exhibit complex molecular genetic alterations that are consistent with the “luminal-complex” phenotype. Some of the identified molecular targets are promising biomarkers; however, functional studies are needed to prove these observations.

Keywords

Breast carcinoma–apocrine carcinoma; Androgen receptor; Immunohistochemistry; Fluorescent in situ hybridization; Multiplex ligation-dependent probe amplification; Next-generation sequencing

1. Introduction

Apocrine carcinoma of the breast is a rare, special histologic subtype of breast carcinoma constituting approximately 1% of all mammary carcinomas [1]. In routine work, they are diagnosed by pathologists using standard hematoxylin and eosin (H&E) staining because of the peculiar cytological features: cells with abundant eosinophilic and granular cytoplasm, large nuclei with prominent nucleoli, and visible cell membrane [1]. These carcinomas are characterized by distinct immunohistochemical (IHC) steroid receptor profile (estrogen receptor [ER]–negative, progesterone receptor [PR]–negative, and androgen receptor [AR]–positive) associated with a frequent overexpression of HER2 [1]. However, although ER, PR, and HER2 are mandatory prognostic/predictive factors and thus are performed in all tumors, the AR is not yet a marker required by the oncologists in routine practice, and the definition of an apocrine tumor is generally confined to the H&E cytological aspect together with the ER negativity.

On the other hand, many works have focused on the "molecular apocrine (MA) subtype" [2, 3, 4, 5, 6, 7] and [8]. Recently, Lehmann-Che et al [8] have described the morphological and immunohistochemical features of a series of MA-defined tumors. From the histological point of view, the retrospective analysis described them all as invasive ductal carcinomas, but only 7% presented with morphological apocrine features. From the IHC point of view, the signature "HER2 score 3+ or the expression of the 15-kDa gross cystic disease glycoprotein (GCDFP-15^{+}) had a sensitivity and a specificity for MA tumors of 94% and 100%, respectively [8].

The frequency and the significance of apocrine differentiation in carcinomas of the breast are uncertain because of the lack of reliable and reproducible criteria for morphological diagnosis [1]. Despite the marked improvement in the understanding of molecular mechanisms and classification of breast cancer, little is known about specific molecular genetic alterations and potentially targetable biomarkers in apocrine carcinomas [1], [9], [10] and [11].

In this study, we performed an extensive IHC and molecular profiling analysis on a subset of invasive apocrine carcinomas of the breast as defined by the standard H&E histopathological criteria to evaluate the correspondance with the specific immunophenotype and to identify the most common molecular genetic alterations.

2. Materials and methods

2.1. Patients and tumor samples

Consecutive cases of breast cancers with a diagnosis of invasive apocrine carcinoma were retrieved from the pathology file of the Azienda Ospedaliera Città della Salute e della Scienza of Turin, Italy. Clinicopathologic characteristics of the cohort were collected from the pathology database and are summarized in Table 1. A local ethical committee approved the study. Informed consents were available and signed by all patients whose tumor samples were sequenced; as at our institution, written informed consent was obtained from patients to use both residual fresh neoplastic and archival tissues [12]. All cases were reviewed, and the apocrine cytomorphology [1] was confirmed (S.V., Z.G., and A.S.). Forty-five formalin-fixed paraffin-embedded (FFPE) blocks were retrieved from the archive, of which 37 samples fulfilled the criteria for apocrine differentiation and had enough tissue for immunohistochemical and molecular profiling.

2.2. Tissue microarray construction

A tissue microarray (TMA) was constructed from the FFPE tissue blocks using the Galileo TMA CK 3500 (Integrated Systems Engineering Srl, Milan, Italy). Briefly, 3 replicate cores (1 mm in diameter) of primary apocrine carcinomas and of 2 normal breast tissue samples were included. A pathologist selected representative H&E areas.

2.3. Immunohistochemistry

The primary antibodies, manufacturers, clones, dilutions, antigen retrieval, and scoring system [13, 14, 15, 16, 17, 18, 19] and [20] details are outlined in Table 2. Immunohistochemistry (IHC) assays were performed using the ultraView Universal DAB Detection Kit and on the BenchMarck XT and BenchMarck ULTRA (Roche/Ventana, Oro Valley, AZ) instruments. Positive and negative controls for each marker were included in each immunohistochemical run.

2.4. Fluorescence in situ hybridization assay

Fluorescence in situ hybridization (FISH) assay was performed on borderline cases (score 2+) using the HER2/CEP17 probe (Vysis Inc, Downers Grove, Illinois, USA) as previously described [21]. The analysis was performed by selection and automated acquisition of representative areas using the Metafer Scanning System (Carl Zeiss MetaSystems GmbH, Baden-Wuttenberg, Germany) and AxioImager epifluorescence microscope. PathVysion V2 software was used to analyze the data, whereas selected cases were also counted manually. Cases were scored according to the recently updated American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines [22].

