High rate of *PIK3CA* mutations but no *TP53* mutations in low-grade adenosquamous carcinoma of the breast

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Aims: Low-grade adenosquamous carcinoma of the breast (LGASC) is a rare variant of metaplastic carcinoma characterised by a favourable outcome and histologically composed of glandular and squamous elements in a spindle cell background typically associated with a lymphocytic stromal reaction. Because of its rarity, the immunophenotypic and genetic profile of LGASC has not been sufficiently characterised. The aim of this study was to gain insights into the molecular and phenotypic characteristics of LGASC.

Methods and results: We reviewed the clinical and morphological features and detailed the immunohistochemical characteristics of a retrospective series of 13 LGASCs. Targeted sequencing of 50 genes was performed in 10 of 13 cases. Identified mutations were further assessed by Sanger sequencing in a validation series of 11 additional cases. All tumours showed a triple-negative immunophenotype, expressed 'basal' keratins, showed variable levels of epidermal growth factor receptor expression, and did not express androgen receptor. Sequencing analysis of the screening set of LGASCs revealed a high rate (seven of 10 cases) of *PIK3CA* mutations, whereas no *TP53* mutations were found. All *PIK3CA* mutations were missense mutations located either in exon 20 ($n = 6$) or in exon 9 ($n = 1$). The global *PIK3CA* mutation rate, including the validation series, was 52% (11 of 21 cases). No disease recurrences were observed. [Correction added on 11 June 2018, after first online publication: The percentage of mutation rate was corrected to 52%]

Conclusions: Our results indicate that LGASC of the breast is a low-grade triple-negative breast cancer that harbours a basal-like phenotype with no androgen receptor expression, and shows a high rate of *PIK3CA* mutations but no *TP53* mutations.

Keywords: androgen receptor, breast cancer, low-grade adenosquamous carcinoma, metaplastic carcinoma, *PIK3CA* mutations

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Introduction

Low-grade adenosquamous carcinomas of the breast (LGASCs) represent a rare histological type of invasive breast cancer that has been included in the latest edition of the World Health Organisation classification,[1] as part of the spectrum of metaplastic carcinomas of the breast. They feature well-developed gland/tubule formation intimately admixed with solid nests of squamous cells in a spindle cell background. Rosen and Ernsberger[2] emphasised that, despite the presence of metaplastic elements, these tumours show a low-grade histological pattern.

Typically, the carcinomatous component is characterised by small glandular structures with rounded rather than angulated contours, and solid cords of epithelial cells that may contain squamous cells, squamous pearls, or squamous cyst formation. The epithelial structures of adenosquamous carcinomas are often dispersed in a highly cellular spindle cell background. These spindle cells appear to merge with the epithelial cells and, in some cases, spindle cell metaplasia may be identified. Some authorities have suggested that the spindle cell background may be considered as part of the neoplastic component.[3] However, it may sometimes be difficult to distinguish between spindle cell metaplasia and ordinary stroma,[4,5] and this fusiform background may be challenging to interpret in core needle biopsy specimens. In agreement with their low-grade morphological features, the majority of LGASCs show an excellent prognosis with a low incidence of lymph node metastasis.[6] A proportion of cases, however, can behave in a locally aggressive manner.[6]

Although several case reports and relatively small cohorts have been reported over the years, LGASC remains poorly defined in terms of phenotype; in addition, its genetic profile is yet to be established.[3,6–16] The immunohistochemical studies published to date have described LGASC as part of the triple-negative breast cancer (TNBC) spectrum.[1,6,17,18]

A genome-wide characterisation of copy number alterations has been performed on two cases, one of which showed a complex profile of copy number changes encompassing focal amplifications.[3] A thorough genomic characterisation of metaplastic carcinomas has been recently provided,[19] but, regrettably, LGASCs were not present in the cohort.

To gain greater insights into the specific biological characteristics of LGASC, in this study we report a detailed morphological and immunophenotypic analysis of 13 cases and the first characterisation of the genomic landscape of LGASC by use of a targeted panel of genuine cancer genes.

Materials and methods

CASES

We retrospectively retrieved 13 LGASCs diagnosed at Institut Curie (Paris and Saint Cloud) and at Centre François Baclesse (Caen) between 1999 and 2013. These carcinomas were originally registered as either 'adenosquamous' or 'metaplastic' carcinomas. Samples were anonymised before analysis. Ethical approval from the Institutional Review Board (Institut Curie breast cancer study group: IRB 03/14) was obtained for the use of all specimens. Clinical data on treatment and follow-up were obtained from the Institut Curie electronic medical records.

