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Original Article

Morphological features and mucin expression profile of breast carcinomas with signet-ring cell differentiation

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ABSTRACT

Signet-ring cells are relatively common in breast cancers but are frequently overlooked. Although previously defined as a subtype of mucin producing carcinomas, breast carcinomas with signet-ring cell (SRC) differentiation nowadays are not considered a distinct entity.

The objective of the present study was to characterize the morphological features and mucin expression profile of breast carcinomas with SRC differentiation. All breast carcinomas diagnosed at Centro Hospitalar S. Joao between 1996 and 2006 in which the pathology report mentioned the presence of SRCs ($n = 11$) and four mucinous carcinomas were included in the study. The frequency of SRCs and immunohistochemistry expression of MUC1/MUC2/MUC5AC/MUC6 were evaluated.

We confirmed that SRC differentiation can occur in different histological types, including ductal, lobular, mucinous and metaplastic carcinomas. The proportion of SRCs was highly variable (range: 8–70%). Tumors encompassed SRCs of intracytoplasmic lumina and goblet-cell type. A higher percentage of SRCs was associated with lymphovascular invasion ($p = 0.047$). All tumors expressed cytoplasmic and membranous MUC1. Secretory mucins were more frequent in mucinous carcinomas and in carcinomas with extensive SRC differentiation.

We conclude that besides the usefulness of mucin immunodetection for the differential diagnosis of carcinomas with SRC differentiation of breast origin, it is important to report SRC differentiation regardless of histological type because of its intrinsic prognostic value.

Introduction

Breast carcinomas with signet-ring cell (SRC) differentiation, although previously defined as a subtype of mucin producing carcinomas, are no longer considered by the World Health Organization (WHO) to represent a distinct entity [1,2]. Nevertheless, SRC differentiation has been associated with a greater frequency of axillary lymph nodal involvement and a higher mortality rate than those observed in other forms of breast cancer [3–5].

SRCs are characterized by a well-defined globule of mucinous material that causes peripheral displacement of the nucleus. SRCs may have different morphological characteristics: intracytoplasmic lumina containing some eosinophilic material or apparently empty – "intracytoplasmic lumina type"; pale, homogeneous eosinophilic cytoplasm – "eosinophilic type"; abundant, finely vacuolated and faintly basophilic cytoplasm – "goblet-cell type" [6].

Mucins are high molecular weight glycoproteins, classified into two main groups: secretory (MUC2, MUC5AC, MUC5B, and MUC6) and transmembrane mucins (MUC1, MUC3A, MUC3B, MUC4, MUC12, MUC16 and MUC17) [7]. Much interest has been paid to the expression of mucins because of their potential role in distinguishing metastasis of breast carcinoma from those of gastrointestinal origin, as well as for their prognostic role in breast cancer [8–10].

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Table 1
Clinical and pathological features of patients with breast carcinomas with signet ring cell (SRC) differentiation.

Case	Age	Tumor site	Surgery	Tumor size (mm)	Histologic type	Grade	LVI	Lymph node status	SRC%	Follow-up (months)	Status	ChT	RT	HT
1	61	L-UOQ	TM	45	Ductal	3	Yes	n/a	24	9	DOD	Yes	Yes	n/a
2	82	R-OQT	Lump	14	Ductal	1	No	0/4(sn)	23	106	AFD	No	Yes	Yes
3	49	L-UOQ	Lump + nd	9	Ductal	1	No	1/7	15	82	AFD	No	Yes	Yes
4	81	L-C	TM	34	Ductal	3	Yes	n/a	51	59	AFD	No	No	Yes
5	35	R	TM + nd	40	Ductal	1	Yes	18/18	61	102	DOD	Yes	Yes	n/a
6	77	L-OQT	TM + nd	23	Ductal	2	Yes	2/7	70	87	AFD	No	Yes	Yes
7	34	R-C	TM + nd	23	Ductal	2	No	1/5	31	68	AFD	Yes	No	Yes
8	62	R-UOQ	Lump	n/a	Lobular	1	No	0/3(sn)	50	112	AFD	No	No	Yes
9	83	R-UQT	Lump + nd	23	Lobular	3	No	0/11	33	87	DOC	No	Yes	Yes
10	54	L-C	TM + nd	85	Lobular	2	Yes	12/12	58	2	DOD	No	Yes	n/a
11	51	R-LOQ	TM + nd	33	Metaplastic	3	No	0/14	[a]	151	AFD	Yes	No	n/a
12	85	R	TM	50	Mucinous	1	No	n/a	15	13	DOC	No	No	Yes
13	64	R-UIQ	Lump	14	Mucinous	2	No	0/2 (sn)	16	84	AFD	No	Yes	Yes
14	82	C	TM + nd	40	Mucinous	1	No	0/8	17	120	AFD	n/a	n/a	n/a
15	81	L-UOQ	TM + nd	32	Mucinous	1	No	0/8	8	136	AFD	n/a	n/a	n/a

n/a – not available/not aplicable. AFD – alive and free of disease; C – central area; Chm – chemotherapy; DOD – dead of disease; DOC – dead of other cause; HT – hormone therapy; L – left; LOQ – lower outer quadrant; LVI – lymphovascular invasion; Lump – lumpectomy; nd – lymph node dissection; OQT – outer quadrant transition; R – right; RT – radiotherapy; sn – sentinel lymph node; TM – total mastectomy; UIQ – upper inner quadrant; UOQ – upper outer quadrant; UQT – upper quadrant transition.

