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Metaplastic breast carcinomas are basal-like tumours

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Aims: Recently, an immunohistochemical panel comprising antibodies against HER2, oestrogen receptor (ER), epidermal growth factor receptor (EGFR) and cytokeratin (CK) 5/6 was reported to identify basal-like breast carcinomas, as defined by cDNA microarrays. Our aim was to analyse a series of metaplastic breast carcinomas (MBCs) using this panel plus two other basal markers (CK14 and p63) and progesterone receptor (PR), to define how frequently MBCs show a basal-like immunophenotype.

Methods and results: Sixty-five cases were retrieved from the pathology archives of the authors' institutions and reviewed by three of the authors. Immunohistochemistry with antibodies for HER2, ER, EGFR, CK5/6, CK14 and p63 was performed according to standard methods. All but six cases (91%) showed the typical immunoprofile of basal-like tumours (ER– and HER2–, EGFR+ and/or CK5/6+). When CK14 and p63 were added to the panel, two additional cases could be classified as basal-like. The majority of MBCs lacked PR, except 4/19 (21%) carcinomas with squamous metaplasia.

Conclusions: Our results demonstrate that MBCs show a basal-like phenotype, regardless of the type of metaplastic elements. Moreover, as these neoplasms frequently overexpress EGFR (57%), patients with MBC may benefit from treatment with anti-EGFR drugs.

Keywords: carcinosarcoma, epidermal growth factor receptor (HER1), immunohistochemistry, myoepithelial, sarcomatoid carcinoma

Abbreviations: CK, cytokeratin; EGFR, epidermal growth factor receptor; ER, oestrogen receptor; MBC, metaplastic breast carcinoma; PR, progesterone receptor

Introduction

cDNA microarray studies are reshaping breast cancer taxonomy. It has been demonstrated that breast cancers can be classified according to their gene expression profiles into four main groups: basal-like, luminal (A and B), HER2+ and normal breast-like breast carcinomas.[1–6] Most importantly, these groups have prognostic and predictive implications.[1–3,5–8] Tumours classified into the basal-like and HER2 groups are reported to have a more aggressive clinical behaviour when compared with carcinomas with luminal and normal breast-like phenotypes.[1–3,5,6]

Recently, Nielsen *et al.*[5] proposed an immunohistochemical panel, comprising oestrogen receptor (ER),

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Table 1. Immunohistochemical panel for breast cancer classification as defined by Nielsen *et al.*

Group	HER2	ER	CK5/6 and/or EGFR
HER2	+	Any	Any
Luminal	–	+	Any
Basal-like	–	–	+
Undetermined	–	–	–

CK, Cytokeratin; EGFR, epidermal growth factor receptor; ER, oestrogen receptor.

epidermal growth factor receptor (EGFR), HER2 and cytokeratin (CK) 5/6, which could be used to identify breast carcinomas with a basal-like phenotype as defined by cDNA microarrays (Table 1). Out of 21 basal-like breast carcinomas by cDNA profiling, 16 were ER– and HER2– and EGFR+ and/or CK5/6+, conferring a sensitivity of 76% and a specificity of 100%.[5]

In a preliminary study to characterize the morphological features of basal-like breast carcinomas,[9] we observed that areas of focal metaplastic change in grade III invasive ductal carcinomas, in the form of spindle and squamous cells, were independent predictors of a basal-like phenotype.

Metaplastic breast carcinoma (MBC) is a descriptive term that refers to a heterogeneous group of tumours characterized by an intimate admixture of adenocarcinoma (i.e. usual types of breast cancer) with metaplastic elements, which can be homologous (squamous or spindle metaplasia) or heterologous (chondroid, osseous or lipomatous differentiation).[10–16] These tumours account for < 1–3.7% of all breast carcinomas, depending on the definition and the type of metaplasia.[10–16] Based upon the immunohistochemical profile and ultrastructural features of MBCs, we[17–20] and others[10,12,21–23] have suggested that these tumours show features of myoepithelial differentiation. However, to the best of our knowledge, a systematic assessment of a large series of MBCs encompassing tumours with homologous and heterologous elements, with the immunohistochemical panel designed to identify basal-like carcinomas, has never been performed. Here we report an analysis of a large series of MBCs for the expression of ER, HER2, EGFR and CK5/6. In addition, we also analysed the distribution of progesterone receptor (PR) and two other basal/myoepithelial markers (CK14 and p63) in these neoplasms.