A second independent cohort of 11 LGASCs that served as a validation series was obtained from the Centre Georges François Leclerc in Dijon, Centre Jean Perrin in Clermont-Ferrand, and Centre Léon-Bérard in Lyon.

MORPHOLOGICAL ANALYSIS

Histopathological review was performed by two experienced breast pathologists (A.V.S. and G.B.) for the purpose of subtyping and grading (using the Elston–Ellis grading system).[20] Specific histological features commonly seen in LGASC, such as degree of glandular, squamous and spindle cell differentiation, were assessed, and tumours were classified according to the predominant component (glandular, squamous, and spindle cell). In addition, stromal characteristics, including the presence/degree of lymphoid aggregates, were recorded. The respective proportions of glandular and squamous tumour cells and the spindle cell component, lymphoid stromal cells and associated normal glands within the tumour were determined. The presence of lymphovascular invasion and lymph node metastasis was also recorded.

IMMUNOHISTOCHEMISTRY

Three-micrometre-thick formalin-fixed paraffin-embedded (FFPE) sections were mounted on silane-coated slides. Immunohistochemical staining was performed with 16 different antibodies directed against oestrogen receptor (ER), progesterone receptor (PR), androgen receptor (AR), human epidermal growth factor receptor 2 (HER2), Ki67, epidermal

growth factor receptor (EGFR), p63, cytokeratin (CK) 5/6, CK7, CK14, CK8/18, α-smooth muscle actin, E-cadherin, CD117, PTEN, and EZH2. Details of antibody sources, dilutions and antigen retrieval methods are summarised in Table S1. Positive and negative controls (omission of the primary antibody and IgG-matched serum) were utilised.

All markers were scored (percentage of positive tumour cells) separately in the glandular, squamous and spindle cell components of the tumours. ER, PR and HER2 were evaluated according to the current American Society of Clinical Oncology/College of American Pathologists guidelines.[21,22] The positive cut-off for expression of all other markers was 10%. Markers were characterised according to the highest percentage of positive cells within each component.

MACRODISSECTION AND DNA EXTRACTION

All tumours were macroscopically dissected to enrich for tumour cell content, by taking out normal breast tissue and any areas of fibrocystic changes and intraductal papillomas. Macrodissection was performed with a sterile scalpel from three consecutive 6-μm-thick sections of FFPE samples after deparaffinisation, guided by the microscopic limits of the tumour on the haematoxylin and eosin-stained section. DNA was extracted with the NucleoSpin 8 tissue Kit (Macherey Nagel, Düren, Germany), according to the manufacturer's recommendations. The DNA level was measured with the Qubit 2.0 fluorometer assay according to the manufacturer's instructions (Life Technologies, Thermo Fisher Scientific, Waltham, MA, USA). A *KRAS* polymerase chain reaction (PCR) was performed with LightMix Kit Kras to assess DNA quality. Following DNA quantification and quality assessment, three of 13 cases were withdrawn because of lack of dissected material (case 6) or excessively low DNA levels (cases 2 and 4).

TARGETED MASSIVE PARALLEL SEQUENCING (MPS)

Given that only FFPE material was available, we focused on mutations affecting known oncogenes and tumour suppressor genes in human cancers. For this purpose, targeted sequencing was performed with the Ion Ampliseq Cancer Hotspot panel v2 in conjunction with the Ampliseq library kit v2.0 and the Ion Torrent Personal Genome Machine (PGM) Analyzer (Life Technologies, Carlsbad, CA, USA). According to the manufacturer's instructions, 10 ng of extracted DNA was used to generate 207 amplicons surveying 2800 COSMIC mutations in 50 well-established oncogenes and tumour suppressor genes that are frequently mutated in different malignancies, including breast cancer (summarised in Table S2).

Extremities of amplicons were partially digested, and Ion adapters (Ion Xpress PCR barcode adapters; Life Technologies), including one with a molecular barcode, were ligated at both ends. After amplification of the construction, the quality of the libraries was checked on a BioAnalyzer (Agilent, Santa Clara, CA, USA) and quantified with Qubit Technology (Life Technologies). Template preparation was performed with the Ion OneTouch System 2 with the Ion PGM Template OT2 200 Kit (Life Technologies). Templates were sequenced on the Ion Torrent PGM with an Ion PGM Sequencing 200 Kit v2 and an Ion 318v2 Chip.

The overall quality of each run was evaluated on the basis of the report generated by the Torrent Server. At least 100 000 reads per sample were required. A sample was considered to be valid only if 95% of targeted positions were covered at >100×, according to the analysis performed by the variant caller plugin of TORRENT SUITE (Life Technologies).

