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Research Article

A fractal analysis of the spatial distribution of tumoral mast cells in lymph nodes and bone marrow

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ABSTRACT

The spatial distribution of mast cells inside the tumor stroma has been little investigated. In this study, we have evaluated tumor mast cells distribution through the analysis of the morphological features of the spatial patterns generated by these cells, including size, shape, and architecture of the cell pattern. We have compared diffuse large B cells lymphoma (DLBCL) and systemic mastocytosis in two different anatomical localizations (lymph nodes for DLBCL and, respectively, bone marrow for mastocytosis). Results have indicated that, despite the high difference in size exhibited by the mast cells patterns in the two conditions, the spatial relationship between the mast cells forming the aggregates resulted similar, characterized by a significant tendency of the mast cells to self-organize in clusters.

1. Introduction

Rudolf Virchow was the first to recognize the presence of inflammatory cells, including macrophages, mast cells, B and T cells, infiltrating tumors and to establish a causative connection between the infiltrate and the development of cancer.

The spatial distribution of mast cells inside the tumor stroma has been little investigated, although it could represent a significant source of information on the biological processes involved in tumor growth and development. In fact, the spatial pattern formed by a cell population in the tissue is intimately linked to a series of interactions each cell can establish with other cells of the same or different type and with the extracellular matrix [1,2]. In a previous research, we have studied the pattern of distribution of mast cells in biopsy samples obtained from four different human tumors, including endometrial adenocarcinoma, advanced primary melanoma, non-small lung carcinoma, and cutaneous mastocytoma [3]. The results have evidenced that a similar spatial arrangement of mast cells was recognizable in all the tumors, despite histopathological differences, and mast cells showed a virtual random spatial distribution, albeit with varying densities.

The aim of this study was to further contribute to our knowledge concerning tumor mast cells distribution through a set of morphometric parameters able to provide a quantitative evaluation of the morphological features of the spatial patterns generated by mast cells, including size of the cell pattern; shape of the cell pattern, and architecture of the cell pattern. We have compared the spatial distribution of mast cells in two different pathological conditions, namely diffuse large B cells lymphoma (DLBCL) and systemic mastocytosis (SM), and two different anatomical localizations (lymph nodes for DLBCL and, respectively, bone marrow for mastocytosis) of mast cells. Thus, the spatial pattern generated by mast cells in a mast cell neoplasm (SM) was compared with the pattern mast cells can form in a different tissue context, namely bone marrow and lymph node microenvironments, where they mainly interact with neoplastic cells of a different type.

2. Materials and methods

2.1. Tissue samples

Tissue specimens were collected from 10 patients affected by DLBCL with lymph node localization and by 10 patients affected by

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mastocytosis with bone marrow localization. The cases were retrospectively selected from the files of the Pathology Section of the Department of Pathology of the University of Bari Medical School. The study was conducted on tissue blocks stored in the archives.

2.2. Immunohistochemistry

Paraffin-embedded tissues representatives of the DLBCL cases were sectioned at 3 μm. The sections were transferred onto poly-L-lysine coated slides and subjected to deparaffinization and rehydratation. After blocking of endogenous peroxidases with a methanol–hydrogen peroxide solution for 30 min a standard heat antigen retrieval in ethylenediaminetetracetic acid (pH 8.0) was performed. The samples were then incubated with antibody against tryptase (dilution 1:150, DAKO, Glostrup, Denmark). The sections were then incubated with biotinylated anti-mouse immunoglobulins, peroxidase-conjugated streptavidin and diaminobenzidine (DAB). Counterstain was performed with Harris hematoxylin. Each immunohistochemistry reaction was coupled with a positive control reaction (reactive lymph node) and a negative control reaction (no primary antibody).

2.3. Aperio scanscope CS whole slide scanning and analysis

For each case, slides stained for tryptase expression were scanned using the whole-slide scanning platform Aperio Scanscope CS (Leica Biosystems, Nussloch, Germany). All the slides were scanned at the maximum magnification available (40 ×) and stored as digital high resolution images on the workstation associated with the instrument. Digital slides were inspected with the Aperio ImageScope v.11 software (Leica Biosystems, Nussloch, Germany) at 20 × and 40 × magnifications and three fields with equal area were selected for the analysis. Tryptase expression was assessed with the Positive Pixel Count algorithm embedded in the Aperio ImageScope software and reported as a percentage of positivity, defined as the number of positively stained pixels on the total of positive and negative pixels of the image.

