Pathology – Research and Practice 210 (2014) 432–439

Contents lists available at ScienceDirect

Pathology – Research and Practice

journal homepage: www.elsevier.com/locate/prp

Original Article

Expression of cancer stem cell markers in basal and penta-negative breast carcinomas – A study of a series of triple-negative tumors

Diego de Mendonça Uchôa [a,c,*], Marcia Silveira Graudenz [a,b,c], Sidia Maria Callegari-Jacques [d,e], Carolina Rigatti Hartmann [a], Bruna Pellini Ferreira [b], Mariana Fitarelli-Kiehl [e], Maria Isabel Edelweiss [a,b,c]

[a] Department of Pathology, Hospital de Clínicas de Porto Alegre (HCPA), Porto Alegre, Brazil
[b] School of Medicine, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil
[c] Post-Graduate Program in Medicine: Medical Sciences, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil
[d] Statistics Department, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil
[e] Post-Graduate Program in Genetics and Molecular Biology, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil

ARTICLE INFO

Article history:
Received 27 September 2013
Received in revised form 15 January 2014
Accepted 10 March 2014

Keywords:
Breast cancer
Basal
Triple-negative
CD44
CD24

ABSTRACT

Purpose: Breast cancer is a heterogeneous disease. Immunohistochemistry has given rise to triple-negative carcinoma (TNC). Concomitantly, biological origins of neoplasia and its heterogeneity has been strongly debated in cancer stem cells (CSC) theme. This study investigates the prevalence of basal (BCC) and penta-negative carcinomas (5NC) in TNC and establishes associations with CSC (CD44CD24).
Materials and methods: 94 TNC were tested for CK5/6, HER1, CD44 and CD24, evaluated by a simple immunohistochemistry score and correlated with clinicopathological and survival data.
Results: BCC had higher tumor grades than 5NC ($p = 0.004$). CD44 negativity ($p = 0.007$) and $CD44^{-}CD24^{+}$ phenotype ($p = 0.013$) were associated with less vascular invasion amongst TNC. CD44 expression was associated with BCC ($p = 0.007$). $CD44^{-}CD24^{-/low}$ phenotype was associated with 5NC. None of the variables were associated with clinical outcome.
Conclusion: BCC and 5NC are closely related tumor subtypes. $CD44^{-}CD24^{-/low}$ phenotype was associated with 5NC and $CD44^{-}CD24^{+}$ phenotype was associated with vascular invasion. These results require histogenetic confirmation in larger studies.

Introduction

Breast cancer is a heterogeneous disease. Its clinical presentation, biological potential, response to treatment and prognosis can differ significantly [1–6]. The diagnostic and prognostic factors traditionally used in breast cancer have proven insufficient in some cases and there is a clear need for more refined diagnostic criteria.

Depending on immunohistochemistry (IH), triple-negative breast cancer (TNC) emerged in the literature due to its defined poor prognosis and lack of important therapeutic modalities, such as anti-estrogen and anti-HER2 therapies [7–17]. Likewise, in the histogenetic reclassification of breast tumors, basal cell carcinoma (BCC) also exhibits aggressive biological potential [18–21]. In recent years, there has been much discussion regarding the need to separate these two tumor subtypes, although approximately 70–80% of them are the same [22–31]. Despite a considerable overlap between these two entities, there is a large component of both tumor groups that is unrelated [22,24,25,31,32]. Recent studies in TNC, on a simplified IH panel, have indicated that BCC exhibit a worse prognosis than "non-basal TNC", i.e., penta-negative carcinomas (5NC) [26,31,33–35].

The biological origin of neoplasia through the theory of breast cancer stem cells (CSC) has been highlighted in the literature [36–41]. The prospective identification of CSC through the $CD44^{+}CD24^{-/low}$ phenotype was demonstrated in breast cancer [42,43] and, because it was also considered a marker of poor prognosis and BCC, this CSC phenotype rapidly gained notoriety [38,44–46]. However, in a few studies, TNC and BCC subtypes have been compared for the different immunohistochemical expression of CSC [47–50].

* Corresponding author at: Department of Pathology, Hospital de Clínicas de Porto Alegre, Rua Ramiro Barcelos, 2350, Bom Fim, CEP 90035-903, Porto Alegre, Brazil. Tel.: +55 51 33598313; fax: +55 51 33598334.
E-mail address: dmudmu@gmail.com (D. de Mendonça Uchôa).

http://dx.doi.org/10.1016/j.prp.2014.03.005
0344-0338/

This is an IH study, designed to evaluate clinicopathological differences between BCC and 5NC and to assess CSC (CD44 and CD24) expression in BCC and 5NC in a series of TNC.

Materials and methods

All TNC were retrieved from the Hospital de Clínicas de Porto Alegre (HCPA) pathology department database between 2001 and 2006. The sample included 119 patients, 14 of whom were excluded because the paraffin blocks were no longer available, nine due to little or no residual tumor in the blocks (core-biopsies) and two due to inappropriate neoplasia for testing (in situ carcinoma and bone marrow metastasis). The final sample consisted of 94 patients. Clinical, anatomopathological and follow-up data were collected by searching HCPA database or from private medical records and by telephone. This study was approved by Research Ethics Committee of HCPA.