VARIANT CALLING

Data from the PGM runs were initially processed with the Ion Torrent platform-specific pipeline software TORRENT SUITE to generate sequence reads, trim adapter sequences, and filter and remove poor signal-profile reads. Initial variant calling from the Ion AmpliSeq sequencing data was generated by TORRENT SUITE v3.0 with a plug-in 'variant caller' program. For mutation analyses, sequencing reads were aligned on the human reference genome (hg19) by the use of TMAP (Life Technologies). Variants were detected with VARIANT CALLER (Life Technologies), and annotated by use of the ANNOVAR pipeline.[23] Variants were filtered according to their frequency [>4% for single-nucleotide variants (SNVs) and >10% for indels], strand ratio (>0.2), and read coverage (>30× for SNVs and >100× for indels). The Bioinformatics platform integrated the different molecular results in a name-blinded technical report, which was then discussed and interpreted by a pathologist and a molecular biologist together (G.B. and I.B.). Alignments were verified visually with the Integrative Genomics Viewer v.2.2 (Broad Institute).

STATISTICAL ANALYSIS

COSMIC (version 64) and the My Cancer Genome database (http://www.mycancergenome.org/) were used to assess recurring mutations in breast cancer.

Statistical correlations were performed with GRAPHPAD PRISM version 6 (GraphPad Software, La Jolla, CA, USA).

Mutations identified in the 10 cases subjected to targeted MPS were validated by Sanger sequencing (LightCycler 480 Real-Time PCR system, kit PIK3CA, Roche Molecular Systems, Indianapolis, IN, USA). The frequency of PIK3CA mutations was further explored by Sanger sequencing, including in the validation series of 11 LGASCs (Table S3).

Results

CLINICAL FEATURES

The clinicopathological features of the cohort are summarised in Table 1. All patients were female, and they were predominantly of postmenopausal age. Tumour size ranged from 3.5 mm to 70 mm (mean, 22 mm; median, 15 mm). One case (case 8) had axillary lymph node metastases showing a ductal morphology reminiscent of a previously diagnosed contralateral breast carcinoma (Table 1), and showed ER and PR expression. Clinical follow-up (range, 3–17 years; mean, 7.5 years) did not reveal any recurrences.

HISTOPATHOLOGY

As summarised in Table 2, all cases had varying degrees of glandular, squamous and spindle cell components (Figure 1).

The epithelial component was organised in small tubules, cell clusters and cords with focal squamous differentiation in a spindle cell background. Neoplastic glands were elongated, ovoid, or compressed, and some infiltrated in and around residual normal glands. Spindle cells and stromal cells (fibroblasts and lymphoid cells) intimately surrounded the epithelial cell nests. Lymphocytic aggregates were present in all tumours. Neither necrosis nor vascular invasion was observed.

Case 3 showed a focal high-grade component, in line with previous descriptions of progression from low-grade adenosquamous carcinoma to high-grade metaplastic carcinoma.[2,24]

IMMUNOPHENOTYPIC ANALYSIS

Immunohistochemistry results are summarised in Figures 2 and 3 and detailed in Table S4. All tumours were of the triple-negative phenotype (ER-negative, PR-negative, and HER2-negative) and lacked AR expression. The proliferation indices assessed according to Ki67 ranged between 2% and 20% (mean, 11%; median, 10%). The immunophenotype was found to be relatively similar across the various cases, depending on their predominant component: glandular, squamous, or spindle cell (Figure 2).

The glandular component was characterised by diffuse (>50%) expression of CK7 and CK14, E-cadherin, and EZH2. The squamous component expressed CK14, E-cadherin, CK7, CK5/6, EZH2, p63, and PTEN, whereas the spindle cell component expressed SMA, PTEN and, to a lesser extent, EZH2. Some of the spindle cells showed expression of CK5/6, CK14, CK7, and p63.

MUTATION FREQUENCY IN KNOWN CANCER GENES

Mutations affecting the 50 genes included in the targeted AmpliSeq Panel V2 are summarised in Table 2 and detailed in Table S5. Ten breast LGASC samples were analysed by MPS. A mean 100× coverage of 90% with a mean read length of 226 bp was obtained in the 10 FFPE samples. The majority of the samples (seven of 10 cases, 70%) harboured missense PIK3CA mutations affecting exon 20 ($n = 6$) or exon 9 ($n = 1$), and corresponding to the kinase (H1047L and H1047R) and helical (E542K) domains, respectively. Two of the samples showing a PIK3CA mutation also harboured mutations in GNAS and KIT (cases 1 and 3, respectively). Co-occurrence of CDKN2A and PTEN mutations was detected in one other case (case 7). TP53 was sequenced in all exons except exon 1, and no TP53 mutations were identified.