Materials and methods

CASE SELECTION

Cases of MBC were retrieved from the pathology files of The Royal Marsden Hospital (London, UK), The Norwegian Radium Hospital (Montebello, Norway), Institute of Molecular Pathology and Immunology, University of Porto (Porto, Portugal) (IPATIMUP) and Laboratório Salomão & Zoppi (São Paulo, Brazil). This project was approved by the Local Ethics Committees.

All cases were initially reviewed by the contributing authors, who performed additional immunohistochemical markers for corroborating the diagnosis.

The cases were centrally reviewed by three of the authors (J.S.R-F., F.M. and F.C.S.) on a multiheaded microscope and classified into four categories according to the criteria proposed by Huvos *et al.*[15] and Wargotz and Norris.[10–14] Briefly, tumours were classified as matrix producing breast carcinomas if chondroid and/or osseous matrix was observed in the absence of spindle and osteoclast, i.e. giant cell components.[12] Neoplasms were classified as spindle cell carcinomas if intraductal or infiltrating ductal or squamous carcinoma of ductal origin was contiguous to or subtly merged with a spindle cell proliferation of neoplastic cells, which comprised at least 50% of the tumour bulk.[10] Carcinomas with heterologous elements were defined as tumours with an intraductal or invasive carcinatomous component intimately admixed or subtly merging with a sarcomatous spindle cell component with evidence of chondroid, osseous or rhabdomyoid differentiation.[11,15,16] Carcinomas with squamous differentiation were predominantly (> 50%) or completely composed of apparent squamous cell components admixed with areas of invasive ductal and/or spindle cell carcinoma, in the absence of involvement of the overlying skin.[14,16] A median of two representative blocks from each case were selected for immunohistochemical and chromogenic *in situ* hybridization analysis.

IMMUNOHISTOCHEMISTRY

Routinely fixed and processed, paraffin-embedded representative tissue sections (4 μm thick) of each case were cut and mounted on silane-coated slides. Immunohistochemistry with antibodies for ER, PR, EGFR, HER2, CK5/6, CK14 and p63 was performed according to the streptavidin–biotin–peroxidase complex or EnVision® (DakoCytomation) methods as described elsewhere.[24] Clone details and antigen retrieval methods are summarized in Table 2. Detection was

Table 2. Antibodies and antigen retrieval methods

Antibody	Source	Clone	Dilution	Antigen retriveal
HER2	Dakocytomation, Glostrup, Denmark	Polyclonal (Herceptest)	Prediluted	41 min, water bath
EGFR	Zymed, South San Francisco, CA, USA	31G7	1 : 50	10 min, protease
ER	Dakocytomation, Glostrup, Denmark	ID5	1 : 40	2 min, pressure cooker
CK5/6	Chemicon, Temecula, CA, USA	D516B4	1 : 600	18 min, microwave oven
CK14	Novocastra, Newcastle-upon-Tyne, UK	LL02	1 : 40	18 min, microwave oven
p63	Santa Cruz Biotechnology, Santa Cruz, CA, USA	4A4	1 : 200	18 min, microwave oven
PR	Dakocytomation, Glostrup, Denmark	PGR636	1 : 150	2 min, pressure cooker

CK, Cytokeratin; EGFR, epidermal growth factor receptor; ER, oestrogen receptor; PR, progesterone receptor.

performed with diaminobenzidine chromogen as per routine protocol.[24] Appropriate positive and negative (omission of the primary antibody and substitution of the primary antibody by non-immune immunoglobulin) controls were included in each slide run. Moreover, internal positive controls were available in the vast majority of the cases (i.e. ER and PR, luminal cells of adjacent ducts and acini; EGFR, CK14, CK5/6 and p63, normal myoepithelial cells of adjacent ducts and acini).

Staining results were assessed by three of the authors (J.S.R-F., F.M., F.S.C.) on a multihead microscope. A threshold of ≥10% of positive neoplastic cells was adopted for ER, CK5/6, CK14 and PR. EGFR and HER2 were scored according to the guidelines for Herceptest®.[25] Only nuclear staining was considered positive for ER, PR and p63, whereas only cytoplasmic staining was considered positive for CK5/6 and CK14. Membranous staining with or without cytoplasmic staining was regarded as specific for EGFR and HER2. A case was considered positive for a given marker only when all observers agreed upon its specificity and distribution.

