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Spatial distribution of mast cells and macrophages around tumor glands in human breast ductal carcinoma

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ABSTRACT

Macrophages and mast cells are usually present in the tumor microenvironment and play an important role as regulators of inflammation, immunological response and angiogenesis in the tumor microenvironment. In this study, we have evaluated macrophage, mast cell, and microvessel density in a selected group of different grade of invasive breast carcinoma tumor specimens. Furthermore, we have investigated the pattern of distribution of CD68-positive macrophages and tryptase-positive mast cells around tumor glands. Results have shown that: A) Macrophages are more numerous in G2 and G3 breast cancer stages respect to controls, the per cent of macrophages in G1 samples was comparable to the controls, and the spatial relationship between macrophages and glands (as indicated by the mean cell-to-gland distance) correlated with CD31-positive vessels. B) Mast cells in G2 and G3 tumor specimens show a significant increase in their number as compared to control samples, and their spatial distribution around the glands did not show any significant difference among groups. Overall, the results of this study confirm the important role of macrophages and mast cells in tumor progression and angiogenesis in human ductal breast cancer, and pointed out the spatial relationship between tumor macrophages and glands, and its correlation with microvascular density.

1. Introduction

Angiogenesis plays a significant role in breast cancer growth and metastasis [1,2]. Many studies have demonstrated that microvascular density correlated with metastatic disease [3]. Moreover, the association with larger tumor size, high grade, lymph node metastasis, and poor prognosis have been demonstrated [4].

Macrophages and mast cells are usually present in the tumor microenvironment and play an important role as regulators of inflammation, immunological response and angiogenesis in the tumor microenvironment [5,6]. Macrophages and mast cells participate to the mammary gland development, both by the clearance of cellular debris and by producing numerous growth factors, angiogenic cytokines, chemokines, and inflammatory mediators regulating the activity of the other stromal cells [7,8].

In this study, we have evaluated macrophage, mast cell, and microvessel density in a selected group of different grade of invasive breast carcinoma tumor specimens. Furthermore, the aim of this study is to investigate the pattern of distribution of tryptase-positive mast cells and CD68-positive macrophages around the blood vessels and glands in human breast ductal carcinoma samples to further contribute to the knowledge about the role of microenvironment during breast cancer progression.

2. Materials and methods

Primary human breast tumors were retrospectively selected from the archive of the Section of Pathology of the Hospital San Paolo, Bari, Italy. Full ethical approval and signed informed consent from individual patients were obtained to conduct the study. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration. All primary tumors were obtained from patients who had undergone breast cancer surgery. Patients had no received neoadjuvant therapies and there were subjected to a modified radical mastectomy. Tumor type and stage were determined according to the

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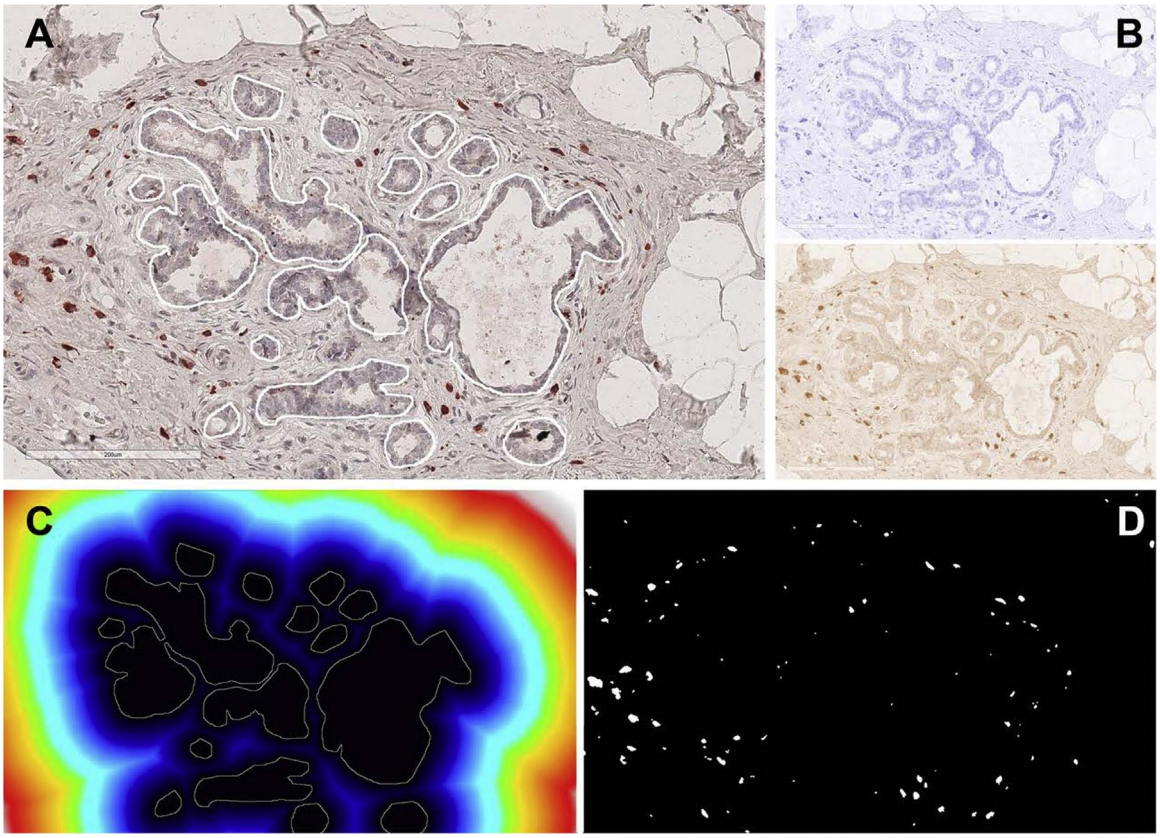