Six of the seven PIK3CA mutations identified by Ion Torrent sequencing analysis were subsequently validated by Sanger sequencing in the index cases. It should be noted that the PIK3CA mutation not detected by Sanger sequencing showed an allele frequency of 6.7% in the Ion Torrent sequencing assay. To confirm the high rate of PIK3CA mutations in this entity, we analysed an independent series of 11 LGASCs and found four tumours (36%) harbouring PIK3CA mutations in exon 20 ($n = 2$) or exon 9 ($n = 2$) (Table S5B). The global PIK3CA mutation rate, including screening and validation series, accounted for 52% (11 of 21 cases) (Table S5C).

Table 1. Clinical characteristics of the cohort of 13 low-grade adenosquamous carcinomas of the breast (LGASCs)

Case	Age (years)	Previous breast lesion	Family history of cancer	Initial clinical presentation	Size (mm)	Lymph node metastasis	Mastectomy	Chemotherapy	Radiotherapy	Disease-free survival (years)
1	50	Benign	None	Palpable mass	25	0/14	Total	No	No	17
2	54	None	Mother (breast)	Palpable mass	20	NA	Partial	No	Yes	13
3	54	None	Father (oesophagus)	Screening mammography	10	0/10	Partial	Yes	Yes	11
4	85	None	None	Palpable mass	12	NA	Partial	No	No	11
5	54	None	Father (colon)	Palpable mass	15	0/4	Partial	No	Yes	9
6	56	Invasive lobular carcinoma	None	Screening mammography	3.5	0/1	Partial	No	Yes	8
7	66	None	None	Palpable mass	20	0/19	Partial	No	Yes	6
8	81	Invasive ductal carcinoma	Mother (breast)	Palpable mass	35	10/15*	Partial	Yes	Yes	5
9	46	None	None	Palpable mass	34	NA	Partial	No	No	5
10	66	None	None	Screening mammography	10	0/12	Partial	Yes	Yes	4
11	62	None	None	Screening mammography	14	0/19	Partial	Yes	Yes	3
12	58	Benign	Mother (breast)	Palpable mass	70	0/10	Partial	Yes	Yes	3
13	28	None	Mother (breast)	Palpable mass	11	0/2	Partial	No	Yes	3

NA, not available.

*Lymph node metastasis of contralateral oestrogen receptor-positive invasive ductal carcinoma (i.e. invasive carcinoma of no special type).

Table 2. Pathological features of 13 low-grade adenosquamous carcinomas of the breast (LGASCs)

Cases	Histological grade*				Tumour components			Stroma			Normal glands (%)†	Other associated lesions	Mutated genes‡
	Gland formation	Nuclear atypia	Mitoses	Final grade	Glandular cells (%)†	Squamous cells (%)†	Spindle cells (%)†	Fibroblasts (%)†	Lymphoid cells (%)†	Lymphoid infiltrate			
1	3	1	2	II	5	30	10	5	25	Intratumour	25	Intraductal papilloma	PIK3CA, GNAS
2	3	1	1	I	0	30	15	10	15	Intratumour	30	–	NA
3	3	2	1	II	40	10	15	5	20	Intratumour	10	Fibrocystic disease, papilloma	PIK3CA, KIT
4	2	2	1	I	15	15	40	5	5	Intratumour	20	–	NA
5	2	2	1	I	15	30	25	15	10	Intratumour	5	Fibroadenoma	PIK3CA
6	3	2	1	II	10	25	40	10	5	Peripheral	10	–	NA
7	3	2	1	II	10	10	25	5	15	Intratumour	40	Fibrocystic disease, papilloma	CDKN2A, PTEN
8	3	3	1	II	25	15	35	10	5	Intratumour	10	Fibrocystic disease, papilloma	PIK3CA
9	2	2	1	I	5	5	40	10	20	Intratumour	20	–	PIK3CA
10	3	2	1	II	15	30	30	10	10	Intratumour	5	Adenosis	PIK3CA
11	2	2	1	I	10	10	30	0	10	Peripheral	40	Fibroadenoma	Wild type
12	2	1	1	I	10	10	15	10	15	Intratumour	40	Intraductal papilloma	Wild type
13	2	1	1	I	25	10	15	30	10	Intratumour	10	Fibrocystic breast disease	PIK3CA

NA, not available.

*According to Elston and Ellis.[20]

†The relative percentages of distinct cell components of the lesions sum up to 100%.