Results

The histological classification and immunohistochemical results are summarized in Table 3.

HER2

All but two MBCs lacked HER2 overexpression (2+ or 3+). Both were carcinomas with squamous metaplasia. Case 14, which showed HER2 2+, was subjected to chromogenic *in situ* hybridization for *HER2*, which demonstrated lack of amplification of *HER2* gene (data not shown). Therefore, this case was considered negative.

OESTROGEN RECEPTOR

One spindle cell carcinoma and one carcinoma with squamous metaplasia were positive for ER. Interestingly, in case 46, the metaplastic spindle cells were positive for ER, whereas in case 12 only the invasive ductal component was ER+, whereas the metaplastic squamous cells were consistently negative.

EPIDERMAL GROWTH FACTOR RECEPTOR

EGFR overexpression, defined as 2+ and 3+ reactivity, was observed in 37/65 (56.9%) of all MBCs, including 12/21 (57.1%) spindle cell carcinomas, 14/19 (73.7%) carcinomas with squamous metaplasia, 5/18 (27.8%) matrix producing breast carcinomas and 5/7 (71.4%) carcinomas with heterologous elements.

CK5/6

Fifty-six of 65 (86.1%) MBCs were positive for CK5/6. Five spindle cell carcinomas, two matrix producing breast carcinomas and two carcinomas with heterologous elements lacked CK5/6 expression.

CK14

Fifty-three of 65 (81.5%) MBCs displayed CK14 positivity. Five spindle cell carcinomas, four matrix producing breast carcinomas, two carcinomas with squamous metaplasia and two carcinomas with heterologous elements lacked CK14 expression.

Interestingly, three cases of spindle cell carcinoma, one matrix producing carcinoma and one carcinoma with heterologous elements lacked both CK5/6

Table 3. Summary of immunohistochemical findings

Case	Diagnosis	Her2	ER	EGFR	CK5/6	CK14	p63	PR
1	Carcinoma with heterologous elements	–	–	–	+	–	+	+
2	Carcinoma with heterologous elements	–	–	1+	+	+	+	–
3	Carcinoma with heterologous elements	–	–	2+	–	+	+	–
4	Carcinoma with heterologous elements	–	–	2+	+	+	+	–
5	Carcinoma with heterologous elements	–	–	3+	–	–	+	–
6	Carcinoma with heterologous elements	–	–	3+	+	+	+	–
7	Carcinoma with heterologous elements	–	–	3+	+	+	+	–
8	Carcinoma with squamous metaplasia	–	–	–	+	+	+	+
9	Carcinoma with squamous metaplasia	–	–	–	+	+	–	+
10	Carcinoma with squamous metaplasia	–	–	1+	+	+	–	–
11	Carcinoma with squamous metaplasia	1+	–	1+	+	+	–	–
12	Carcinoma with squamous metaplasia	–	+	1+	+	+	+	+
13	Carcinoma with squamous metaplasia	–	–	2+	+	–	+	–
14	Carcinoma with squamous metaplasia	2+	–	3+	+	+	+	–
15	Carcinoma with squamous metaplasia	–	–	3+	+	+	+	–
16	Carcinoma with squamous metaplasia	–	–	3+	+	+	+	–
17	Carcinoma with squamous metaplasia	–	–	3+	+	+	+	–
18	Carcinoma with squamous metaplasia	–	–	3+	+	+	+	–
19	Carcinoma with squamous metaplasia	–	–	3+	+	+	+	–
20	Carcinoma with squamous metaplasia	–	–	3+	+	+	+	–
21	Carcinoma with squamous metaplasia	–	–	3+	+	+	+	–
22	Carcinoma with squamous metaplasia	–	–	3+	+	+	+	–
23	Carcinoma with squamous metaplasia	–	–	3+	+	+	+	–
24	Carcinoma with squamous metaplasia	–	–	3+	+	+	–	–
25	Carcinoma with squamous metaplasia	3+	–	3+	+	+	+	–
26	Carcinoma with squamous metaplasia	–	–	3+	+	–	+	+
27	Matrix producing carcinoma	–	–	–	–	+	+	–
28	Matrix producing carcinoma	–	–	–	+	+	+	–
29	Matrix producing carcinoma	–	–	–	+	+	+	–
30	Matrix producing carcinoma	–	–	–	+	+	+	–
31	Matrix producing carcinoma	–	–	–	+	+	–	–

Table 3. (*Continued*)