Tissue microarray (TMA) was performed in all cases where there was sufficient tumor for extraction with the removal of a 3-mm diameter cylinder from each tumor. The IH technique was initiated on a Dako automated device (PT Link model) and then was processed on an automated Dako Autostainer Link 48 (Dako, Carpinteria, CA, USA). CK5/6 Dako antibody (D5/16B4 clone, ready to use, retrieval in a high-pH buffer for 20 min at 98 °C), Zymed HER1 antibody (EGFR) (31g7 clone, ready to use, with Pepsin Digest-all 3, retrieval for 5 min at 37 °C), Cell Marque CD44 antibody (MRQ-13 clone, 1:200 dilution, retrieval in a high-pH buffer for 20 min at 98 °C) and Neomarquers CD24 antibody (SN3b clone, 1:50 dilution, retrieval in a low-pH buffer for 20 min at 98 °C) were used. The slides were assessed by two experienced breast pathologists based on a consensus of diagnostic observations and without prior knowledge of the clinicopathological characteristics of the studied tumors. All cases were evaluated with respect to the quantity of positive tumor cells, coding as 0 (0%), 1 (0.1–1%), 2 (1.1–10%), 3 (10.1–33%), 4 (33–66%) and 5 (>66%). TNC were considered BCC when there was expression of CK5/6 and/or HER1; 5NC were those that were negative for both markers. For CK5/6, cytoplasmic staining was taken into account; for HER1, membrane staining was considered. Expressions with score ≥1 were considered positive. For CD44 and CD24, membrane and cytoplasmic staining of neoplastic cells was considered. CD44 expression was considered positive if score ≥1. For CD24 scores of 4 and 5 were considered positive and scores of 0, 1, 2 and 3 were considered negative or low (*−/low*).

Data is presented as the means and standard deviations or absolute numbers and percentages. The categorical variables were compared using a Chi-squared test, and an exact *p*-value was obtained for the test. Whenever necessary, an analysis of residuals was also performed. The analyses were performed using SPSS (PASW Statistics 18.0 [2009]) available at www.spss.com.hk/statistics and WinPEPI [51]. We considered *p*-values <0.05 to be statistically significant.

Table 1
Clinicopathological parameters of the triple-negative series.

	n	%
Histologic type		
Ductal	80	85.1
Lobular	2	2.1
Atypical medullary	1	1
Metaplastic	1	1
Mixed (ductal + lobular)	1	1
NA	9	9.5
Tumor size		
≤2.0 cm	21	22.3
2.0–4.9 cm	35	37.2
≥5.0 cm	15	15.9
NA	23	24.4
Tumor grade		
Grade 1	3	3.1
Grade 2	26	27.6
Grade 3	43	45.7
NA	22	23.4
Lymphovascular invasion		
Present	21	22.3
Absent	26	27.6
NA	47	50
Lymph nodes		
Negative	34	36.8
1–3 LN+	18	19.1
≥4 LN+	22	23.4
NA	20	21.3
Recurrence		
No	26	27.7
Yes	37	39.4
NA	31	33.0
Condition at the end of the study		
Alive	46	48.9
Deceased	31	33.0
NA	17	18.1
Type of material analyzed		
TMA (surgery and nodulectomy)	71	75.5
Biopsy (core-needle)	23	24.4

NA, not available.

Results

Clinicopathological characteristics are shown in Table 1. Eighty-five percent of TNC were ductal not otherwise specified (NOS). The majority of the tumors (46%) were grade 3. Tumor size greater than 2.0 cm was identified in 50 cases (53%), of which 15 (16%) were larger than 5.0 cm. Some cases (17/94) were received for second opinion and immunohistochemical examination, but without data regarding the age of patients. The mean age was 55.4 years, ranging from 25 to 81. The mean follow-up time was 71.5 months (2–353).

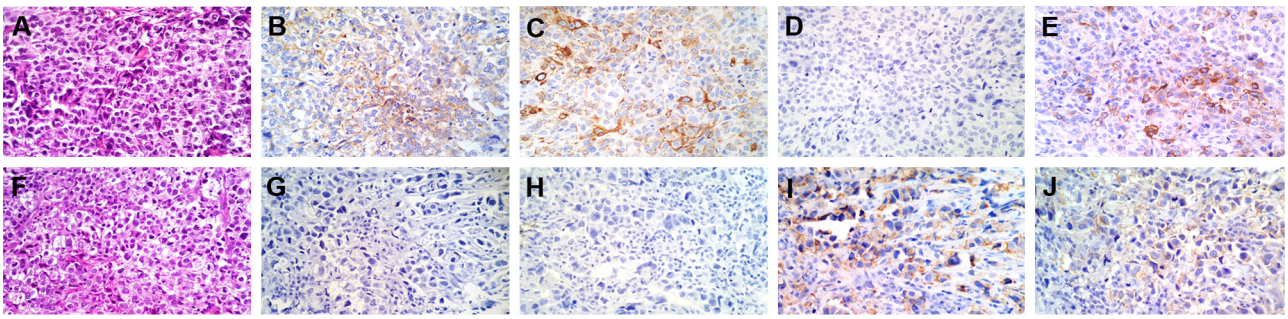

Fig. 1. BCC (a) HE staining; (b) membranous HER1 expression; (c) cytoplasmic CK5/6 expression; (d) CD44 negative; (e) CD24 mainly cytoplasmic staining (400×). 5NC (f) HE staining; (g) HER1 negative; (h) CK5/6 negative; (i) CD44 mainly membranous staining; (j) CD24 mainly membranous staining (400×).