‡The mutational status reported here includes details on the hotspot mutations investigated in 50 cancer genes.
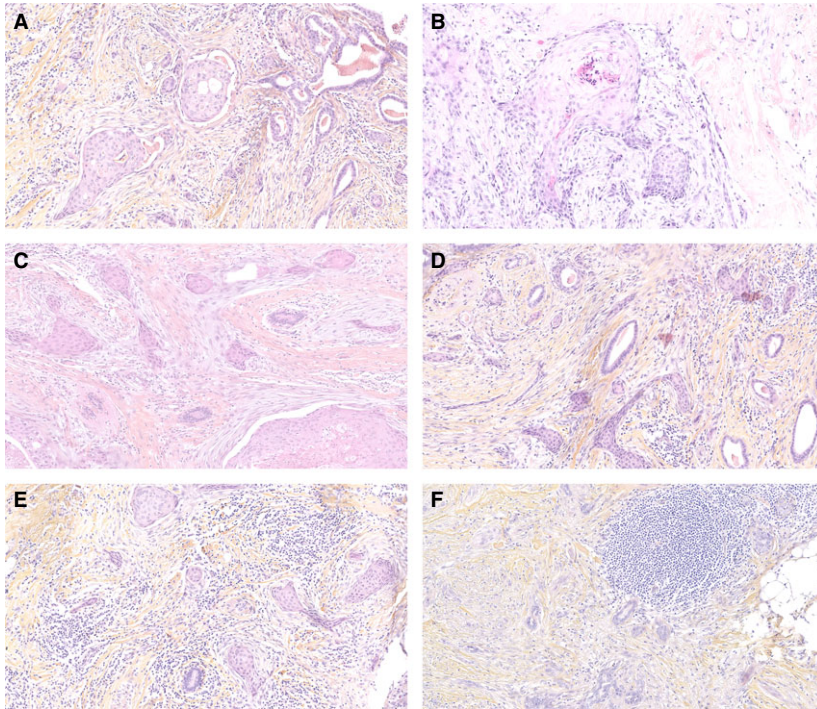
Figure 1. Histological features of low-grade adenosquamous carcinomas of the breast (Hematoxylin-Eosin-Saffron). This entity is composed of infiltrating glandular structures (**A**) with variable degrees of squamous differentiation (**B,C**) in a spindle cell background (**D**) entrapping residual normal glands (**C–E**). Lymphocytic aggregates were present in all tumours (**D–F**).

Discussion

In this study, we show that LGASC represents an indolent form of TNBC with basal-like features characterised by high rates of *PIK3CA* mutations and lack of *TP53* mutations. These data on LGASC provide insights into the genomic landscape of metaplastic breast cancer, and may help in the understanding of the possible evolutionary pathways of low-grade forms of TNBC, by representing a starting point for future studies of putative precursors of LGASC and other metaplastic carcinomas. These findings may also be of help in the diagnostic setting, because, although LGASC can be relatively easily recognised on morphological grounds in surgical specimens, it may be a challenging entity to be diagnosed on core biopsy samples with respect to other fusiform proliferations, especially those of low grade.

LGASC is an unusual form of metaplastic carcinoma featuring a variable admixture of well-formed glands and solid cords of cells with squamous features arranged in a haphazard, infiltrative pattern and typically showing relatively little cytological atypia and low levels of proliferation, to the extent that differential diagnosis with radial scar (RS)/complex sclerosing lesions (CSLs) can be challenging. The extension of

the lesion in a spindle cell background that can stand for the presence of desmoplastic stroma and the presence of a triple-negative phenotype may represent helpful features for the differential diagnosis.