Case	Diagnosis	Her2	ER	EGFR	CK5/6	CK14	p63	PR
32	Matrix producing carcinoma	–	–	–	+	+	–	–
33	Matrix producing carcinoma	–	–	–	+	+	–	–
34	Matrix producing carcinoma	–	–	–	+	+	–	–
35	Matrix producing carcinoma	–	–	–	+	+	–	–
36	Matrix producing carcinoma	–	–	1+	–	–	–	–
37	Matrix producing carcinoma	–	–	1+	+	–	+	–
38	Matrix producing carcinoma	–	–	1+	+	+	+	–
39	Matrix producing carcinoma	–	–	1+	+	+	–	–
40	Matrix producing carcinoma	–	–	2+	+	+	+	–
41	Matrix producing carcinoma	–	–	2+	+	+	+	–
42	Matrix producing carcinoma	–	–	3+	+	–	+	–
43	Matrix producing carcinoma	–	–	3+	+	+	+	–
44	Matrix producing carcinoma	–	–	3+	+	+	+	–
45	Spindle cell carcinoma	–	–	–	–	+	–	–
46	Spindle cell carcinoma	–	+	–	–	–	+	–
47	Spindle cell carcinoma	–	–	–	+	+	+	–
48	Spindle cell carcinoma	–	–	–	+	+	+	–
49	Spindle cell carcinoma	–	–	–	+	+	+	–
50	Spindle cell carcinoma	–	–	–	+	+	+	–
51	Spindle cell carcinoma	–	–	–	+	+	+	–
52	Spindle cell carcinoma	–	–	1+	+	+	+	–
53	Spindle cell carcinoma	–	–	1+	+	+	+	–
54	Spindle cell carcinoma	–	–	2+	–	–	+	–
55	Spindle cell carcinoma	–	–	2+	–	–	+	–
56	Spindle cell carcinoma	–	–	2+	+	+	+	–
57	Spindle cell carcinoma	–	–	3+	–	–	+	–
58	Spindle cell carcinoma	–	–	3+	+	–	–	–
59	Spindle cell carcinoma	–	–	3+	+	+	+	–
60	Spindle cell carcinoma	–	–	3+	+	+	+	–
61	Spindle cell carcinoma	–	–	3+	+	+	+	–
62	Spindle cell carcinoma	–	–	3+	+	+	+	–

Table 3. (*Continued*)

Case	Diagnosis	Her2	ER	EGFR	CK5/6	CK14	p63	PR
63	Spindle cell carcinoma	–	–	3+	+	+	+	–
64	Spindle cell carcinoma	–	–	3+	+	+	+	–
65	Spindle cell carcinoma	–	–	3+	+	+	+	–

CK, Cytokeratin; EGFR, epidermal growth factor receptor; ER, oestrogen receptor; PR, progesterone receptor.

and CK14 expression. However, these cases either expressed other keratins (CK8/18, CK19 or 34βE12, data not shown) and/or were associated with high-grade ductal carcinoma *in situ*.

p63

Fifty-two out of 65 (80%) MBCs showed p63 expression. In seven matrix producing breast carcinomas, four carcinomas with squamous metaplasia and two spindle cell carcinomas < 10% of p63+ neoplastic cells were identified, rendering these cases negative. Interestingly, all but one of the cases that lacked both CK5/6 and CK14 were positive for p63. This case was a matrix producing breast carcinoma which showed positivity for CK8/18 and CK19.

PROGESTERONE RECEPTOR

Four of 19 (21.0%) carcinomas with squamous metaplasia and one of seven (14.3%) carcinomas with heterologous elements expressed PR, which was largely restricted to areas with squamous metaplasia. Interestingly, in cases 8 and 26, PR decorated only the non-metaplastic elements. The remaining cases consistently lacked PR expression.

CLASSIFICATION OF METAPLASTIC BREAST CANCER INTO HER2, LUMINAL AND BASAL-LIKE GROUPS

Following the immunohistochemical panel proposed by Nielsen *et al.*, 59 out of 65 MBCs (90.8%) displayed the typical immunophenotype of basal-like breast carcinomas (Table 4 and Figures 1, 2 and 3). Three cases, two matrix producing breast carcinomas (cases 27 and 36) and one spindle cell carcinoma (case 45), were negative for ER and HER2, but also lacked EGFR and CK5/6 expression.