2.5. Multiplex ligation-dependent probe amplification assay

For multiplex ligation-dependent probe amplification (MLPA) analysis, cases had to fulfill the following criteria: (a) minimal/no necrosis; (b) minimal/absent in situ component; (c) high tumor cellularity (≥70%). One or two 4-μm-thick FFPE sections were dewaxed and microdissected manually to select invasive tumor areas. As a reference DNA, we used normal breast tissue. In each experiment, at least 3 reference samples were used to enable data normalization. Purified genomic DNA (50-200 ng) (Invitrogen PureLink Genomic DNA extraction kit) was used following manufacturer's instructions. Samples were tested with two probemixes: P004-C1 ERBB2 (22 genes on chromosome 17, ESR1, EGFR, and BRCA2) and P078-C1 Breast Tumour kit (22 genes located at 8 different chromosomes) (MRC-Holland, Amsterdam, Netherlands) (Supplementary Table 1 and Supplementary Table 2). MLPA reactions were performed on PTC-100 MJ Research thermal cycler (MJ Research BIORAD). Polymerase chain reaction products were separated on an ABI 3130 capillary sequencer (Applied Biosystems), and results were analyzed using the GeneMapper (Applied Biosystems) and Coffalyser (Version: v.130112.0327, MRC-Holland) software. Genes tested by multiple probes were reported as a mean value [23] (Supplementary Table 2). Values between 0.7 and 1.5 were considered normal, values less than 0.7 as loss, greater than 1.5 as gain, and greater than 2 as an amplification [23.]

2.6. Next-generation sequencing assay

Direct sequence analysis (Caris Life Sciences, Phoenix, Arizona) was performed on genomic DNA isolated from FFPE samples of 8 apocrine carcinomas (all ER−/PR−/AR+/GCDFP-15+) using the Illumina MiSeq platform (samples of the patients that signed informed consent). Specific regions of the genome were amplified using the Illumina TruSeq Amplicon Cancer Hotspot panel. All variants reported here are detected with >99% confidence based on the frequency of the mutation present and the amplicon coverage. The sequencing included 44 genes (Supplementary Table 1).

2.7. Statistical analysis

The $\chi 2$ test/Fisher exact tests were used for comparisons of the variables and groups. Spearman correlation rank was applied for the correlation between the variables. The κ statistics was used to compare the HER2 results obtained by IHC/FISH and MLPA. These analyses were carried out using the Statistical Package for the Social Sciences version 19.0 (SPSS, Chicago, IL, USA).

For MLPA results, unsupervised hierarchical cluster analysis (Euclidean distance, average linkage analysis) was performed using open-source R statistical software (http://www.r-project.org). To assess the uncertainty in hierarchical cluster analysis we used the pvclust package as is implemented in R. For each cluster in hierarchical clustering, P values were calculated via multiscale bootstrap resampling.

3. Results

3.1. Hormone receptor profile

Results of the immunohistochemical tests of morphologically diagnosed apocrine carcinoma are summarized in Table 3 and Fig. 1A-L. By using the steroid receptor profile, 27 cases (73%) were ER−/PR−/AR+ (Fig. 1B and C). Only one case was negative for the three markers (2.7%). Six cases (16%) were ER+/AR+, of these 1 case was also PR+. Three other cases (8%) were ER+/PR−/AR−. All but 1 case (96%) with the ER−/PR−/AR+ immunophenotype exhibited GCDFP-15 expression (Fig. 1D). In contrast, only 5 (55.6%) of 9 tested cases with diverse steroid receptor profile expressed GCDFP-15 (P = .012).

HER2 overexpression score 3+ and score 2+ (FISH amplified) was observed in 32.4% of cases (11/34) (Fig. 1F). Of these, 63.3% were ER−/PR−/AR+, and 75% were GCDFP-15+ as well. So, at the end, we could identify IHC-apocrine tumors (ER−/PR−/AR+ and GCDFP-15+ and/or HER2+) in 100% of cases, whereas the remaining cases were only H&E-apocrine tumors.

3.2. Comparison of IHC-apocrine and H&E-apocrine carcinoma immunoprofile

On a total of 35 cases evaluated for E-cadherin status, 2 cases of IHC-apocrine and 2 H&E-apocrine cases were negative (11.4%).

The mean Topo2α overexpression was 13% (range, 1%-50%), without significant differences (P = .68) between the two subgroups. Topo2α status correlated with Ki-67 (P = .005, rs = 0.481) and PPH3 expression (P = .038, rs = 0.362). The mean Ki-67 positivity was 16% (range, 1%-40%).

EGFR protein was overexpressed (score 2+/3+) exclusively in 14 cases (56%) of IHC-apocrine and none of H&E-apocrine cases (P = .004).

MUC-1 was overexpressed in 88.2% of cases with a moderate to strong intensity at the membrane and within the cytoplasm (score 2+/3+) (Fig. 1H). No significant differences were observed between the two subgroups (P = .65). A strong positive association was observed between HER2 and MUC-1 expression (P = .002), whereas a trend toward positive correlation was found with Topo2α expression (P = .086).

Regarding basal markers expression, CK14 was never expressed, p63 was rarely expressed, and CK5/6 was expressed only in 4 high-grade IHC-apocrine cases (P = .026) (Fig. 1I and J). No significant differences were observed between IHC-apocrine and H&E-apocrine tumors considering each of the three proliferation markers. Ki-67 showed a wide range (1%-40%; mean, 16%) of proliferative activity (Fig. 1K). Considering 14% as the cutoff point of proliferative activity, 70% of the H&E-apocrine cases showed high proliferation rate. PPH3, a marker of mitotic activity, correlated with Ki-67 expression (P = .003, rs = 0.471). No significant association was found between cyclin D1 expression and the other two proliferation markers.