So far, relatively small cohorts characterising this entity have been reported, and almost exclusively at the immunophenotypic level. In agreement with previous studies,[6,18] all of the cases pertaining to our series were of the triple-negative phenotype. In addition, AR expression was not observed. This is in contrast to the report of the large majority of metaplastic carcinomas being AR-positive, except for squamous cell carcinomas.[25] We also confirmed in the epithelial neoplastic component the consistent expression of basal CKs and of markers usually expressed in normal myoepithelial cells. In a subgroup of cases, a minor proportion of the spindle cells also expressed p63, CKs, and EZH2, which is a protein that is overexpressed in a wide range of solid tumour types, including breast cancer.[26] Taken together, these preliminary observations may indirectly suggest that the spindle cell background observed in LGASC could be an integral part of the neoplastic process. This hypothesis should be further explored in studies aimed at analysing the molecular features of the distinct cell components of LGASCs. Interestingly Geyer
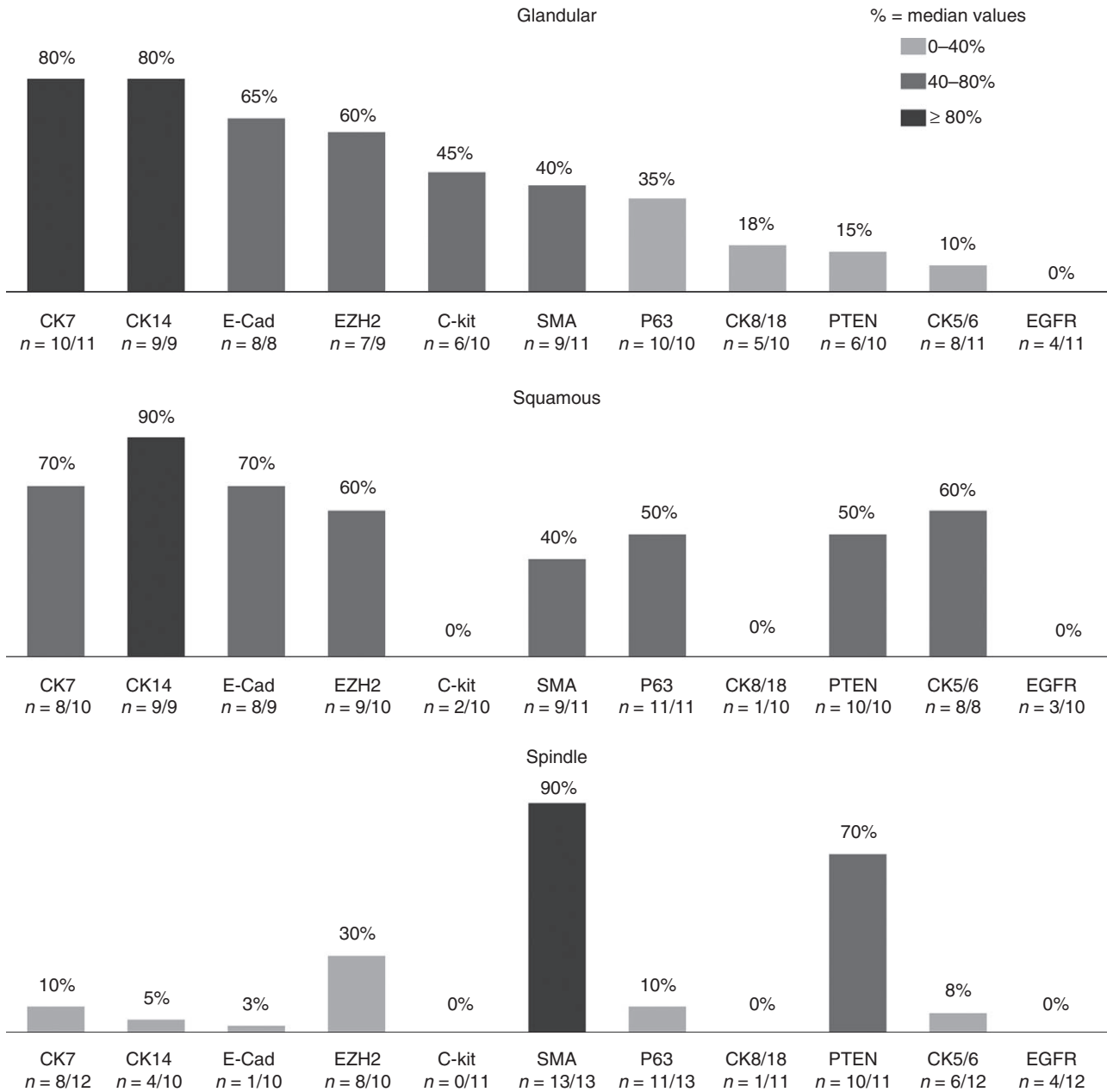
Figure 2. Immunophenotype of glandular, squamous and spindle cell components. Median values of percentages of positive cells (grey bar plots) within the glandular, squamous and spindle cell components are shown. Numbers of positive cases relative to the total number of cases available for analysis per marker (the threshold for positivity was set at 10%) are indicated at the bottom of the columns.

et al.[3] have reported on the molecular karyotype obtained in two cases of LGASC by microarray comparative genomic hybridisation, and have shown a rather simple genome in one case and a higher degree of complexity of copy number changes in the other case, which harboured a focal *EGFR* amplification. By means of chromogenic *in-situ* hybridisation analysis, the authors were able to demonstrate that the *EGFR* amplification found in the epithelial cells

was also present in a minority of the spindle cells of the stromal component, in particular in those near the epithelial clusters.