Fig. 1. Main steps of the image analysis procedure. A. Field image showing CD68-stained cells and hematoxylin-stained gland profiles (outlined in white). B. By applying a color deconvolution procedure (see text) the two stains can be efficiently separated and a binary image of the positive cells (shown in D) can be obtained by conventional thresholding. C. Distance transform of the image shown in A: background pixels are labeled according to their distance from the nearest gland profile boundary as indicated by the color-coded map where a blue-to-red scale is used to represent increasing distances. The mean distance of the cell pattern from the glands can then be estimated by averaging the pixel values of the map at the points corresponding to the white pixels in the binary image D.

World Health Organization classification [9] using conventional histology. Bioptic tumor specimens were collected from 30 ductal invasive carcinomas grade 1 (G1), 10 grade 2 (G2), 10 grade 3 (G3), and 10 healthy tissues, histologically confirmed, from patients who undergone breast cancer surgery. Histologic tumor grading, based upon he tumor cell cytology, degree of glandular differentiation and the mitotic count have been estimated accordingly to Nottingham Grading System [10] in G1 (corresponding to low combined histologic grade, slow growing and well-differentiated), G2 (corresponding to intermediate combined histologic grade, moderately differentiated) and G3 (corresponding to highly combined histologic grade, poorly-differentiated and highly proliferative). Tissues were collected at the time of export of the tumor, fixed in 10% buffered formalin for 24 h and paraffin-embedded.

2.1. CD31, CD68 and tryptase immunohistochemistry

Histological sections of 4 μm thickness, collected on poly- L-lysine-coated slides (Sigma Chemical, St Louis, MO, USA), were deparaffinized. The sections were rehydrated in a xylene-graded alcohol scale and then rinsed for 10 min in 0.1 M PBS. Sections were pre-treated with sodium citrate pH 6.1 or pH 9 (Bcl-6) (Dako Corporation, Milan, Italy) in Dako PT Link for antigen retrieval solution for 30 min at 98 °C and then incubated with rabbit polyclonal anti-CD31 (ab28364, Abcam, Cambridge, UK)o stain endothelial cells (vessel quantification through CD31 staining is a standard method for determining intra-tumoral microvessel density and its validity as a prognostic indicator has been established in several malignant tumors [11]), mouse monoclonal anti-tryptase (NB-100–64820, Novus Biologicals, Littleton, CO, USA) to stain mast cells, mouse monoclonal anti-CD68 (NCL-CD68-KP1, Novocastra Laboratories Ltd Newcastle, United Kingdom) stain macrophages, diluted 1:60, 1:1000, 1:50 respectively. Thereafter, the sections were counter-stained with Mayer hematoxylin and mounted in synthetic medium. Specific pre-immune serum (Dako), replacing the primary antibodies, served as negative control.

2.2. CD31, CD68 and tryptase immunohistochemistry quantification

Ten sections from each experimental group were scanned using the whole-slide morphometric analysis scanning platform Aperio Scanscope CS (Leica Biosystems, Nussloch, Germany). All the slides were scanned at the maximum available magnification (20×) and stored as digital high resolution images on the workstation associated with the instrument. Digital slides were inspected with Aperio ImageScope v.11 software (Leica Biosystems, Nussloch, Germany) at 10× magnification and ten fields with an equal area were selected for the analysis at 20× magnification. Fields with an equal area were selected for the analysis by systematic random sampling [12] of the glandular region. The protein expression was assessed with the Positive Pixel Count algorithm embedded in the Aperio ImageScope software and reported as positivity percentage, defined as the number of positively stained pixels on the total pixels in the image. This approach provides a reliable automatic estimation of the amount of stained structures in the tissue [13,14] and is less sensitive to errors linked to high cell densities when compared to methods involving direct cell counting.

2.3. Morphometric characterization of gland tissue, tryptase- and CD68-positive mast cells in the tissue samples

Computer-assisted image analysis was performed to morphometrically characterize gland tissue, tryptase- and CD68-positive mast cells