Overexpression of p53 protein was observed in 11 of 33 cases, without significant difference between the two subgroups (P = .21).

In 29 (82.9%) of 35 PTEN, protein expression was completely lost (Fig. 1L) including the cases with confirmed PTEN gene mutation.

3.3. Comparison of HER2 immunohistochemical/FISH results and MLPA assay results

Of 26 tested cases, 24 had interpretable results (Fig. 2). A good concordance was obtained between the ERBB2 status by MLPA and the results by IHC and FISH in all but 1 case ($\kappa = 0.915$, $P < .001$). This case exhibited ERBB2 gain by MLPA (average 1.83) and a monosomic pattern (HER2 gene, 1.4; CEP17, 1.3) by FISH. Gain of the GRB7, a gene within the HER2 amplicon, was seen in all ERBB2-positive cases. Gain of other genes along the chromosome 17q region coexisted frequently (IKZF3 [77.8%], NEUROD2 and CDC6 [66.6%], MED1 [55.6%], and RARA [44.4%]). On the opposite, only 1 case with ERBB2 gain showed TOP2A gain (11.1%), whereas another case with ERBB2 gain exhibited TOP2A loss. TOP2A loss was also observed in 4 additional cases (total number of cases with TOP2 deletion = 5). A trend toward significant positive correlation was observed between TOP2A status and Topo2α protein expression (P = .073, rs = 0.399).

None of the tumors exhibited CDH1 gene (encoding E-cadherin) loss, whereas a gain of exon 1 was observed in 13 (54%) of 24 cases. Gain of both exon 1 and 9 was seen in 2 cases. Two cases negative for E-cadherin showed CDH1 gain as well.

One case (score 3+ by IHC) showed gain of EGFR gene (~4%) and 2 cases with EGFR loss had score 0 by IHC (P = .082). A loss of EGFR exon 2 was observed in 11 additional cases without loss in the 3 other exons (exons 8, 23, and 25). Three of these cases were further tested by next-generation sequencing (NGS) that revealed no EGFR gene mutations.

Unsupervised hierarchical cluster analysis revealed two distinct clusters: HER2-positive and HER2-negative (P = .03 and .04, respectively). In addition, four small subclusters (A1/A2, B1/B2), deriving from loss or gain of genes in both 17p and 17q chromosome regions, were identified within the 2 main clusters (Fig. 3).

Some of the most commonly altered genes (MYC, CCND1, ZNF703, BRCA1, BRCA2, and ADAM9) in tissue samples were distributed along all clusters and appeared to be a more general feature of the apocrine carcinomas (Fig. 2; Supplementary Fig. 1). Similarly, we also observed frequent alterations in some reference genes that were consistent along the clusters (CASP1, POR, TGIF, and SNCA).

3.4. NGS assay results

NGS assay was successful in 7 of 8 tested apocrine carcinomas. Mutations were found in 5 (11.4%) of 44 tested genes including PTEN (3/7), PIK3CA (2/7), TP53 (2/7), KRAS (1/7), and BRAF (1/7) (Table 4). Cases 3 and 5 harbored mutations of both PIK3CA/KRAS and PTEN/TP53, respectively, while case 7 had 2 PTEN gene mutations (at 16% for both). Case 6 harbored a frameshift PTEN gene mutation that is frequently seen in patients with Cowden syndrome. In addition, four cases (cases 1-4) that harbored other driving mutations exhibited low levels of PTEN and/or PIK3CA gene mutations (all present at <5%).

4. Discussion

In the present study we demonstrated that the cytomorphological appearance of apocrine differentiation in a breast tumor does not always correspond to a classical apocrine immunophenotype reported in the literature as specific for apocrine tumors [1] and [11]. In fact, only 73% of these carcinomas fulfill the immunohistochemical criteria of true apocrine breast tumors (ie, ER−/PR−/AR+/) [1]. However, when GCDFP-15 and HER2 were added, we increased the sensitivity to 100%. On the other hand, with the exception of basal markers such as EGFR and CK5/6 observed in a small subset of IHC-apocrine tumors, all the other markers were similarly expressed in both IHC-apocrine and H&E-apocrine carcinomas [previous studies that explored the expression of basal markers in apocrine carcinoma are reviewed in Supplementary Table 3].

In analogy to IHC, no significant differences in gene copy number changes were observed by MLPA between the two subgroups. Until recently, only the histological features were used by pathologists to diagnose an apocrine tumor. To define MA carcinomas, Lehmann-Che et al recently proposed a transcriptional and IHC approach. They suggested that HER2 and GCDFP-15 IHC expression is specific and sensitive enough to differentiate MA carcinomas from molecular basal-like carcinomas [8]. However, both HER2 and GCDFP-15 were not expressed in the totality of MA carcinomas [8]. Similarly, FOXA1 expression was present in most MA carcinomas, but it may not be considered a good marker to differentiate apocrine tumors from other ER-positive tumors, because it is a transcription factor related to steroid receptors [25].