3. Image analysis procedures, image acquisition and preprocessing

All the image analysis procedures were performed by using the Image J software [4], freely available at http://rsb.info.nih.gov/ij/. They can be summarized as follows. Bright-field images of the immunohistochemical preparations were acquired by using a Leica DMR microscope (Leica Microsystems, Wetzlar, Germany) and a high resolution digital camera (DC 200, Leica Microsystems). At a primary magnification of × 20 three fields randomly chosen within each of two sections per biopsy were selected and their image acquired in full colors (RGB, 24-bit), processed to correct shading, then filed TIFF (Fig. 1A). To identify the pattern generated by mast cells, each image underwent a greyscale (8-bit) conversion, that discards color information and maintains only intensity information. A thresholding operation was then applied in which the threshold to discriminate between tryptase-positive structures and background was automatically identified as the gray level maximizing the inter-class entropy [4]. It was followed by a morphological "opening" [5] to eliminate small artifacts and residual noise. As illustrated in Fig. 1B, this procedure allowed a quite accurate identification of the immune-positive structures.

3.1. Morphometry

The obtained binary images (Fig. 1B) represented the input data for procedures aimed at estimating indices able to capture the following morphological features of the spatial patterns generated by mast cells:

3.1.1. Size of the cell pattern

The amount of tissue tryptase-positive structures account for was directly estimated from the corresponding binary image by evaluating the area fraction occupied by the binary pattern [6].

3.1.2. Shape of the cell pattern

To globally describe the complexity of form in quantitative terms the 'fractal dimension' (D) can be a valuable parameter [7–9]. It measures the rate of addition of structural detail with

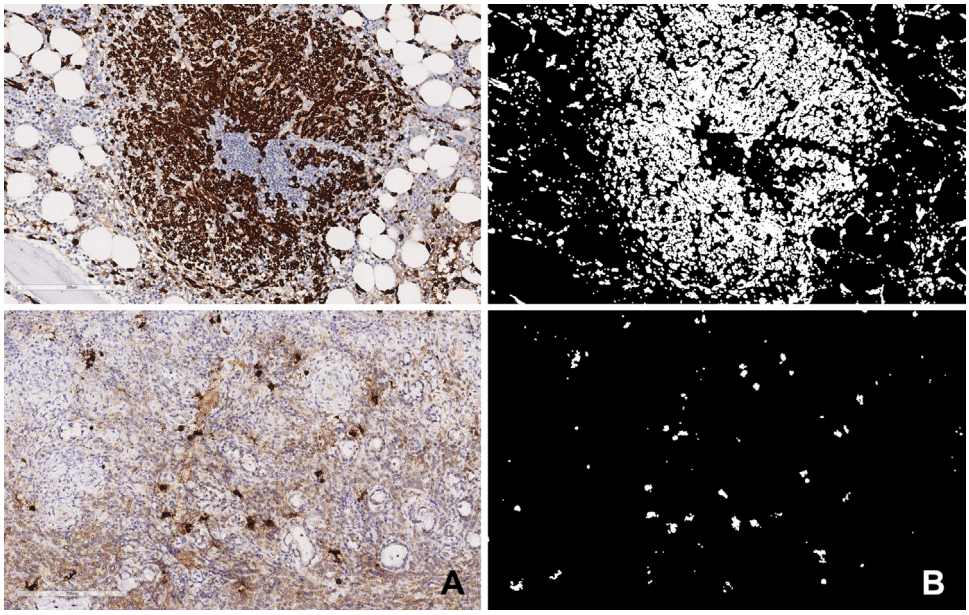

Fig. 1. (A) Tryptase-positive mast cells in bioptic specimens from mastocytosis (upper panel) and lymphoma (lower panel). (B) Binary images of the patterns of immunoreactivity shown in (A). They were identified by an image analysis procedure involving 'maximum enthropy thresholding' followed by morphological filtering to remove small artifacts and residual noise (see text).