Two cases showed the immunophenotype of luminal tumours (one carcinoma with squamous metaplasia and one spindle cell carcinoma) and another case of carcinoma with squamous metaplasia was classified into the HER2 group. Interestingly, all of these cases expressed at least one basal/myoepithelial marker.

Furthermore, one matrix producing breast carcinoma (case 27) and one spindle cell carcinoma (case 45) that were both HER2–, ER–, CK5/6– and EGFR– were positive for p63 and/or CK14. Hence, these cases could be considered of basal-like phenotype if these antibodies were included in the immunohistochemical panel for identifying basal-like tumours.

Discussion

We have demonstrated that 90.8% of MBCs show a basal-like immunophenotype as defined by Nielsen *et al.*[5] By including p63 and CK14 in the immunohistochemical panel, 61 out 65 (93.8%) MBCs were classified as basal-like tumours.

In previous studies addressing clinicopathological characteristics of basal-like carcinomas, it has been reported that these tumours are usually of high grade, lack well-formed ductal structures, harbour high proliferation rates, have centrally necrotic/sclerotic zones and show a proclivity to disseminate to the brain and lungs, sparing regional nodes, liver and

Table 4. Classification of 65 metaplastic breast carcinomas according to the immunohistochemilcal panel proposed by Nielsen *et al.*

Histological type	*N*	HER2	Luminal	Basal-like	Undetermined
Spindle cell carcinoma	21	0	1	19	1
Carcinoma with squamous metaplasia	19	1	1	17	0
Matrix producing breast carcinoma	18	0	0	16	2
Carcinoma with heterologous elements	7	0	0	7	0