In our cohort, we observed a common MUC-1 expression associated with HER2, which was either overexpressed or amplified in about 30% of the cases. MUC-1 has been frequently described in breast cancer including subsets of HER2+ and triple-negative (TN) cases [26] and [27]. MUC-1 expression has been linked to trastuzumab resistance; however, targeting of MUC-1 may down-regulate HER2 activation and overcome the resistance in breast cancer cells [28]. Thus, our study may suggest that testing MUC-1 by IHC in apocrine HER2+ carcinomas may be a valid option to better predict response to targeted therapy. Cluster analysis of the MLPA data revealed HER2 as a key discriminator along with GRB7 (HER2 amplicon) and other genes in the 17q12-21 region. Also, HER2+ cluster was associated with higher proliferation activity, which may indicate a more aggressive clinical behavior. In contrast, HER2− (triple negative) apocrine cluster was associated with EGFR expression and lower proliferation as previously confirmed in both apocrine and nonapocrine mammary carcinomas [11] and [29].

MLPA assay revealed extensive gene copy number changes in apocrine tumors, many of which have not been previously described in this cancer. Concurrent amplification of ERBB2 and TOP2A has been frequently described in breast cancer [24]. The present results showed instead that apocrine carcinomas tend to harbor TOP2A loss, which may confer anthracycline resistance [28]. We observed also a frequent BRCA2 loss. This alteration has been associated with ER+ (luminal) phenotype [30]. Previous studies defined a genetic profile of “luminal-complex” tumors that characterizes subsets of BRCA2-related, luminal A and luminal B tumors [31]. This profile includes alterations at 8p, 8q, 11q, 13q, and 17q. Some of these alterations (eg, ZNF703 and ADAM9 [8p], MYC [8q], CCND1 [11q], BRCA2 [13q], ERBB2 [17q]) are present in apocrine tumors. A gain of ZNF703 was particularly common. ZNF703 is a recently described oncogene, specific for luminal B tumors, involved in mammary epithelium differentiation [32]. In contrast to previous reports, in apocrine tumors, we found a loss of ADAM9 with only one case harboring ADAM9 gain [33].

In our study, apocrine tumors frequently exhibited a gain of CDH1 gene affecting predominantly the exon 1. This observation was previously reported by Lacle et al in breast cancer [34]. Two apocrine cases that were negative for E-cadherin showed neither loss of CDH1 (by MLPA) or CDH1 mutations (by NGS), which may indicate alternative mechanisms of the CDH1 silencing in apocrine tumors.

Mutational analysis revealed mutations within the mTOR signaling pathway including PIK3CA and PTEN genes (followed by the loss of PTEN protein expression). PI3KCA mutations are typically associated with ER+ and HER2+ breast cancers (COSMIC, accessed: August 19, 2014), whereas PTEN aberrations usually affect ER− breast tumors. PIK3CA mutations have been previously described in a small series of benign and malignant apocrine lesions [1], whereas germline PTEN mutations have been described in patients with Cowden syndrome [OMIM #158350] that are prone to develop breast cancer with apocrine differentiation. Despite the limited number of tested cases, PIK3CA/PTEN alterations in apocrine tumors may be relevant for targeted therapy using PIK3CA/mTOR inhibitors. A subset of apocrine tumors also harbored KRAS and BRAF gene

mutations. Both genes are infrequently mutated in breast cancer, although 2 recent studies indicated that a subset of TN and HER2-positive breast carcinomas might harbor these genetic alterations [35] and [36]. TP53 mutations and p53 expression in apocrine tumors are in line with previous data [1] and [7].

We conclude that apocrine carcinomas as defined by morphology identify a group of tumors that may express different markers predictive of response to targeted therapies. Biological studies are needed to prove these alterations as functional, but the molecular results we obtained confirm that apocrine carcinomas diagnosed by standard H&E are tumors that exhibit complex molecular genetic alterations characteristic for luminal-complex phenotype.

Acknowledgment

We thank Sandra Milena Rondon-Lagos for technical assistance. The preliminary results of this study were presented at the 25th European Congress of Pathology 2013, Lisbon, Portugal.

References

1 S. Vranic, F. Schmitt, A. Sapino, et al. Apocrine carcinoma of the breast: a comprehensive review Histol Histopathol, 28 (2013), pp. 1393–1409

2 P. Farmer, H. Bonnefoi, V. Becette, et al. Identification of molecular apocrine breast tumours by microarray analysis Oncogene, 24 (2005), pp. 4660–4671

3 A. Doane, M. Danso, P. Lal, et al. An estrogen receptor–negative breast cancer subset characterized by a hormonally regulated transcriptional program and response to androgen Oncogene, 25 (2006), pp. 3994–4008

4 B. Kreike, M. van Kouwenhove, H. Horlings, et al. Gene expression profiling and histopathological characterization of triple-negative/basal-like breast carcinomas Breast Cancer Res, 9 (2007), p. R65

5 S. Sanga, B. Broom, V. Cristini, M. Edgerton Gene expression meta-analysis supports existence of molecular apocrine breast cancer with a role for androgen receptor and implies interactions with ErbB family

6 M. Guedj, L. Marisa, A. de Reynies, et al. A refined molecular taxonomy of breast cancer Oncogene, 31 (2013), pp. 1196–1206