With the exception of the study above, the molecular underpinning of LGASC is largely unknown. The advent of MPS has contributed to a thorough characterisation of the genetic landscape of breast cancer.[27] Recently, it has been demonstrated that, although metaplastic carcinomas and common forms of TNBC
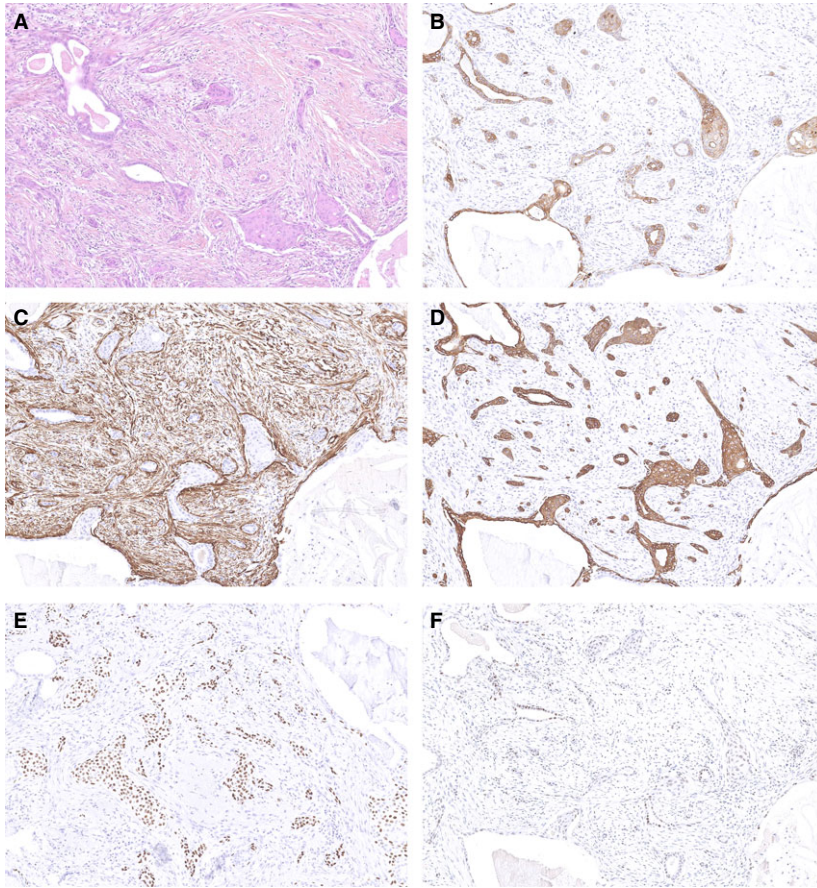
Figure 3. Immunophenotypic profile of low-grade adenosquamous carcinomas of the breast (LGASCs). **A**, LGASC with glandular, squamous and spindle components (Hematoxylin-Eosin-Saffron). **B**, LGASC is a triple-negative carcinoma showing cytokeratin (CK) 5/6 expression. **C**, The spindle cells are positive for α-smooth muscle actin. **D–F**, The glandular and squamous components express CK14 (**D**), p63 (**E**), and EZH2 (**F**).

harbour similar mutational frequencies for *TP53*, they are genetically distinct entities.[19] Metaplastic carcinomas show more frequent mutations in *PIK3CA*, *PIK3R1*, *PTEN* (57% of cases), and Wnt pathway genes (51% of cases). *PIK3CA* mutations are significantly more frequently observed in spindle cell metaplastic carcinomas than in other metaplastic carcinomas.[19] In this context, our results provide another piece of evidence for heterogeneity within the spectrum of metaplastic carcinomas. We observed that LGASCs seem to lack *TP53* mutations and to harbour a relatively high frequency of *PIK3CA* mutations (52%), in stark contrast to high-grade spindle cell metaplastic carcinomas, which harbour both *TP53* and *PIK3CA* mutations.