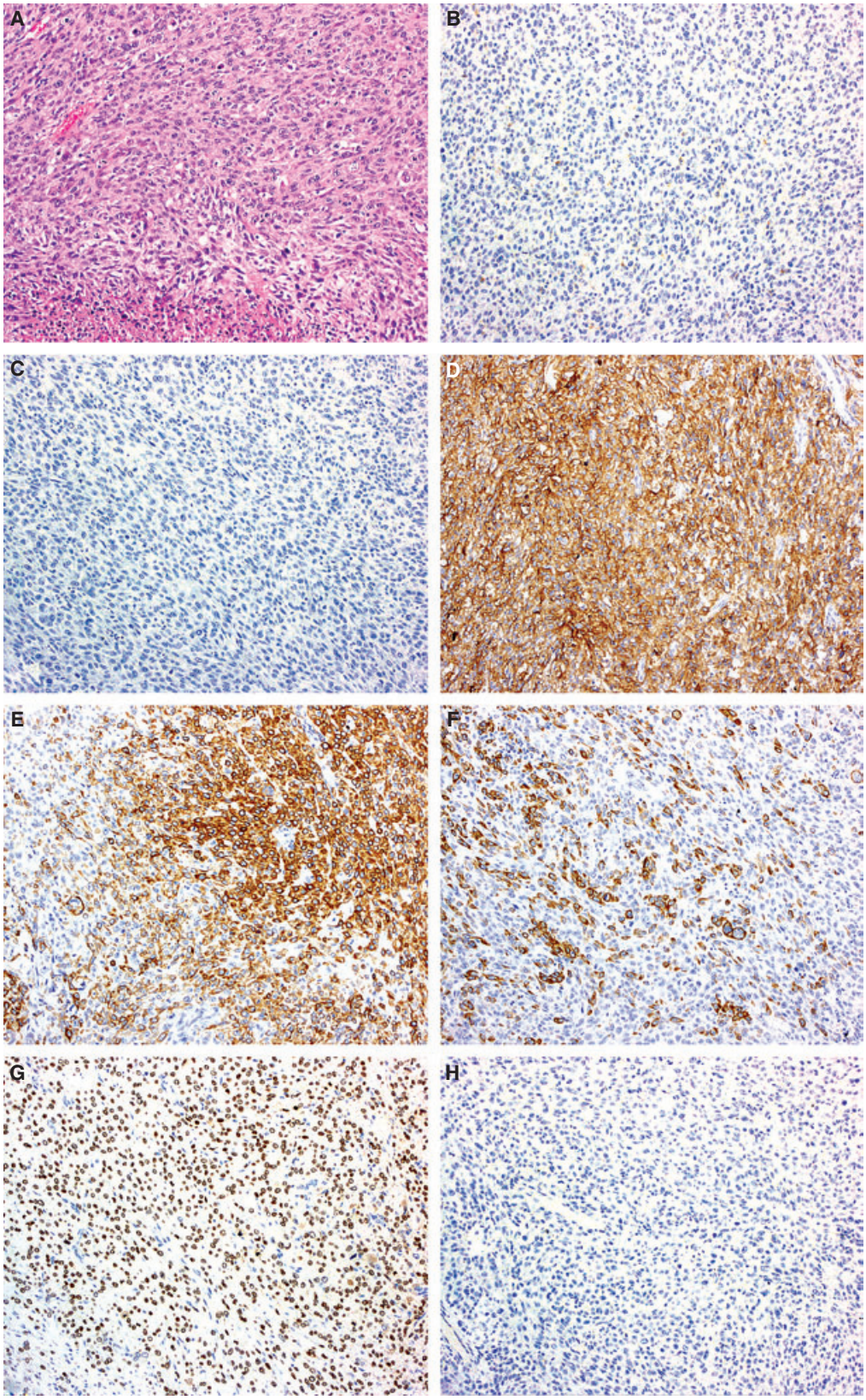

Figure 1. Spindle cell carcinoma (case 63): **A,** H&E; **B,** HER2; **C,** ER; **D,** EGFR; **E,** CK5/6; **F,** CK14; **G,** p63; **H,** PR.