7 A. Dumay, J.P. Feugeas, E. Wittmer, et al. Distinct tumor protein p53 mutants in breast cancer subgroups Int J Cancer, 132 (2013), pp. 1227–1231

8 J. Lehmann-Che, A.S. Hamy, R. Porcher, et al. Molecular apocrine breast cancers are aggressive estrogen receptor negative tumors overexpressing either HER2 or GCDFP15 Breast Cancer Res, 15 (2013), p. R37

9 R. Lininger, Z. Zhuang, Y. Man, W. Park, M. Emmert-Buck, F. Tavassoli

Loss of heterozygosity is detected at chromosomes 1p35-36 (NB), 3p25 (VHL), 16p13 (TSC2/PKD1), and 17p13 (TP53) in microdissected apocrine carcinomas of the breast Mod Pathol, 12 (1999), pp. 1083–1089

10 C. Jones, S. Damiani, D. Wells, R. Chaggar, S. Lakhani, V. Eusebi Molecular cytogenetic comparison of apocrine hyperplasia and apocrine carcinoma of the breast Am J Pathol, 158 (2001), pp. 207–214

11 S. Vranic, O. Tawfik, J. Palazzo, et al. EGFR and HER-2/neu expression in invasive apocrine carcinoma of the breast Mod Pathol, 23 (2010), pp. 644–653

12 L. Annaratone, C. Marchiò, R. Russo, et al. A collection of primary tissue cultures of tumors from vacuum packed and cooled surgical specimens: a feasibility study PLoS One, 8 (2013), p. e75193

13 M.E. Hammond, D.F. Hayes, M. Dowsett, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer Arch Pathol Lab Med, 134 (2010), pp. 907–922

14] E.A. Rakha, T.K. Teoh, A.H. Lee, C.C. Nolan, I.O. Ellis, A.R. Green Further evidence that E-cadherin is not a tumour suppressor gene in invasive ductal carcinoma of the breast: an immunohistochemical study Histopathology, 62 (2013), pp. 695–701

15 A. Sangoi, J. Higgins, R. Rouse, A. Schneider, J. McKenney Immunohistochemical comparison of MUC1, CA125, and Her2Neu in invasive micropapillary carcinoma of the urinary tract and typical invasive urothelial carcinoma with retraction artifact Mod Pathol, 22 (2009), pp. 660–667

16 E.A. Rakha, M.E. El-Sayed, A.R. Green, et al. Breast carcinoma with basal differentiation: a proposal for pathology definition based on basal cytokeratin expression Histopathology, 50 (2007), pp. 434–438

17 A.K. Witkiewicz, A. Dasgupta, S. Sammons, et al. Loss of stromal caveolin-1 expression predicts poor clinical outcome in triple negative and basal-like breast cancers Cancer Biol Ther, 10 (2010), pp. 135–143

18 A. Goldhirsch, E.P. Winer, A.S. Coates, et al. Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013 Ann Oncol, 24 (2013), pp. 2206–2223

19 D.J. Williams, C. Cohen, M. Darrow, A.J. Page, B. Chastain, A.L. Adams Proliferation (Ki-67 and phosphohistone H3) and oncotype DX recurrence score in estrogen receptor-positive breast cancer Appl Immunohistochem Mol Morphol, 19 (2011), pp. 431–436

20 B. Weigelt, H. Horlings, B. Kreike, et al. Refinement of breast cancer classification by molecular characterization of histological special types J Pathol, 216 (2008), pp. 141–150

21 C. Marchiò, M. Lambros, P. Gugliotta, et al. Does chromosome 17 centromere copy number predict polysomy in breast cancer? A fluorescence in situ hybridization and microarray-based CGH analysis J Pathol, 219 (2009), pp. 16–24

22 A.C. Wolff, M.E. Hammond, D.G. Hicks, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update Arch Pathol Lab Med, 138 (2014), pp. 241–256

23 C.W. Elston, I.O. Ellis Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up Histopathology, 19 (1991), pp. 403–410

24 C.B. Moelans, R.A. de Weger, M.T. van Blokland, E. van der Wall, P.J. van Diest Simultaneous detection of TOP2A and HER2 gene amplification by multiplex ligation-dependent probe amplification in breast cancer Mod Pathol, 23 (2010), pp. 62–70

25 R. Watters, P. Benos, S. Oesterreich To bind or not to bind—FoxA1 determines estrogen receptor action in breast cancer progression Breast Cancer Res, 14 (2012), p. 312

26 D. Raina, Y. Uchida, A. Kharbanda, et al. Targeting the MUC1-C oncoprotein downregulates HER2 activation and abrogates trastuzumab resistance in breast cancer cells Oncogene, 33 (2014), pp. 3422–3431

27 B. Sinn, G. von Minckwitz, C. Denkert, et al. Evaluation of mucin-1 protein and mRNA expression as prognostic and predictive markers after neoadjuvant chemotherapy for breast cancer Ann Oncol, 24 (2013), pp. 2316–2324

28 W. Jacot, M. Fiche, K. Zaman, A. Wolfer, P.J. Lamy The HER2 amplicon in breast cancer: topoisomerase IIA and beyond Biochim Biophys Acta, 2013 (1836), pp. 146–157