Recently Geyer *et al.*[28,29] have reported on the existence of a 'low-grade triple-negative breast neoplasia family' featuring microglandular adenosis/atypical microglandular adenosis as non-obligate precursors of TNBC, and acinic cell carcinomas as low-grade forms

of TNBC with the potential to transform into conventional high-grade TNBCs. Both microglandular adenosis/atypical microglandular adenosis associated with a carcinoma and acinic cell carcinomas have been shown to harbour *TP53* mutations, and the acquisition of a *TP53* mutation seems to represent the key event in the possible progression of microglandular adenosis/atypical microglandular adenosis to a carcinoma.[29] In this respect, the lack of *TP53* mutations and the presence of *PIK3CA* mutations in LGASC may suggest that these lesions follow a molecular evolutionary pathway that is distinct from that of conventional TNBC. Interestingly, lesions such as papillomas,[30] RS/CSLs[31,32] and infiltrating epitheliosis[33] have also been shown to harbour *PIK3CA* mutations, and the causative role of *PIK3CA* mutations in these entities remains unclear. As a further level of complexity, an association between RS/CSLs and LGASCs has also been documented.[24,34] In this scenario, one could hypothesise that LGASC of the breast may represent an indolent

carcinomatous lesion whose non-obligate precursor may be identified in RS/CSLs, at least in a subgroup of cases. To unravel the causative role within this association, comparative molecular analyses of synchronous RS/CSLs and LGASCs are warranted. At present, a single report has documented a case with synchronous CSL featuring infiltrating epitheliosis and an LGASC that shared the same *PIK3CA* and *SF3B1* mutations, thus providing evidence that the LGASC most likely originated from the CSL.[33]

Another low-grade variant of metaplastic carcinoma, i.e. fibromatosis-like metaplastic carcinoma, may arise in direct association with sclerosing papillary lesions.[34] Molecular studies dissecting the genetic landscape of this lesion would be of help in ascertaining whether or not these lesions share a genetic background with LGASC.

Our study has some limitations. The first is the limited number of cases analysed by MPS; nevertheless, it has to be acknowledged that LGASC represents a rare entity, and the major finding of the sequencing related to *PIK3CA* mutations was validated in an independent cohort. Second, the targeted panel used was composed of a limited number of genes, and covered either hotspots or a limited number of exons. It is rather remarkable, however, that none of the cases showed *TP53* mutations, as this gene was fully covered in the sequencing assay except for exon 1. Notwithstanding this, we cannot exclude the possibility that more comprehensive analyses in larger series may add to the real frequency of *PIK3CA* mutations, or unveil the presence of *TP53* mutations in a subgroup of cases and provide evidence of additional recurrent mutations characterising this entity. Finally, four of the 13 patients were followed up for <5 years, a time interval that may be suboptimal for investigating the behaviour of a low-grade form of invasive carcinoma of the breast. Nevertheless, no disease recurrence events were observed in patients with longer follow-up.

However, even if we take these limitations into account, our study not only confirms the morphological and immunohistochemical data already reported in the literature concerning this entity, but also provides evidence suggesting that LGASCs constitute a distinct genetic entity among metaplastic carcinomas, characterised by high rates of *PIK3CA* mutations and a lack of *TP53* mutations, a triple-negative phenotype, and no AR expression. Although further studies are needed to confirm and expand our findings, this observation seems to corroborate the concept that LGASC should be distinguished from high-grade forms of metaplastic carcinoma and other triple-negative/basal-like carcinomas that are associated with an aggressive behaviour.

At present, the clinical management of LGASC requires complete local and regional treatment, including surgery and radiotherapy. In our experience, axillary lymph node dissection and adjuvant therapies do not appear to be warranted, because of the favourable prognosis of these cancers. Additional analyses are required to assess the inflammatory stromal reaction in terms of antitumour immunity. Finally, further studies analysing the distinct cell components of LGASC are warranted to gain insights into the neoplastic nature of the spindle cells constituting the background of these tumours.

Acknowledgements

Conflicts of interest

The authors have no conflicts of interest to declare.

Author contributions

G. Bataillon characterised the cohort at the morphological and immunophenotypic levels, performed experiments, analysed data, and wrote the manuscript. L. Fuhrmann curated the immunophenotypic characterisation of the cohort and participated in data analysis. E. Girard analysed molecular data. E. Menet and M. Laé participated in cohort review and in the interpretation of immunohistochemical results. M. Capovilla, I. Treilleux, L. Arnould and F. Penault-Llorca provided samples and reviewed cases. R. Rouzier performed the clinical data analysis. C. Marchiò participated in data analysis and wrote the manuscript. I. Bieche performed the molecular analysis, analysed data, and critically reviewed the manuscript. A. Vincent-Salomon conceived and supervised the study, provided samples, interpreted results, and wrote the manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. List of antibodies used.

Table S2. Ion Ampliseq cancer Panel target genes.

Table S3. Primers used for Sanger sequencing.

Table S4. Immunophenotypic analysis of the distinct components.

Table S5. Summary of *PIK3CA* mutations in LGASC (MPS and Sanger sequencing).