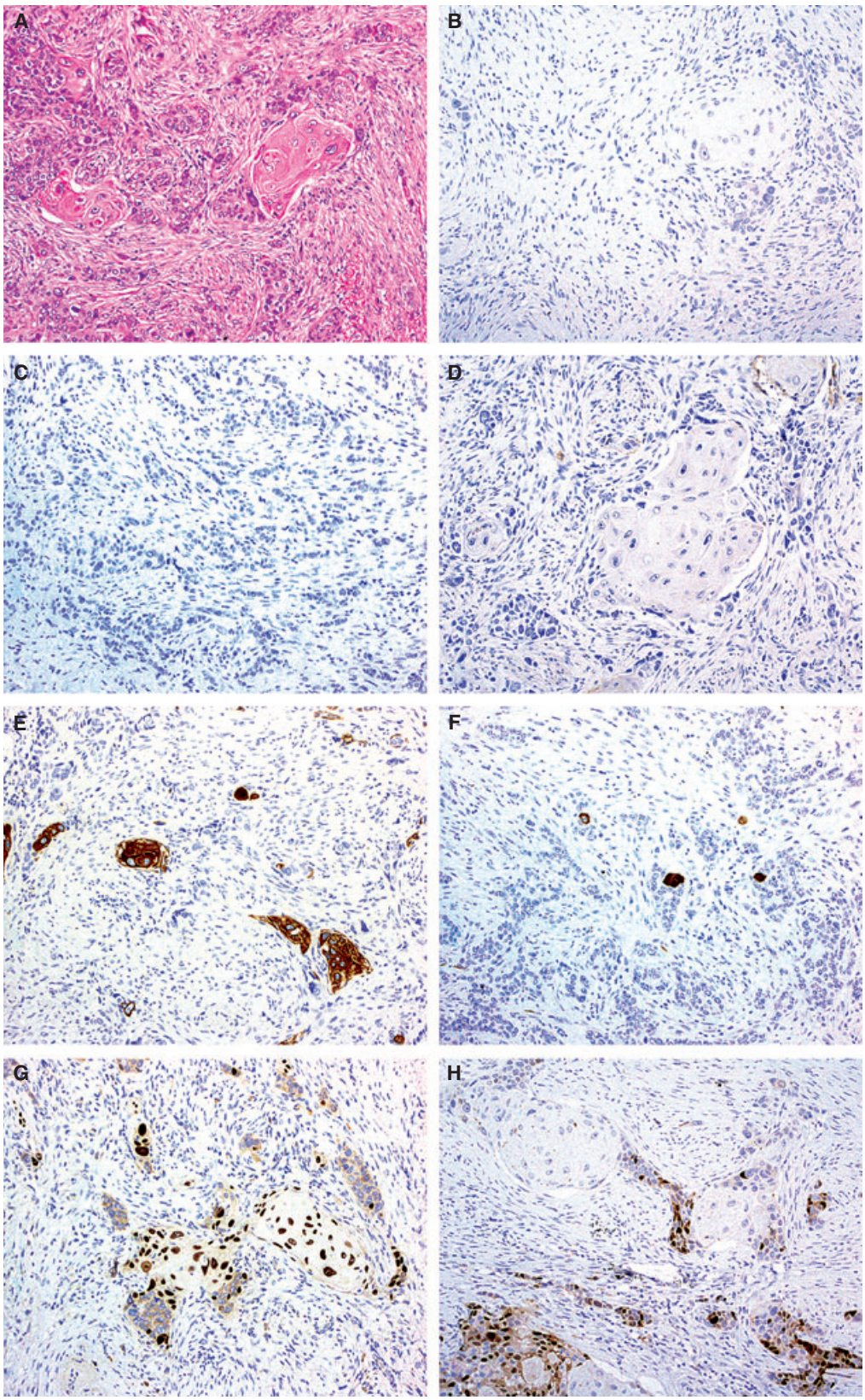

Figure 2. Carcinoma with squamous metaplasia (case 8): **A,** H&E; **B,** HER2; **C,** ER; **D,** EGFR; **E,** CK5/6; **F,** CK14; **G,** p63; **H,** PR.