29 K.M. McNamara, T. Yoda, Y. Miki, et al. Androgenic pathway in triple negative invasive ductal tumors: its correlation with tumor cell proliferation Cancer Sci, 104 (2013), pp. 639–646

30 N. Pécuchet, T. Popova, E. Manié, et al. Loss of heterozygosity at 13q13 and 14q32 predicts BRCA2 inactivation in luminal breast carcinomas Int J Cancer, 133 (2013), pp. 2834–2842

31 P.M. Wilkerson, J.S. Reis-Filho The 11q13-q14 amplicon: clinicopathological correlations and potential drivers Genes Chromosomes Cancer, 52 (2013), pp. 333–355

32 F. Sircoulomb, N. Nicolas, A. Ferrari, et al. ZNF703 gene amplification at 8p12 specifies luminal B breast cancer EMBO Mol Med, 3 (2011), pp. 153–166

33 C. O'Shea, N. McKie, Y. Buggy, et al. Expression of ADAM-9 mRNA and protein in human breast cancer Int J Cancer, 105 (2003), pp. 754–761

34 M.M. Lacle, R. Kornegoor, C.B. Moelans, et al. Analysis of copy number changes on chromosome 16q in male breast cancer by multiplex ligation-dependent probe amplification Mod Pathol, 26 (2013), pp. 1461–1467

35 D. Craig, J. O'Shaughnessy, J. Kiefer, et al. Genome and transcriptome sequencing in prospective metastatic triple-negative breast cancer uncovers therapeutic vulnerabilities Mol Cancer Ther, 12 (2013), pp. 104–116

36 C. Pereira, M. Leal, C. de Souza, et al. Prognostic and predictive significance of MYC and KRAS alterations in breast cancer from women treated with neoadjuvant chemotherapy PLoS One, 8 (2013), p. e60576

This study was supported by Associazione Italiana per la Ricerca sul Cancro grants (IG10787 to A.S. and MFAG13310 to C.M.) and by Ricerca Sanitaria Finalizzata (Rome, Italy) (RF-2010-2310674 to AS). Dr Semir Vranic (University of Sarajevo, Bosnia and Herzegovina) is the recipient of a scholarship for postdoc research at the Department of Medical Sciences, University of Turin (Turin, Italy) under the Erasmus-Western Balkans joint mobility program (ERAWEB, Erasmus Mundus Action 2 program, Contract Project Reference: 2011-2586-EM Action 2). Both Universities were partners in this project. ERAWEB has been funded with support from the European Commission. The Eracol (Erasmus-Colombus, the Netherlands) funded César Payán-Gómez.

Correspondence to: S. Vranic, Department of Pathology, Clinical Center, University of Sarajevo, Bolnička 25, BA-71000 Sarajevo, Bosnia and Herzegovina.

Correspondence to: A. Sapino, Department of Medical Sciences, University of Turin, Via Santena 7, 10126, Turin, Italy.

Tables and Figures

Table 1.

Clinicopathologic characteristics of the apocrine cohort

Table 1.
Clinicopathologic characteristics of the apocrine cohort
Characteristic,Frequency (%)
Gradea,1,0 (0)
2,21 (56.8)
3,16 (43.2)
pT,1 (â‰¤2 cm),20 (54)
2 (2-5 cm),13 (35.1)
3 (>5 cm),2 (5.4)
4,1 (2.7)
Unknown,1 (2.7)
pN,0,18 (48.6)
1a,12 (32.4)
2a,3
(8.1)
3a,2
(5.4)
Unknown,2 (5.4)
Surgery,Mastectomy,20 (54)
Quadrantectomy,13 (35)
Lumpectomy,4 (11)

Table 2. Antibodies for TMA IHC study and score for assessing positive results

Table 1.
Clinicopathologic characteristics of the apocrine cohort
Characteristic,Frequency (%)
Gradea,1,0 (0)
2,21 (56.8)
3,16 (43.2)
pT,1 (â‰¤2 cm),20 (54)
2 (2-5 cm),13 (35.1)
3 (>5 cm),2 (5.4)
4,1 (2.7)
Unknown,1 (2.7)
pN,0,18 (48.6)
1a,12 (32.4)
2a,3
(8.1)
3a,2
(5.4)
Unknown,2 (5.4)
Surgery,Mastectomy,20 (54)
Quadrantectomy,13 (35)
Lumpectomy,4 (11)

Table 3. Results of the immunohistochemical profiling of apocrine carcinoma of the breast

Table 3.
Results of the immunohistochemical profiling of apocrine carcinoma of the breast