[a] Signet ring cell percentage was not calculated because they were present only in a small focal area.

The aim of the present study was to characterize the morphological features and mucin expression profile of breast invasive carcinomas with SRC differentiation.

Materials and methods

Following ethical approval by the Ethical Committee for Health of Centro Hospitalar S. João (CHSJ), breast invasive carcinomas diagnosed between 1996 and 2006, in which the pathology report mentioned the presence of SRC ($n = 11$), were retrieved from CHSJ surgical pathology database. These represented 1.2% of all breast invasive carcinomas diagnosed in the same period of time. Four representative mucinous carcinomas were randomly selected for the study. Clinical information was collected from the medical records, and the pathological material was reviewed according to the current WHO guidelines [2]. In each case, the tumor tissue had been fixed in 10% buffered formalin and embedded in paraffin; 3-μm thick sections were stained with hematoxylin–eosin. Representative sections of the tumors were stained with periodic acid-Schiff with diastase digestion (PAS-D). The presence of SRC in each tumor was evaluated quantitatively by two observers (CB and CE) that independently determined the percentage of SRCs among the total number of tumor cells assessed in ten high power fields centered in SRCs hot spots.

Immunohistochemical assays were carried out using the avidin–biotin method as previously described [11]. MUC1, MUC5AC, MUC6, and MUC2 were detected using monoclonal antibodies HMFG [12], CLH2 [13], CLH5 [14], and PMH1 [15], respectively. Estrogen receptor (ER) (clone: SP1, Neomarkers, Fremont, CA, USA), E-cadherin (clone: 4A2C7, Zymed/Invitrogen, Carlsbad, CA, USA), CD56 (clone: 1B6, Novocastra, Newcastle, UK), synaptophysin (27G12, Novocastra, Newcastle, UK) and GCDFP-15 (clone: 23A3, Thermo Scientific, Fremont, CA, USA) expression were also evaluated. A case was considered ER positive when more than 1% of the tumor cells were positive. For the remaining antibodies, a 5% cut-off was used [16].

Data was analyzed using STATA (STATACorp, Texas, USA), version 12.1. Mann–Whitney and Kruskal–Wallis rank tests were used to compare mean SRC percentage between tumors with and without lymphovascular invasion, lymph node metastases, and tumor grade. Spearman correlation coefficient was used to test the association between SRC percentage and tumor size. A p value equal or less than 0.05 was considered significant.

Results

The clinical and pathological features of the 15 cases are summarized in Table 1. The mean and median ages were 65.4 and 64 years, respectively, with a range of 34–85 years. The most frequent location of tumors was the upper outer quadrant of the breast. All patients were submitted to diagnostic fine needle aspiration or core biopsy. Of these only two core biopsies were available for review and showed signet ring cells representation. Of the remaining biopsies, three also mentioned signet rings in the report. Lumpectomy ($n = 5$) or total mastectomy ($n = 10$) was performed, with sentinel node biopsy ($n = 12$) followed by nodal dissection whenever appropriate ($n = 9$). Three patients did not undergo lymph node evaluation. Four patients were treated with chemotherapy, eight patients underwent local radiotherapy and nine received hormone therapy. For two patients, data on adjuvant treatment were not available, and for six, there was no information concerning hormone treatment. The median follow-up time was 87 months, ranging from 2 months to 12.6 years. The three patients who died of disease had distant tumor spread at diagnosis.

The size of the tumors ranged from 0.9 to 8.5 cm (mean: 3.3 cm) in maximum diameter. The gross specimens of the 11 cases identified as displaying SRCs (Table 1 – cases 1–11) were invariably described as whitish-gray, ill-defined masses. In the mucinous carcinoma cases, the lesions were described as gelatinous masses. Histologically, seven of the 11 tumors with SRCs were classified as ductal carcinoma (one of which rich in osteoclast-like giant cells), three as lobular carcinoma (two pleomorphic and one classical) and one as metaplastic carcinoma (with mixed squamous cell and spindle cell components). All but one (the metaplastic carcinoma) of these 11 tumors had easily recognizable areas of SRC that constituted variable proportions of the total of tumor cells, ranging from 15% to 70%. SRCs were also identified in mucinous carcinomas, ranging from 8% to 17%. Two types of SRCs were observed: SRCs of the intracytoplasmic lumina type and of goblet cell type (Fig. 1). Intracellular mucin was confirmed by PAS-D staining in all tumors. Extracellular mucin was only seen in mucinous carcinomas.

In ductal carcinomas, the areas containing the highest concentrations of signet-ring cells were characterized by a more diffuse growth pattern, frequently with a solid, cohesive aspect (Fig. 2A). Areas of well-formed neoplastic acini containing SRCs were also seen. In ductal carcinomas, the SRCs had a variable appearance from case to case, some being of goblet cell and others of intracytoplasmic lumina type. Two ductal carcinomas had ductal carcinoma