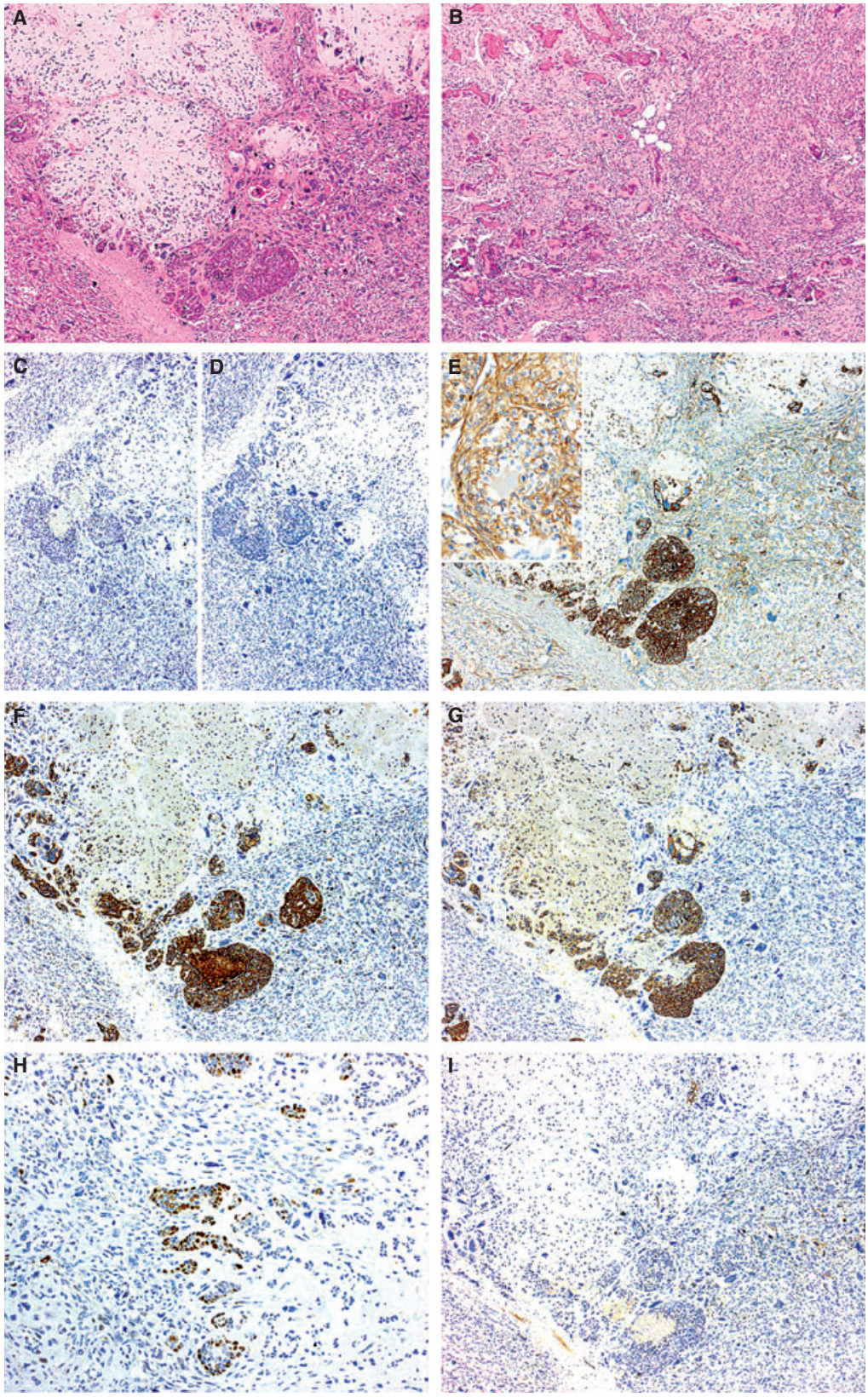

Figure 3. Carcinoma with heterologous elements (case 7): **A,B,** H&E; **C,** HER2; **D,** ER; **E,** EGFR, inset EGFR; **F,** CK5/6; **G,** CK14; **H,** p63; **I,** PR.

bone.[7,26–28] Recently, Jacquemier *et al.* demonstrated that medullary carcinomas also show a basal-like immunophenotype.[29] Our results suggest that the spectrum of basal-like breast carcinomas is wider than previously appreciated and that metaplastic elements are also features of basal-like breast cancers.

We and others have demonstrated that matrix producing breast carcinomas, spindle cell carcinomas and carcinomas with heterologous elements consistently show features of basal/myoepithelial differentiation.[10,12,17–19,21,22,30,31] It has been suggested that breast carcinomas with squamous metaplasia should not be considered within the category of tumours with myoepithelial differentiation.[23] Although these tumours consistently express basal markers (CK5/6, Ck14 and p63),[18,19,23,32] expression of myoid markers is usually not found.[19,23] However, there are several lines of evidence to suggest that carcinomas with squamous metaplasia should also be considered part of the spectrum of tumours with basal/myoepithelial differentiation: (i) Raju[33] and Reddick *et al.*[34] have demonstrated a transition between myoepithelial cells and squamous cells in benign breast lesions at histological, immunohistochemical and ultrastructural levels; (ii) sorted breast myoepithelial cells, and not breast luminal cells, undergo squamous metaplasia when cultured in specific media;[35] (iii) foci of squamous metaplasia are frequently found in spindle cell carcinomas and spindle cell metaplasia is not rare in breast carcinomas with squamous metaplasia;[10,11,16,31,36,37] and (iv) matrix producing breast carcinomas and carcinomas with heterologous elements frequently harbour foci of squamous differentiation.[12] Therefore, the classification proposed by Leibl *et al.*[23] in which MBCs are grouped into (i) MBCs with squamous differentiation and (ii) MBCs with a myoepithelial immunophenotype seems to be artificial and unjustified.

The fact that up to 93.8% of all MBCs display a basal-like phenotype has a significant impact on our understanding of the biology and management of patients diagnosed with these lesions. Basal-like breast carcinomas are reported to have a more aggressive clinical behaviour and a less significant response to anthracycline-based adjuvant chemotherapy than luminal and normal-like breast carcinomas.[37a] Furthermore, Rouzier *et al.*[38] have recently demonstrated that up to 45% of basal-like breast carcinomas show a pathological complete response after 12 weeks of paclitaxel followed by four courses of neoadjuvant chemotherapy with 5-fluorouracil, doxorubicin and cyclophosphamide.[38]

A surprising finding in this study is that 7.7% of MBCs showed expression of PR. Interestingly, PR positivity was almost restricted to the metaplastic squamous cells of the carcinoma with heterologous elements and the four carcinomas with squamous metaplasia. However, PR is not part of the 'intrinsic gene list' used in cDNA microarray studies to classify breast carcinomas into the five main groups.[2–4,38] Although PR expression *per se* would not render the classification of these tumours as basal-like invalid, it suggests that basal-like carcinomas are not homogeneous. In fact, there are several lines of evidence to suggest that basal-like carcinomas are not homogeneous in terms of their expression profiles[6,8,29,39] and molecular genetic features.[40]

Unlike the majority of invasive ductal breast carcinomas, MBCs are unlikely to respond to conventional hormone therapy and anti-Her2 therapeutic schemes, as these lesions consistently lack ER expression and HER2 overexpression/gene amplification.[41–43] In a recent study, our group has shown that up to 25% of MBCs harbour *EGFR* gene amplifications.[41] Given that there are compelling data to suggest that tumours harbouring *EGFR* amplification may respond to EGFR inhibitors,[44] studies addressing the efficacy of these agents for the treatment of patients with MBCs are warranted.

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