IHC marker(expression),IHC-apocrine(n = 27),H&E-apocrine (n = 10),Total(N = 37),P
E-cadherin,+,23 (92%),8 (80%),31 (88.6%),.56
âˆ',2 (8%),2 (20%),4 (11.4%)
HER2,+,7 (29.2%),4 (40%),11 (32.4%),.31
âˆ',17 (70.8%),6 (60%),23 (70.3%)
Topo2Î±,<13%a,17 (62.9%),6 (75%),23 (65.7%),.68
>13%,10 (37.1%),2 (25%),12 (34.3%)
EGFR,2+/3+,14 (56%),0 (0%),14 (41.2%),.004
0/1+,11 (44%),9 (100%),20 (58.8%)
MUC-1,0/1+,3 (12%),1 (11.1%),4 (11.8%),.65
2+/3+,22 (88%),8 (88.9%),30 (88.2%)
CK5/6,+,4 (16%),0 (0%),4 (11.4%),.026
âˆ',21 (84%),10 (100%),31 (88.6%)
CK14,+,0 (0%),0 (0%),0 (0%),NA
âˆ',25 (100%),10 (100%),35 (100%)
p63,+,1 (4%),1 (10%),2 (5.7%),.49
âˆ',24 (96%),9 (90%),33
(94.3%)
Cav-1,+,2 (8%),0 (0%),2 (5.9%),1.0
âˆ',23 (92%),9 (100%),32 (94.1%)
Ki-67,<20%,14 (56%),6 (60%),20
(57%),.56
â‰¥20%,11 (42%),4 (40%),15 (43%)
PHH3,<2%,17 (68%),5 (50%),22 (62.9%),.41
2%-5%,7 (28%),5 (50%),12 (34.3%)
>5%,1 (4%),0 (0%),1 (2.8%)
Cyclin D1,Low,14 (56%),3 (33.3%),17 (50%),.44
High,11 (44%),6 (66.7%),17 (50%)
p53,>10%,10 (41.7%),1 (11.1%),11 (33.3%),.21
<10%,14 (58.3%),8 (88.9%),22 (66.7%)
PTEN,+,3 (12%),3 (0%),6 (17.1%),.50
âˆ',22 (88%),7 (100%),29 (82.9%)

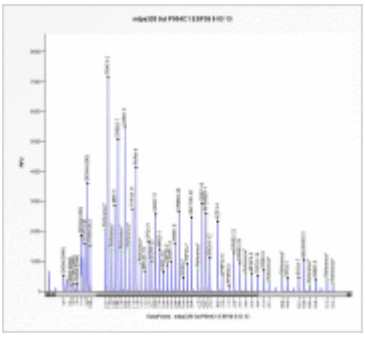

Table 4. Results of the next-generation sequencing analysis performed on apocrine carcinomas of the breast

Table 4.
Results of the next-generation sequencing analysis performed on apocrine carcinomas of the breast
Apocrine carcinoma,HER2 status,Mutation,Additional alterations (low level mutations)
Case 1,Not amplified,BRAF (D594G),PIK3CA (L100F)PTEN (S287L)
Case 2,Gain (MLPA),PIK3CA (H1047R),PTEN (D19G)
Case 3,Not amplified,KRAS (G12D)PIK3CA (E542K),PTEN (P213L)
Case 4,Not amplified,TP53 (R175H),PIK3CA (R555G)
Case 5,Not amplified,PTEN (P213L)TP53 (C141Y),â€“
Case 6,Amplified (FISH),PTEN(frameshift mutation),â€“
Case 7,Not amplified,PTEN (P248_V249del) PTEN (Q214X),â€“

Fig. 1. A comprehensive immunohistochemical survey of a case of invasive apocrine carcinoma (A, 20×) showing a characteristic steroid receptor profile: ER-negative (B) and AR-positive (C); positivity for GCDFP-15 (D) and E-cadherin (E); overexpression of HER2 (F); moderate positivity for Topo2α (G); strong expression of MUC-1 (H); the lack of expression for basal markers: CK5/6 (I) and Cav-1 (J); moderate expression of Ki-67 (K), and loss of PTEN protein (L) (all at 10×).

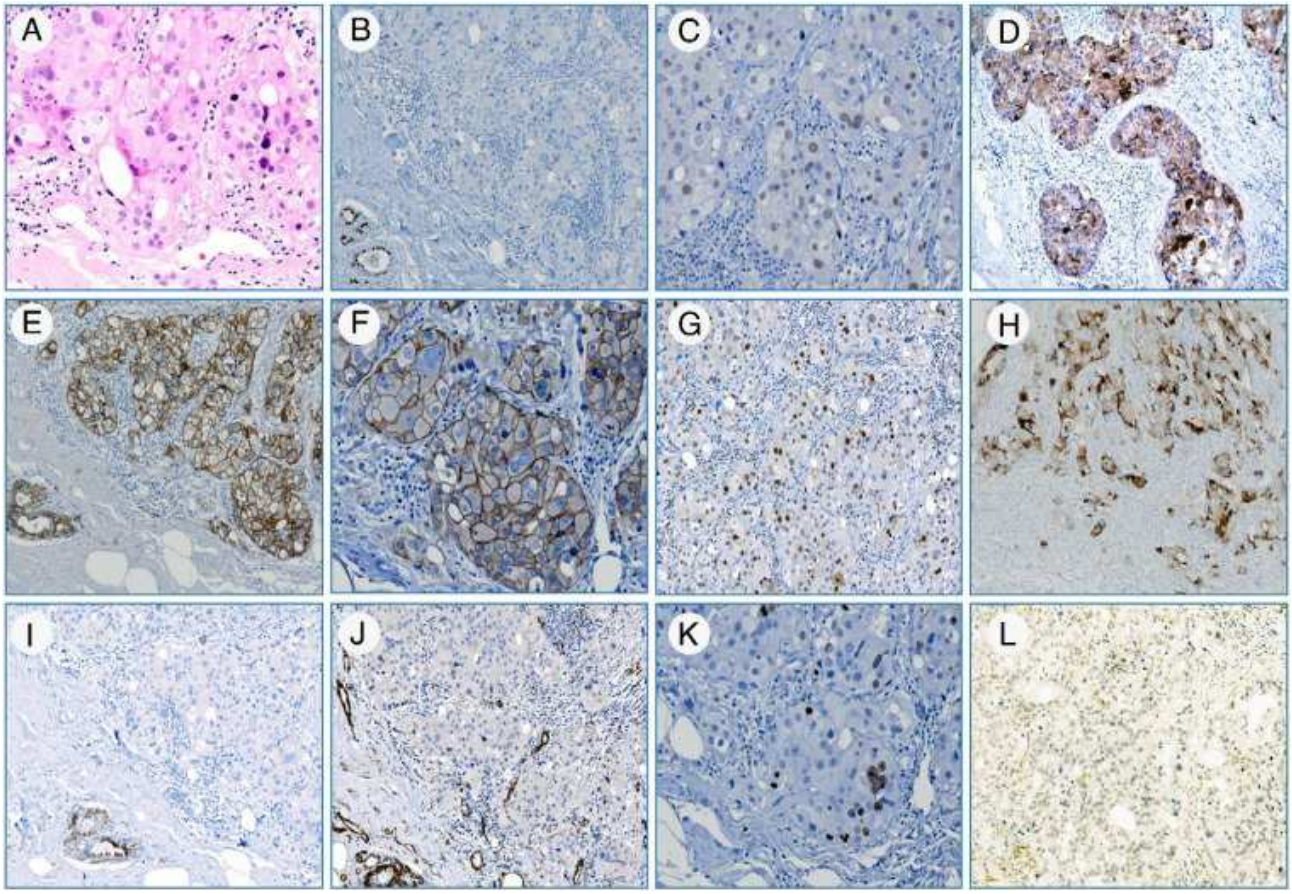

Fig. 2.

Numerous gene copy number changes detected by MLPA in 24 apocrine carcinomas of the breast. Forty-one genes, located at 9 chromosomes, were analyzed using 2 different MLPA probe mixes: the P004-C1 ERBB2 and P078-C1 Breast Tumour kit. The values less than 0.7 were defined as a gene loss (blue), whereas values greater than 1.5 as a gene gain (yellow).

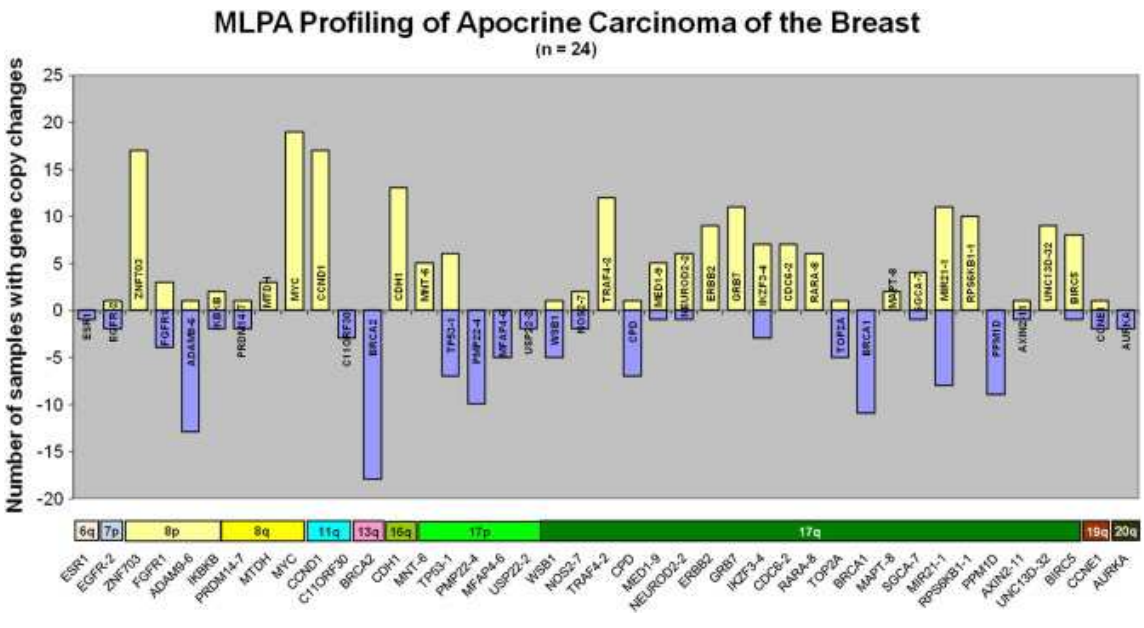

Fig. 3. Unsupervised hierarchical cluster analysis revealed two distinct clusters: cluster A that represents HER2-negative subset (17 cases), whereas cluster B contains HER2-positive cases (7 cases). Four small subclusters (A1/A2, B1/B2), deriving from loss or gain of genes in both 17p and 17q chromosome regions, were identified within the 2 main clusters.